

Physiological, Morphological Changes and Storage Root Yield of Sweetpotato [*Ipomoea batatas* (L.) Lam.] under PEG-Induced Water Stress

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Abstract

Sweetpotato is an important tuberous root crop rich in nutrients such as vitamins and carbohydrates, and can grow well in arid regions with less water consuming crop. The aim of this research was to evaluate the storage root yields, physiological, biochemical and morphological traits in sweetpotato cv. 'Japanese Yellow' subjected to polyethylene glycol (PEG)-induced water deficit. At harvest (4 months after planting) the number of storage roots per plant and storage root fresh weight in sweetpotato treated with 5% PEG (-0.54 MPa) in nutrient solution of hydroponic culture declined by 20.0% and 47.4% compared to the control without PEG, respectively. Leaf area and leaf dry weight significantly decreased by 85.6% and 95.3%, respectively when exposed to water deficit stress. Sucrose content (114.7 mg g⁻¹ dry weight; DW) in storage roots of sweetpotato grown under PEG-induced water deficit conditions was enriched by 2.2 fold of control (52.5 mg g⁻¹ DW) and was greater than in storage roots derived from soil culture (70.3 mg g⁻¹ DW). Total soluble sugar in the root and storage root tissues was enriched and may play a key role as osmotic adjustment (OA) in PEG-induced water stressed plants. Free proline and sucrose contents were also dominated in the leaf tissues to maintain the leaf osmotic potential in water stressed plants. In addition, chlorophyll degradation, chlorophyll fluorescence diminution and stomatal closure were found in plants grown under PEG-induced water deficit conditions, leading to reduction in net photosynthetic rate (P_n) and subsequently lesser amounts of glucose and fructose contents in the leaf tissues. Sucrose and free proline in the roots of sweetpotato play a key role as major osmotic adjustment when subjected to PEG-induced water deficit condition. Basic knowledge gained from this research will further be investigated the drought defense mechanism in sweetpotato via osmoregulation system.

Keywords: net photosynthetic rate, osmotic adjustment, polyethylene glycol (PEG), sucrose, total soluble sugar.

Introduction

Sweetpotato is used as a major carbohydrate resource in the developing countries where it is the fifth most important food crop (Mukhopadhyay *et al.*, 2011). Apart from carbohydrates, the storage roots are rich in proteins, β-carotene, anthocyanins, ascorbic acid, potassium and calcium (Truong *et al.*, 2010; Mukhopadhyay *et al.*, 2011; Mohanraj and Sivasankar, 2014; Motsa *et al.*, 2015). The green leaves are rich in anthocyanins, polyphenolic acid and essential oils (Islam *et al.*, 2002; Islam, 2006; Wang *et al.*, 2010). Orange-flashed sweetpotato, when consumed daily, can prevent vitamin A deficiency (Burri, 2011; Fitzpatrick *et al.*, 2012). Storage roots of sweetpotato are rich in

starch, vitamins and minerals. In general, storage root yield ranging from 20 to 25 tons ha⁻¹ is a minimal requirement to validate the crop as an elite variety in a field trial (Nedunchezhiyan *et al.*, 2012). The productivity of sweetpotato in P.R. China and United States of America (USA) has been bench-marked as 21.0 and 22.5 tons ha⁻¹, respectively (Mukhopadhyay *et al.*, 2011). In contrast, the productivity of sweetpotato in African countries such as Uganda, Nigeria, Tanzania, Angola, Burundi, Mozambique, Madagascar, Rwanda, Ethiopia, Kenya and Cameroon is very low (<10 tons ha⁻¹), and hence improvement in the yield traits of sweetpotato is of prime importance to plant breeders (Tekalign, 2007; Placide *et al.*, 2013; Andrade *et al.*, 2016).

In sweetpotato production, water shortage in arid zone i.e. Africa, is a critical problem, especially in rainfed areas (< 500 mm yr⁻¹ precipitation), resulting in lower productivity (6.6 tons

ha⁻¹ in rainfed) when compared to other regions (12.5 tons ha⁻¹ in 30% full irrigation) (Önder *et al.*, 2015). Sweetpotato has been identified as a moderate drought tolerant crop and it is very sensitive to water deficit in storage root initiation stage (10–40 days after planting) (Mukhopadhyay *et al.*, 2011; Villordon *et al.*, 2012). Earlier studies indicate that the physiological responses including water potential, photosynthetic pigment contents, chlorophyll fluorescence, net photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency of sweetpotato cvs. 'Beauregard' and 'Evangeline' grown under reduced soil moisture (0.107 m³ H₂O m⁻³ soil) were significantly dropped. This led to growth reduction in sweetpotato which was identified by low number of leaf, reduced leaf area and retarded vine length which resulted in delayed storage root formation and storage root yield reduction (Gajanayake *et al.*, 2013, 2014). Screening drought tolerant trait in sweetpotato in the field trial of arid regions has been evaluated using storage root yield as major criteria (Laurie *et al.*, 2013; Maquia *et al.*, 2013; Kivuva *et al.*, 2015; Andrade *et al.*, 2016). The field trial evaluation is performed at least two crop cycles to collect the data following validated recommendation before release of the candidate varieties to the farmers. Fifty-nine genotypes of orange-fleshed sweetpotato have been screened for drought tolerant traits (shoot height, shoot fresh weight, shoot dry weight, root dry weight and leaf area) in plant tissue culture using PEG₆₀₀₀-induced water deficit in the culture medium. Of these, 8 candidate genotypes, 194515.5, 194539.36, 441724, 441538, 189135.9, 41768, 192033.5 and 440429, were identified as drought tolerant (Agilis *et al.*, 2015). In addition, sweetpotato cv. 'TWA1' has been identified as water deficit tolerant by shoot height, leaf fresh weight, root length and root dry weight using PEG₈₀₀₀-induced water deficit (Gopal and Iwama, 2007).

Recently, hydroponic culture system for storage root development of sweetpotato has been investigated with controlled environments i.e. plant spacing (Mortley *et al.*, 1991), photoperiod (Mortley *et al.*, 2009), light intensity (Mortley *et al.*, 1996), substrate moisture content (Eguchi *et al.*, 1995), ambient relative humidity (Mortley *et al.*, 1994; Eguchi *et al.*, 1998) and planting depth (Islam *et al.*, 2006). The size of tuberous storage root enlarges depending on the hydroponic culture period and the number of leaves (Eguchi *et al.*, 1996). Therefore, the basic knowledge on osmoregulation defense mechanisms in water deficit tolerance, cv. 'Japanese Yellow' (Yooyongwech *et al.*, 2013) hydroponically grown under 5% PEG-induced water deficit in a long term period from planting to harvesting is still limited. The aim of this study was to establish the responses of sweetpotato cv. 'Japanese Yellow' to PEG-induced water deficit in term of storage root yield traits, physiological, biochemical and morphological characteristics.

Materials and Methods

Plant materials and PEG-induced water deficit condition

'Japanese Yellow' cultivar of sweetpotato obtained from Agricultural Extension Group, Phichit province, Thailand, was used as a master stock material. Single vine (10±1 cm in length) cutting without leaf blades was propagated and planted into plastic bags of 15 cm of diameter and 20 cm of length, respectively, containing 0.5 kg mixed soil with EC = 2.69 dS m⁻¹;

pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%, and total potassium = 1.19%. The vine cuttings planted in the plastic bag were incubated in a greenhouse at 500–1,000 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD), 10 h d⁻¹ photoperiod, 28±2 °C temperature, and 80±5% relative humidity. One-week-old vine cuttings were washed to remove the soil particles with tap water and divided into two groups: (1) positive check to be grown in the plastic pot of 20 cm of diameter and 25 cm of length, containing 2 kg mixed soil, (2) modified nutrient solution (Ingestad and Lund, 1986; Göransson, 1998) with hydroton clay pellets as supporting material. The nutrient solution was prepared and replaced at two days-interval using ebb and flow technique (15 min incubation) for 2 weeks. Then, the 0% polyethylene glycol (PEG) (control) and 5% PEG-induced water deficit stress ($\Psi = -0.54$ MPa) in the nutrient solution were adjusted. Plant grown under well watering in soil culture was validated as positive check. Sweetpotato plants in each treatment were incubated in a greenhouse for 4 months. Growth performances, photosynthetic abilities, water potential, soluble sugar, free proline and storage root yield traits in each treatment were measured.

Growth measurement

Vine length, leaf, shoot, root dry weight, leaf area, root length, number of root; shoot and root fresh weight of sweetpotato were determined. Leaf area per plant was measured by Root/Leaf Area DT-Scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., UK). Vine, leaves and roots were dried at 80 °C in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of vine, leaf and root dry weight.

Biochemical assay

Free proline in the leaf tissues was extracted and analyzed according to the method of Bates *et al.* (1973). In brief, fifty milligrams of fresh leaf tissues was ground with liquid nitrogen in a mortar. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3%, w/v) and filtered through filter paper (Whatman #1, 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 H₃PO₄) and incubated at 95 °C for 1 h. The reaction was terminated by placing the container in an ice bath. The reaction mixture was mixed vigorously with 2 mL of toluene. After cooling to 25 °C, the chromophore was measured at 520 nm by spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) using L-proline as a calibration standard. Soluble sugar (sucrose, glucose and fructose) in the root, leaf and storage root tissues were assayed following the method of Karkacier *et al.* (2003). In brief, fifty-milligram of plant sample was ground in a mortar with liquid nitrogen. One mL of nanopure water was added and centrifuged at 12,000 rpm for 15 min. The supernatant was collected and filtered through a 0.45 μm membrane filter (VertiPure™, Vertical®). Twenty micro-liters of the filtrate was injected into a Waters HPLC equipped with a MetaCarb 87C column and a guard column. Deionized water was used as the mobile phase at a flow rate of 0.5 mL min⁻¹. The online detection was performed using a Waters 410 differential refractometer detector and the data was analyzed by Empower® software. Sucrose, glucose and fructose (Fluka, USA) were used as the standards.

Physiological analysis

Osmotic potential in the roots, leaves and storage roots of sweetpotato was measured, according to Lanfermeijer *et al.* (1991). Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC) and total carotenoids contents in the leaf tissues were assayed according to the method of Shabala *et al.* (1998), as well as total carotenoids (C_{x+c}) content was assayed following Lichtenthaler (1987) method. One hundred milligrams leaf tissue was homogenized in glass vials using 10 mL of 99.5 % acetone, and blended using a homogenizer. The glass vials were sealed with Parafilm® to prevent evaporation, and then stored at 4°C for 48 h. Chl_a and Chl_b concentrations were measured at 662 nm and 644 nm whereas C_{x+c} concentration measured at 470 nm using UV-VIS spectrophotometer (HACH DR/4000; Model 48000, HACH Company, Loveland, Colorado, USA) against acetone (95.5%) as a blank. Chlorophyll fluorescence emission was measured from the adaxial surface on the leaf using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode (Loggini *et al.*, 1999; Maxwell and Johnson, 2000). Net photosynthetic (P_n; μmol m⁻² s⁻¹) and transpiration rate (E; mmol m⁻² s⁻¹) were measured using a Portable Photosynthesis System with an Infra-red Gas Analyzer (Model LI 6400, LI-COR Inc., Lincoln, Nebraska, USA). Both parameters were measured continuously by monitoring the content of the air entering and existing in the IRGA headspace chamber, according to Cha-um *et al.* (2007).

Experiment design and statistical analysis

The experiment was arranged as Completely Randomized Design (CRD) with eight replicates (*n* = 8). The mean values obtained from three treatments were compared using Tukey's HSD and analyzed with SPSS software. In addition, the *t*-test mean comparison in two treatments with PEG or without PEG was validated for 1, 2 and 3 months after cultivation.

Results*Storage root traits and morphological characteristics*

Storage root, vine and root morphological characters of sweet potato plants cv. 'Japanese Yellow' grown under 0% PEG, 5% PEG and soil culture for 4 months were demonstrated (Fig. 1). The 5% PEG treatment induced water deficit stress, leaf chlorosis and toxicity symptoms in plants. Green leaf area and leaf dry weight in plants grown under hydroponic culture were better than those in the soil culture depending on the full nutrient support. In PEG-induced water deficit, leaf dry weight and green leaf area significantly declined by 85.59% and 95.25%, respectively (Fig. 2A and 2B). Number of storage roots per plant and storage root fresh weight in plants grown under hydroponic culture were lower than those grown

in soil culture. Moreover, number of storage roots per plant and storage root fresh weight in plants grown under 5% PEG-induced water deficit condition dropped by 20% and 47.38%, respectively (Fig. 2C and 2D). Vine length, number of roots, shoot fresh weight, root fresh weight and root dry weight in plants grown under hydroponic culture were higher than those grown in soil culture, while root length was shorter (Table 1). Under water deficit stress, vine length, root length, number of roots, shoot fresh weight, shoot dry weight and root fresh weight were significantly decreased by 52.02%, 22.33%, 28.57%, 67.12%, 47.99% and 51.81% of control (without PEG), respectively (Table 1).

Osmotic potential and osmotic solutes in the cellular levels

Osmotic potential in the leaf tissues significantly declined by 44.83%, 17.43%, 31.63% and 36.95% of control when plants were exposed to 5% PEG-induced water deficit for 1, 2, 3 and 4 months, respectively (Fig. 3A). Free proline levels in the leaf tissues of PEG-induced water stress for 2, 3 and 4 months continuously increased for 2.40, 2.54 and 4.14 folds over



Fig. 1. Storage roots and morphological characteristics of sweetpotato cv. 'Japanese Yellow' grown under hydroponic culture (A) 0, (B) 5% PEG and in the soil (C) for 4 months

Table 1. Vine length (VL), root length (RL), number of roots (NR), shoot fresh (SFW), shoot dry weight (SDW), root fresh (RFW) and root dry weight (RDW) of sweetpotato cv. 'Japanese Yellow' grown under 0 (control) and 5% PEG-induced water deficit in the hydroponic culture and, in the soil (positive check) for 4 months

Treatment	VL (cm)	RL (cm)	NR	SFW (g)	SDW (g)	RFW (g)	RDW (g)
0% PEG	269.67a	33.90b	14.0a	121.52a	13.44a	54.01a	6.75a
5% PEG	129.40b	26.33c	10.0b	39.96b	6.99b	25.99b	5.91a
Soil	74.00c	65.33a	5.33c	25.02b	5.41b	7.93c	2.33b
ANOVA	**	**	**	**	**	**	*

Different letters in each column show significant difference at $p \leq 0.01$ (**) and $p \leq 0.05$ (*) by Tukey's HSD

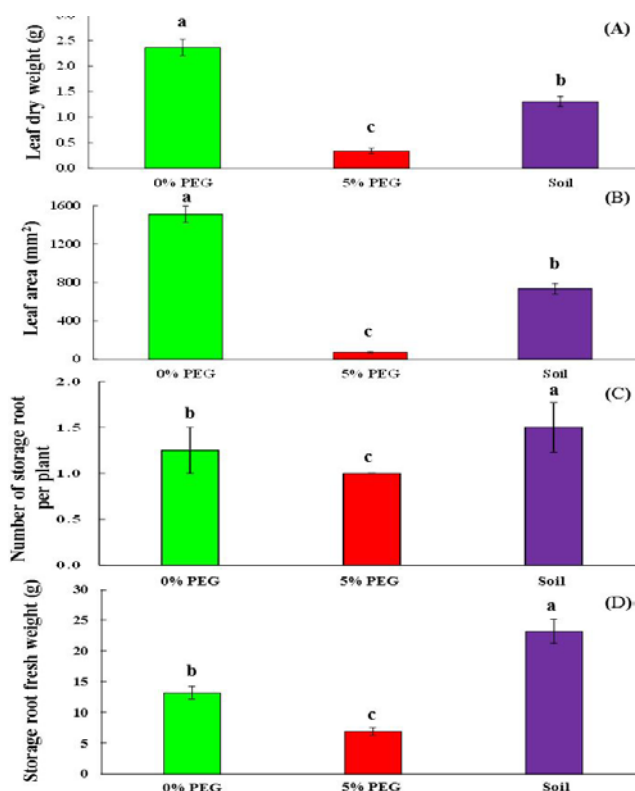


Fig. 2. Leaf dry weight (A), leaf area (B), number of storage roots per plant (C) and storage root fresh weight (D) of sweetpotato cv. 'Japanese Yellow' grown under 0 or 5% PEG in the hydroponic culture and in the soil for 4 months. Error bars represent ±SE. Different letters in each bar show significant difference at $p \leq 0.01$ by Tukey's HSD

control, respectively (Fig. 4A). Sucrose content in the leaf tissues was enriched by 2.18, 3.28 and 3.58 folds, when plants were exposed to 5% PEG-induced water stress for 2, 3 and 4 months, respectively (Fig. 4B). In contrast, osmotic potential in the adventitious fibrous root and tuberous root of sweetpotato was unchanged in either with or without PEG for 1, 2, 3 and 4 months as well as in the soil culture for 4 months (Fig 3B and 3C). Total soluble sugar in root tissues were stimulated by 1.69, 1.91 and 2.43 folds when plants were subjected to 5% PEG-induced water stress for 2, 3 and 4 months, respectively (Fig. 4C). Moreover, total soluble sugar in storage root tissues was moved up to 1.40, 1.37 and 2.11 folds (Fig. 4D).

Photosynthetic abilities and sugar primary products

Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and total chlorophyll (TC) in both PEG-induced water deficit and soil culture were lower than those in hydroponic culture without PEG. In water deficit condition, Chl_a, Chl_b and TC levels decreased by 39.21%, 48.77% and 43.07% of control (Table 2). Maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) in both without PEG and soil culture of sweetpotato were unchanged in each growth stages. In contrast, F_v/F_m was significantly decreased for 18.56%, 21.88% and 36.55% when plants were exposed to 5% PEG-induced water deficit for 2, 3 and 4 months, respectively (Fig. 5A). Correspondingly, Φ_{PSII} decreased by 15.87 % and 38.97% when subjected to 5% PEG-induced water deficit for 3 and 4 months, respectively (Fig. 5B). Stomatal conductance (g_s) and

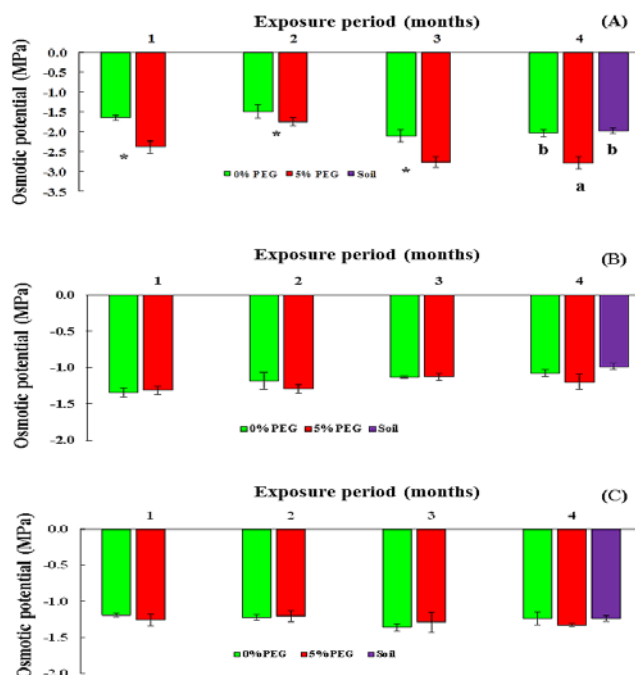


Fig. 3. Osmotic potential (MPa) in leaves (A), root (B) and storage root (C) of sweetpotato cv. 'Japanese Yellow' grown under 0 or 5% PEG in the hydroponic culture and in the soil for 4 months. Error bars represent ±SE. Different letters in each bar show significant difference at $p \leq 0.01$ by Tukey's HSD. *Represents significant difference at $p \leq 0.01$ by *t*-test

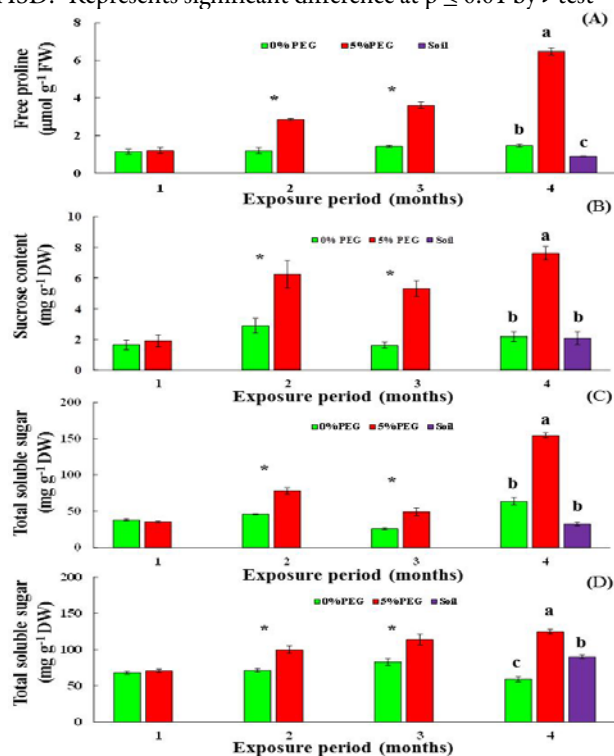


Fig. 4. Free proline (A), sucrose in the leaves (B), total soluble sugar in root (C) and storage root (D) of sweetpotato cv. 'Japanese Yellow' grown under 0 or 5% PEG in the hydroponic culture and in the soil for 4 months. Error bars represent ±SE. Different letters in each bar show significant difference at $p \leq 0.01$ by Tukey's HSD. *Represents significant difference at $p \leq 0.01$ by *t*-test

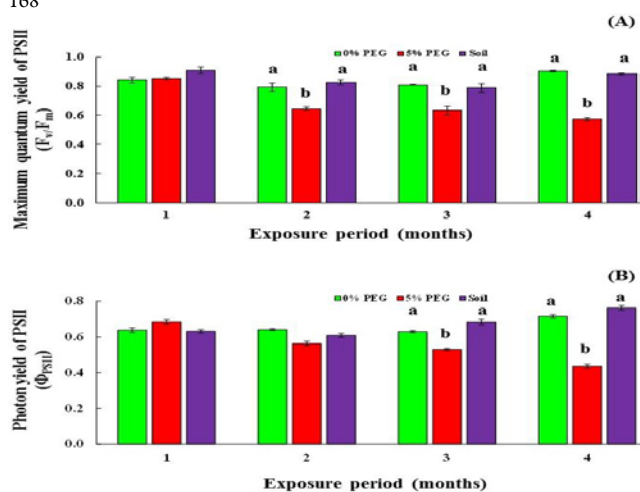


Fig. 5. Maximum quantum yield of PSII (F_v/F_m) (A) and photon yield of PSII (Φ_{PSII}) in the leaves of sweetpotato cv. 'Japanese Yellow' grown under 0 or 5% PEG in the hydroponic culture and in the soil for 4 months. Error bars represent \pm SE. Different letters in each bar show significant difference at $p \leq 0.01$ by Tukey's HSD

transpiration rate (E) in water deficit stressed plants significantly dropped for 68.90% and 60.16% over control, respectively (Table 2). Subsequently, net photosynthetic rate (P_n) in plants grown under PEG-induced water deficit declined by 21.96%, 13.15%, 57.78% and 67.42%, when cultured for 1, 2, 3 and 4 months, respectively (Fig. 6A). Glucose and fructose, a primary product derived from photosynthesis in the leaf tissues significantly decreased when exposed to PEG-induced water deficit (Fig. 6B and 6C). Glucose in the plants grown under PEG-induced water deficit dropped by 65.72%, 33.13%, 51.81% and 8.11% when cultured for 1, 2, 3 and 4 months, respectively. Similarly, fructose content declined by 22.68%, 13.77%, 34.61% and 9.94% (Fig. 6C). In contrast, sucrose, glucose and fructose levels in roots of water deficit stressed plant were enriched by 1.94, 2.63 and 3.35 folds over control, respectively (Table 3). In tuberous root, sucrose ($114.69 \text{ mg g}^{-1} \text{ DW}$) dominated as major soluble sugar, especially in PEG-induced water deficit condition. Sucrose, glucose and fructose

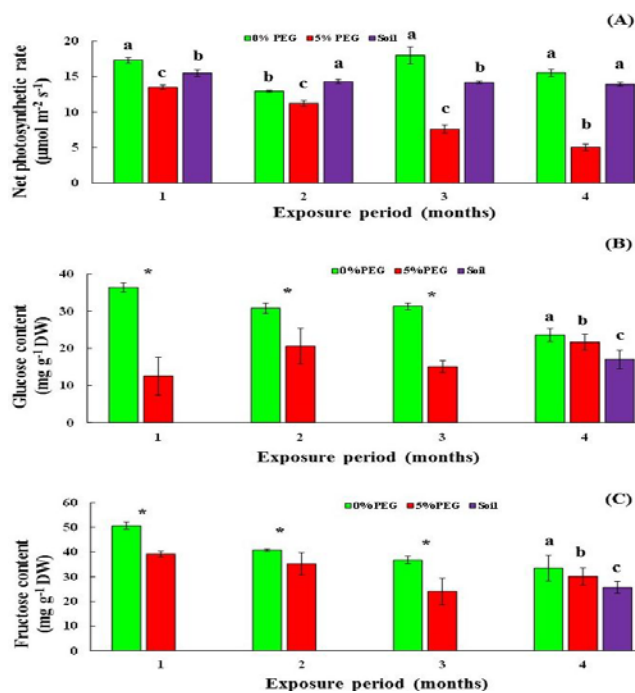


Fig. 6. Net photosynthetic rate (A), glucose (B) and fructose content (C) in leaves of sweetpotato cv. 'Japanese Yellow' grown under 0 or 5% PEG in the hydroponic culture and in the soil for 4 months. Error bars represent \pm SE. Different letters in each bar show significant difference at $p \leq 0.01$ by Tukey's HSD. *Represents significant difference at $p \leq 0.01$ by t -test

levels in tuberous roots enhanced by 2.19, 1.84 and 1.40 folds over control, respectively (Table 3).

Discussion

Overall growth performances of sweetpotato grown under 5% PEG-induced water deficit were significantly dropped (Table 1), leading to reduced storage root yields when compared to controls (without PEG and in the soil culture; Fig. 2). In sweetpotato, storage root per plant and number of

Table 2. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC), stomatal conductance (g_s) and transpiration rate (E) in the leaves of sweetpotato cv. 'Japanese Yellow' grown under 0 (control) or 5% PEG-induced water deficit in the hydroponic culture and in the soil (positive check) for 4 months

Treatment	Chl _a ($\mu\text{g g}^{-1}\text{FW}$)	Chl _b ($\mu\text{g g}^{-1}\text{FW}$)	TC ($\mu\text{g g}^{-1}\text{FW}$)	g_s ($\text{mmol m}^{-2}\text{s}^{-1}$)	E ($\text{mmol H}_2\text{O mol}^{-1}$)
0% PEG	161.2a	109.5a	270.7a	0.164a	2.46a
5% PEG	98.0b	56.1b	154.1b	0.051c	0.98c
Soil	95.0b	57.7b	152.7b	0.095b	1.74b
ANOVA	**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Tukey's HSD.

Table 3. Sucrose, glucose and fructose contents in root and storage root tissues of sweetpotato cv. 'Japanese Yellow' grown under 0 (control) or 5% PEG-induced water deficit in the hydroponic culture and in the soil (positive check) for 4 months

Treatment	Root			Storage roots		
	Suc ($\text{mg g}^{-1} \text{DW}$)	Glu ($\text{mg g}^{-1} \text{DW}$)	Fruc ($\text{mg g}^{-1} \text{DW}$)	Suc ($\text{mg g}^{-1} \text{DW}$)	Glu ($\text{mg g}^{-1} \text{DW}$)	Fruc ($\text{mg g}^{-1} \text{DW}$)
0% PEG	33.39b	16.47b	13.87b	52.47c	1.28c	5.33c
5% PEG	64.81a	43.34a	46.47a	114.69a	2.35b	7.48b
Soil	22.09c	3.47c	6.76c	70.33b	5.38a	14.22a
ANOVA	**	**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Tukey's HSD

storage root on 33% full irrigation significantly decreased by 59.61% and 29.63% when compared with 100% irrigation. The yield traits, storage root per plant (77.02%) and number of storage root (44.44%) were very sensitive to water shortage, especially in rain fed conditions (Önder *et al.*, 2015). In agreement, leaf fresh, leaf dry, root fresh and root dry weight of *Sesuvium portulacastrum* grown under water deficit for 12 days were significantly dropped (Slama *et al.*, 2007). In soybean, shoot height was declined by 17.84% when plants were subjected to 8% PEG for 14 days, leading to reduce yield traits, number of pods per plant (39.72% of control), pod dry weight (51.85% of control) and seed yield per plant (54.25% of control) (Hamayun *et al.*, 2010). Growth characters such as shoot and root fresh weight, shoot and root dry weight of sunflower cv. 'Musala' under -0.8 MPa PEG-mediated drought stress decreased more than 50% of control without PEG (Baloğlu *et al.*, 2012). Aerial biomass, root length and root biomass of *Populus simonii* grown under 5% PEG induced drought stress for 28 days deteriorated by 26.76%, 42.37% and 38.10%, respectively (Meng *et al.*, 2016). In sorghum, plant height, leaf blade biomass and root biomass dropped by 37.63%, 55.56% and 40% when plants subjected to 20% PEG for 28 days (O'Donnell *et al.*, 2013). Leaf area is a good indicator to identify the toxicity of PEG-induced drought stress, in *Eucalyptus camaldulensis* (Utkhao and Yingjajaval, 2015), pistachio (Khoyerdi *et al.*, 2016), *Ipomoea pes-caprae* (Sucre and Suárez, 2011) and sorghum (O'Donnell *et al.*, 2013).

Leaf osmotic potential of sweetpotato declined relating to 5% PEG-incubated period (Fig. 3). In potato, leaf water potential of plants grown under 10% PEG-induced water stress decreased by -1.1, -1.4, and -1.6 MPa when plants were exposed to stress for 1, 2 and 7 days, respectively (Büssis *et al.*, 1998), leading to free proline, glucose and fructose enrichments for osmotic adjustment (Büssis and Heineke, 1998). Free proline and sucrose in cell suspension culture of sweetpotato under 0.6 M mannitol-induced osmotic stress, improved by 5.25 and 3.5 folds over control, respectively, to maintain the cell osmolarity (Wang *et al.*, 1999). In addition, free proline in chickpea cvs. 'ICC4958' and 'Vijay' grown under -1.2 MPa PEG-induced osmotic stress increased by 1.37 and 1.34 folds, relating to stimulate P5CS (Δ^1 -pyrroline-5-carboxylate synthetase, a rate-limiting enzyme for proline biosynthesis) activity for 133.7% and 122.1% over control, respectively (Pawar *et al.*, 2015). Likewise, free proline osmolyte in sorghum seedlings cvs. 'RSLG-262' and 'RSV-1366' grown under -0.5 MPa PEG-induced water stress was increased by 5.21 and 7.59 folds over control, for P5CS activity for 158% and 187%, respectively (Damame *et al.*, 2014). In sugar beet, sucrose and free proline in the leaf blade and storage root increased to control the water relation when subjected to -0.5 MPa sorbitol-induced osmotic stress for 7 d (Wu *et al.*, 2016). Sucrose and free proline in the leaf blade of plants under PEG-induced water stress generally increased relating the incubation period (Fig. 4). Similarly, free proline and soluble sugar levels in PEG-induced water stress on potato (Büssis and Heineke, 1998), pistachio (Khoyerdi *et al.*, 2016), cassava (Fu *et al.*, 2016), *Sesuvium portulacastrum* (Slama *et al.*, 2007), *Bauhinia variegata* (Sinhababu and Kar, 2003), lentil (Muscolo *et al.*, 2014) and *Apocynum venetum* L. (Zhao and Dai, 2015) were increased as major osmotic adjustment.

Chlorophyll photosynthetic pigment degradation and green leaf area reduction were evidently found in plants grown under PEG-induced water stress (Table 2), leading to diminish the F_v/F_m and Φ_{PSII} (Fig. 5) as well as inhibit the g_s and E (Table 2) consequently resulting in P_n reduction and low levels of glucose and fructose in the leaf tissues (Fig. 6). In the leaf organ, the ADP-glucose pyrophosphorylase (AGPase) gene expression was dominated for transitory starch biosynthesis (source) in the day time and then translocated to the storage root organ (sink) in the night time, relating to the storage root enlargement in the night period (Eguchi *et al.*, 1998; Li and Zhang, 2003). Moreover, sucrose is a major sugar in the leaves, especially in water deficit stress, relating to absent invertase (INV) gene expression subsequently to low glucose and fructose contents (Li and Zhang, 2003). Chl_a and Chl_b in the leaves of apple seedlings grown under 10% PEG were significantly degraded, causing to reduction of P_n (Kautz *et al.*, 2015). In addition, Chl_a degradation in *Pistacia lentiscus* seedlings under -2.47 MPa PEG-induced water stress was correlated with F_v/F_m (2.41%) and Φ_{PSII} (8.57%) reduction as well as P_n inhibition (60.54%) (Vasques *et al.*, 2016). Sucrose in adventitious fibrous root and storage root under water deficit condition was evidently enriched to control the osmotic potential in the cellular level. Expression of sucrose (sucrose synthase; SuSy 1 and SuSy 2) and starch (AGPase) biosynthesis related-genes in developed storage root was up-regulated while expression level of INV gene (invertase) was very low in storage root initiation stage and absent in medium/mature storage root. This lead to the accumulation of sucrose and starch in the fibrous and tuberous root tissues (Firon *et al.*, 2013; Li and Zhang, 2003).

Conclusions

In conclusion, osmotic potential in the leaf tissues of sweetpotato plants grown under 5% PEG-induced water stress was maintained by free proline and sucrose enrichment. Chlorophyll pigments, photosynthetic abilities as well as net photosynthetic rate were significantly declined when plants were exposed at 5% PEG-induced water deficit, leading to growth inhibition and storage root yield reduction. In the adventitious and tuberous roots, total soluble sugar plays a key role as an osmotic adjustment in plants when subjected to water deficit stress in both short and long term responses.

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