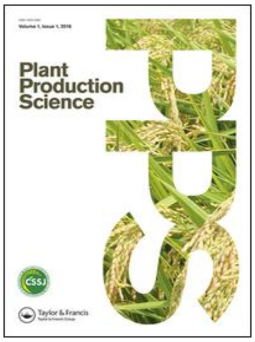

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# Physio-Biochemical Responses of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings to Mannitol- and Polyethylene Glycol-Induced Iso-Osmotic Stresses

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**Abstract: The aim of this investigation was to comparatively examine the physio-biochemical responses of oil palm seedlings to mannitol- and PEG-induced iso-osmotic stresses. The water content of osmotically stressed oil palm seedlings decreased, but the proline content and the electrolyte leakage of the seedlings increased with decreasing water potential ($\Psi_w$). However, the responses varied with the strength of osmotic stress and type of osmotic agent. Relative electrolyte leakage (REL) was negatively correlated to chlorophyll content in the osmotically stressed leaves. Chlorophyll a ($Chl_a$), chlorophyll b ($Chl_b$), total carotenoids ($C_{x+c}$) and total chlorophyll (TC) in the seedlings were significantly reduced by osmotic stress, subsequently reducing maximum quantum yield of PSII ($F_v/F_m$) and photon yield of PSII ($\Phi_{PSII}$), thereby lowering net-photosynthetic rate ($P_n$) and inhibiting growth. Physio-biochemical parameters, including REL, $F_v/F_m$, and $\Phi_{PSII}$ in oil palm seedlings were reduced more greatly by PEG-induced osmotic stress than by mannitol-induced stress. A deterioration in morphological characters, including leaf chlorosis, leaf burn, and green leaf area reduction were demonstrated in oil palm seedlings under osmotic stress induced by either mannitol or PEG. However, the toxic symptoms in oil palm seedlings under PEG-induced stress were severer than in those under mannitol-induced iso-osmotic stress, especially under severe osmotic stress.**

**Key words: Chlorophyll fluorescence, Net-photosynthetic rate, Osmotic stress, Photosynthetic pigment, Proline, Relative electrolyte leakage.**

Southeast Asia, including Malaysia, Indonesia and Thailand, is a tropical zone, and is the main area of cultivation of the oil palm, one of the most important oil production plants in the world (Yusof and Chan, 2003; Wahid et al., 2005). Oil yield and productivity are major concerns of oil palm breeders (Jalani et al., 1997; Cochard et al., 2005). The oil yield trait is not only dependent on genetic factors, but is also affected by environmental conditions, such as relative humidity, water availability, soil structure, fertilizer application, agricultural management and light conditions (Henson and Dolmat, 2003; Kallarackal et al., 2004; Henson and Harun, 2005). Another issue is tolerance to abiotic stress, such as water-deficit, extreme temperature, mineral deficiency, heavy metal toxicity and ultraviolet irradiation. This is an alternative target for oil palm breeding. The water content of soil plays a key role in oil palm growth and development (Henson and Harun, 2005) and may also trigger female sex representation. In arid zones, the proportion of male flowers is high, which retards growth, leading to low productivity (Jones, 1997).

Water deficit stress is a major problem worldwide, limiting plant growth and leading to low productivity in many crop species, especially in arid zones (>1.2 billion hectares) (Chaves and Oliveira, 2004; Kijne, 2006; Passioura, 2007). The response of plants to water stress, such as decreased Rubisco (ribulose-1,5-bisphosphatase carboxyase / oxygenase) activity, reduced photochemical efficiency, enhanced accumulation of stress metabolites (proline, glycinebetaine, polyamine, glutathione, polyamines, sugars, sugar alcohols and $\alpha$-tocolpherol), and increased antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) has been well discovered (Chaves et al., 2002; Parry et al.,

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2002; Flexas et al., 2006). Proline accumulation in plants exposed to water deficit conditions has been generally regarded as an osmotic adjustment or osmoregulation defense mechanism (Gomes et al., 2010), which has been investigated as a biochemical marker of water deficit tolerance screening in many plant species (Ashraf and Foolad, 2007). The physiological changes, including loss of membrane stability, reduced leaf water potential, pigment degradation, decreased stomatal conductance, reduced internal $CO_2$ concentration, net photosynthetic rate ($P_n$) reduction and growth inhibition have been studied intensively (Chaves and Oliveira, 2004; Reddy et al., 2004; Cattivelli et al., 2008). Generally, biochemical and physiological responses of plants to water deficit stress have been studied in field trials and hydroponic systems. In the present study, an environmental control system for photoautotrophic *in vitro* culture was developed (Cha-um et al., 2003) and applied as a prototype for salt tolerance screening in rice (Cha-um et al., 2010a).

Mannitol-induced water stress has been widely applied to many plant species i.e. tomatoes (Weng, 2000), sugarcane (Cha-um and Kirdmanee, 2008), rice and sorghum (Cha-um et al., 2009). Polyethylene glycol (PEG) is an osmoticum for inducing water deficit (Ionenko and Anisimov 2001), and an agent of membrane injury (Ahmad et al., 2007). Moreover, PEG not only plays a role as an osmoticum but also reduces oxygen-dissolution in the culture medium (Hiller and Dexter, 1997; Verslues et al., 1998). The physio-biochemical responses of oil palm seedlings under either mannitol or PEG-induced iso-osmotic stress have not been reported previously. In the Arecaceae family, ecophysiological expression in coconut palms, including membrane lipid composition (Repellin et al., 1997), photosynthetic ability and growth performance under water deficit stress, using PEG solution, has been investigated (Gomes and Prado, 2007; Gomes et al., 2008). Also, we have investigated the physio-biochemical responses of oil palm seedlings grown under PEG-induced water deficit (Cha-um et al., 2010b). In the present study, water content, relative electrolyte leakage (REL), proline accumulation, photosynthetic pigment content, chlorophyll fluorescence, net photosynthetic rate ($P_n$) and growth performance of oil palm seedlings under mannitol- and PEG-induced iso-osmotic stresses were evaluated.

## Materials and Methods

### 1. Plant materials

Oil palm fruits were obtained from Suksomboon Palm Oil Co Ltd., Chonburi province, in the eastern region of Thailand. The kernel of the fruit was removed. The seeds, with the seed coat, were dried in a hot air oven at 45°C for 12 h, and the seed coat scarified. The embryos, along with the endosperm, were surface-disinfected once in 15% Clorox for 20 min and once in 5% Clorox for 30 min. The embryos were then excised and allowed to germinate in MS media (Murashige and Skoog, 1962) containing 3% (w/v) sucrose without plant growth regulators. The media were adjusted to pH 5.7 before autoclaving. Oil palm seedlings were cultured *in vitro* under conditions of 25±2°C ambient temperature, 60±5% relative humidity (RH) and 60±5 $\mu$mol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD) provided from fluorescent lamps with a 16 h $d^{-1}$ photoperiod. After two months, the seedlings were transferred aseptically to MS-liquid sugar-free media (photoautotrophic growth). The uncovered vessels containing the seedlings were transferred aseptically to culture box chambers (Carry Box Model P-850, size 26×36×19 cm, Eastern Premium Co. Ltd., Bangkok, Thailand) with RH controlled at 65±5% with 1.5 L saturated NaCl solution. The number of air exchanges in the culture box chambers was increased to 5.1±0.3 $\mu$mol $CO_2$ $hr^{-1}$ by punching the side of the plastic chambers with 32 holes and placing gas-permeable microporous polypropylene film (0.22 $\mu$m pore size) over the holes (Cha-um et al., 2003). Oil palm seedlings were acclimated for 14 days by placing the chambers in a Plant Growth Incubator controlled at 28±2°C/25±2°C (light/dark), 500±100 $\mu$mol $CO_2$ $mol^{-1}$ concentration, 60±5% RH, 120±5 $\mu$mol $m^{-2}$ $s^{-1}$ PPFD provided from fluorescent lamps with a 16 hr $d^{-1}$ photoperiod. Osmotic potential in the culture media was adjusted to −0.238 (control), −0.674 MPa (mannitol), −0.674 MPa (PEG6000), −0.939 MPa (mannitol), or −0.939 MPa (PEG6000) and the seedlings grown on for 30 days. Water content (WC), relative electrolyte leakage (REL), proline content, photosynthetic pigments, chlorophyll fluorescence, net-photosynthetic rate ($P_n$) and growth characters were measured.

### 2. Data collection

REL (%) was determined according to the Dionisio-Sese and Tobita (1998) method. Leaf tissue was cut into pieces 5.0±0.2 mm in length, and placed in glass vessels (Opticlear; KIMBLE, Vineland, New Jersey, USA) containing 10 mL deionized water. The glass vessels were capped and maintained at room temperature (25°C) for 15 min. Initial electrical conductivity ($EC_0$) was measured using an electrical conductivity meter. The leaf tissue was then incubated at 100°C in a water bath for 15 min, cooled down to 25°C and electrical conductivity ($EC_1$) was measured.

Proline in the leaf tissues was extracted and analyzed according to the method of Bates et al. (1973). The fresh material (50 mg) 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

Table 1. Water content (WC), and contents of chlorophyll a (Chl$_a$), chlorophyll b (Chl$_b$), total chlorophyll (TC) and total carotenoids (C$_{x+c}$) in oil palm grown under mannitol- and PEG-induced iso-osmotic stresses.

| Water potential (MPa) | WC (%) | Chl$_a$ ($\mu$g g$^{-1}$ FW) | Chl$_b$ ($\mu$g g$^{-1}$ FW) | TC ($\mu$g g$^{-1}$ FW) | C$_{x+c}$ ($\mu$g g$^{-1}$ FW) |
|---|---|---|---|---|---|
| –0.238 (Control) | 79.6 a | 242.1 a | 149.2 a | 391.3 a | 73.1 a |
| –0.674 mannitol | 78.1 a | 220.9 a | 108.5 b | 329.4 b | 66.1 a |
| –0.674 PEG | 75.4 b | 110.2 b | 42.4 c | 152.6 c | 36.0 b |
| –0.939 mannitol | 72.9 c | 51.7 c | 20.6 cd | 72.3 d | 19.2 c |
| –0.939 PEG | 70.3 d | 37.0 c | 15.0 d | 52.0 d | 13.4 c |
| ANOVA | ** | ** | ** | ** | ** |

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

glacial acetic acid and 20 mL 6 M $H_3PO_4$) and incubated at 95°C for 1 hr. The reaction was terminated by placing the container in an ice bath. The reaction mixture was mixed vigorously with 2 mL toluene. After cooling to 25°C, the chromophore was measured by UV-visible spectrophotometer (model DR/4000, HACH, Loveland, Colorado, USA) at 520 nm using L-proline as a standard.

Chlorophyll a (Chl$_a$), chlorophyll b (Chl$_b$) and total chlorophyll (TC), were analyzed following the methods of Shabala et al. (1998) and total carotenoid (C$_{x+c}$) concentrations were assayed according to Lichtenthaler (1987). The leaf material (100 mg) was collected and placed in a 25 mL glass vial, along with 10 mL 95.5% 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 using a UV-visible spectrophotometer at 662 nm and 644 nm wavelengths. The C$_{x+c}$ concentration was also measured by spectrophotometer at 470 nm. A solution of 95.5% acetone was used as a blank.

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). A leaf, adapted to dark conditions for 30 min using leaf-clips, was initially exposed to the modulated measuring beam of far-red light (LED source with a peak at wavelength 735 nm). Original ($F_0$) and maximum ($F_m$) fluorescence yields were measured under weak modulated red light (<0.5 $\mu$mol m$^{-2}$ s$^{-1}$) with 1.6 s pulses of saturating light (>6.8 $\mu$mol m$^{-2}$ s$^{-1}$ PAR) and calculated using FMS software for Windows. The variable fluorescence yield ($F_v$) was calculated by the equation of $F_m$-$F_0$. The ratio of variable to maximum fluorescence ($F_v/F_m$) was calculated as maximum quantum yield of PSII photochemistry. The photon yield of PSII ($\Phi_{PSII}$) in the light was calculated by $\Phi_{PSII} = (F_m'-F)/F_m'$ after 45 s of illumination, when steady state was achieved. In addition, non-photochemical quenching (NPQ) was calculated as described by Maxwell and Johnson (2000).

Net photosynthetic rate ($P_n$) was calculated by comparing the concentrations of $CO_2$ inside ($C_{in}$) with those outside ($C_{out}$) the glass vessel containing the oil palm seedlings. The $CO_2$ concentrations at steady state were measured by gas chromatography (GC; Model GC-17A, Shimadzu Co. Ltd., Tokyo, Japan). The $P_n$ of *in vitro* cultivated seedlings was calculated according to the method of Fujiwara et al. (1987).

Shoot height (SH), root length (RL), leaf area (LA), fresh weight (FW) and dry weight (DW) of oil palm seedlings were measured. Oil palm seedlings were dried at 80°C in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of dry weight. The leaf area of oil palm seedlings was measured using a Leaf Area Meter DT-scan (Delta-T Scan®, Delta-T Devices Ltd., Cambridge, UK).

### 3. Experimental design and statistical analysis

The experiment was arranged in a completely randomized design (CRD) with eight replicates ($n$=8). The mean values obtained were compared by Duncan's New Multiple Range Test (DMRT) and analyzed using SPSS software.

## Results

### 1. Water content, proline content and relative electrolyte leakage

The water content in the leaf tissues of oil palm seedlings decreased, relating to water potential in the culture media and the type of osmoticum (Table 1). Water content dropped significantly in response to mild PEG-induced osmotic stress (–0.674 MPa) for 75.4%, while it decreased significantly in severe mannitol-induced osmotic stress conditions (–0.939 MPa) for 72.9%. Proline content and relative electrolyte leakage in the leaf tissues of oil palm seedlings showed a similar trend and increased following water potential reduction under either PEG- or mannitol-induced stress. The proline level and relative electrolyte leakage in extreme PEG-induced osmotic stress ($\Psi_w$=–0.939 MPa) were 5.25 $\mu$mol g$^{-1}$ FW and 40.57%,

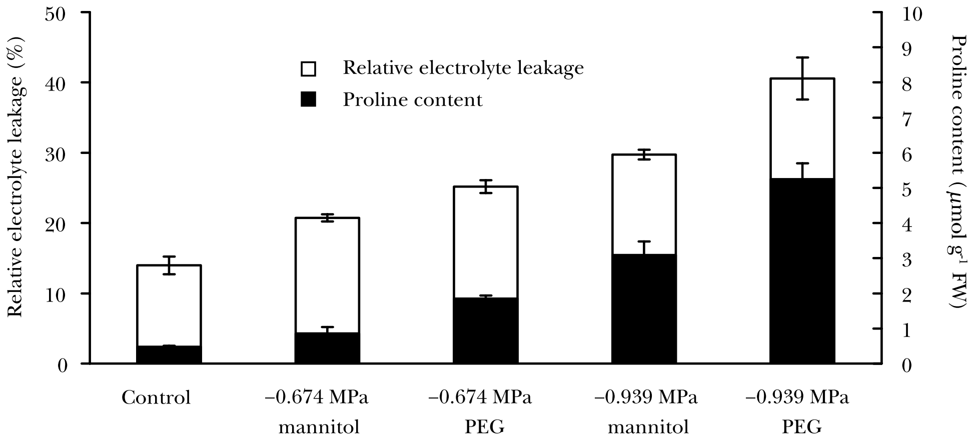

Fig. 1. Relative electrolyte leakage and proline content of oil palm grown under mannitol- and PEG-induced iso-osmotic stresses. Error bars indicate ±SE.

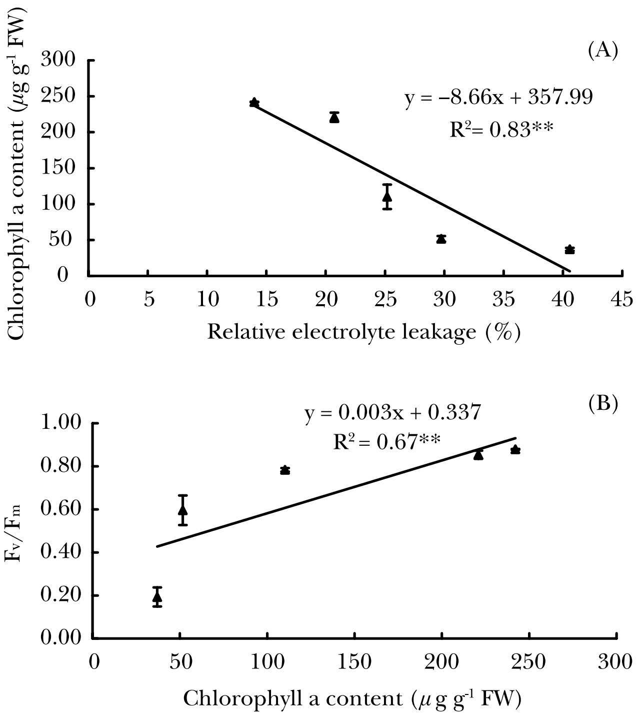

Fig. 2. Relationship between relative electrolyte leakage and chlorophyll a content (A) and between chlorophyll a content and maximum quantum yield of PSII ($F_v/F_m$) (B) in oil palm grown under mannitol- and PEG-induced iso-osmotic stress. Error bars indicated by ±SE. ** represents a highly significant difference.

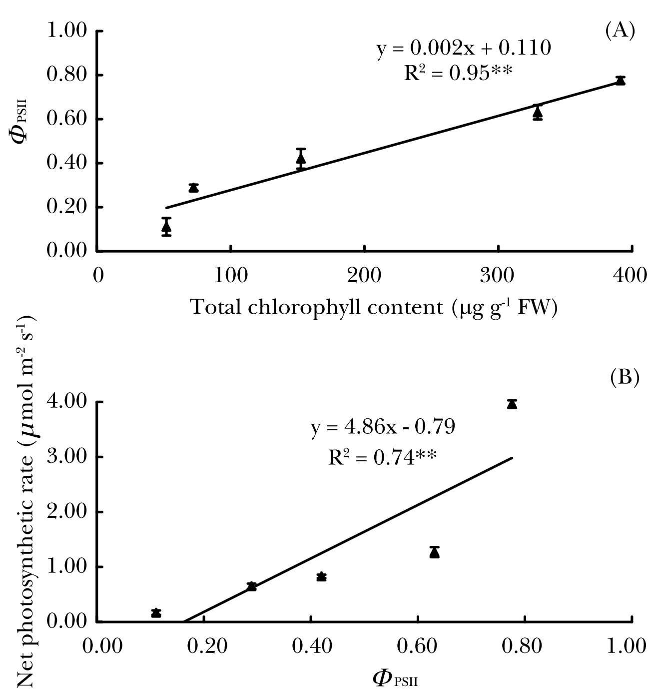

Fig. 3. Relationship between total chlorophyll content and quantum efficiency of PSII ($\Phi_{PSII}$) (A) and between quantum efficiency of PSII ($\Phi_{PSII}$) and net photosynthetic rate ($P_n$) (B) in oil palm grown under mannitol- and PEG-induced iso-osmotic stresses. Error bars indicate ±SE. ** represents a highly significant difference.

respectively which were 10.94 and 2.90 times lager than the values of the control (Fig. 1). Electrolyte leakage showed a correlation with $Chl_a$ damage (Fig. 2A).

## 2. Photosynthetic pigment, chlorophyll fluorescence and net photosynthetic rate

$Chl_a$ contents of the leaf tissues under mild and severe PEG-induced stresses were 54.48 and 84.72% lower that of the control seedlings, respectively. In contrast, $Chl_a$ content under mild mannitol-induced stress was only 8.75% lower than in the control. A similar pattern of $C_{x+c}$ decrease was demonstrated, as shown in Table 1. $Chl_b$ and TC contents of the leaf tissues were also greatly decreased by osmotic stress (Table 1). The degradation of $Chl_a$, $Chl_b$, TC and $C_{x+c}$ in the leaf tissues by osmotic stress was significant, especially in PEG solution. For example, the $Chl_b$ content under mild and severe mannitol-induced stresses was 27.28 and 86.19% lower, respectively, and that in PEG solution

Table 2. Maximum quantum yield of PSII ($F_v/F_m$), photon yield of PSII ($\Phi_{PSII}$), non-photochemical quenching (NPQ) and net-photosynthetic rate ($P_n$) of oil palm grown under mannitol- and PEG-induced iso-osmotic stresses.

| Water potential (MPa) | $F_v/F_m$ | $\Phi_{PSII}$ | NPQ | $P_n$ ($\mu$mol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| −0.238 (Control) | 0.879 a | 0.776 a | 0.003 d | 3.96 a |
| −0.674 mannitol | 0.854 a | 0.631 b | 0.011 d | 1.27 b |
| −0.674 PEG | 0.784 a | 0.420 c | 0.029 c | 0.83 b |
| −0.939 mannitol | 0.596 b | 0.289 c | 0.051 b | 0.65 b |
| −0.939 PEG | 0.193 c | 0.111 d | 0.068 a | 0.17 b |
| ANOVA | ** | ** | ** | ** |

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

Table 3. Growth characters, shoot height (SH), root length (RL), leaf area (LA), fresh weight (FW) and dry weight (DW) of oil palm grown under mannitol- and PEG-induced iso-osmotic stresses.

| Water potential (MPa) | SH (cm) | RL (cm) | LA ($cm^2$) | FW (mg) | DW (mg) |
|---|---|---|---|---|---|
| −0.238 (Control) | 21.88 a | 21.21 a | 76.38 a | 1864 a | 380 a |
| −0.674 mannitol | 21.03 a | 18.50 ab | 40.59 b | 1172 b | 257 b |
| −0.674 PEG | 15.00 b | 17.20 ab | 13.83 c | 495 c | 122 c |
| −0.939 mannitol | 13.90 bc | 14.23 b | 11.80 c | 461 c | 125 c |
| −0.939 PEG | 11.23 c | 14.10 b | 2.40 c | 316 c | 94 c |
| ANOVA | ** | * | ** | ** | ** |

Different letters in each column show significant difference at $p \leq 0.01$ (**) and $p \leq 0.05$ (*) by Duncan's New Multiple Range Test (DMRT).

was 71.58 and 89.94% lower, respectively, than in the control. Degradation of $Chl_a$ under water deficit stress was correlated with $F_v/F_m$ (Fig. 2B), consequently affecting $\Phi_{PSII}$ (Fig. 3A). The values of $F_v/F_m$ and $\Phi_{PSII}$ in the leaf tissues of plants declined significantly under osmotic stress, while NPQ increased to a greater extent, depending on the degree of stress and the type of stressor (Table 2). There was a positive correlation between $\Phi_{PSII}$ and $P_n$ (Fig. 3B). $P_n$ dropped significantly, depending on the degree of osmotic stress (Table 2). We identified $\Phi_{PSII}$ and $P_n$ as sensitive parameters of photosynthetic ability, and dropped significantly under mild water deficit (−0.674 MPa). In contrast, $F_v/F_m$ was identified as an insensitive parameter, maintaining a high value under mild water deficit (−0.674 MPa) and decreasing significantly under severe water deficit (−0.939 MPa). In addition, the reduction percentage of $P_n$ under mild mannitol- and PEG-induced iso-osmotic stresses was 67.93% and 79.04%, respectively, and that under severe mannitol- and PEG-induced iso-osmotic stresses was 83.59 and 95.71%, respectively.

### 3. Growth performance

The growth characters, leaf area (LA), fresh weight (FW) and dry weight (DW) of the seedlings were drastically inhibited by osmotic stress (Table 3). In contrast, shoot height (SH) and root length (RL) were maintained under mild osmotic stress ($\Psi_w$=−0.674 MPa), but decreased significantly under severe osmotic stress, −0.939 MPa (Table 3). The reduction percentage of plant dry weight under −0.674 MPa mannitol- and PEG-induced iso-osmotic stresses was 32.37 and 67.89%, respectively, and that under −0.939 MPa mannitol- and PEG-induced iso-osmotic stresses was 67.11 and 75.26%, respectively. There was a similar trend in leaf area and plant fresh weight. Leaf chlorosis, leaf burn and reduction of leaf expansion were evident in the severe osmotic stress induced either by PEG or mannitol (Fig. 4).

## Discussion

In general, the water available for the plant is reduced by adding an osmoticum such as PEG or sugar alcohol (sorbitol and mannitol), leading to a slower rate of water absorption, uptake and translocation, as identified by low water content and low water potential (Gomes et al., 2010). Proline accumulation in higher plants is an indicator of stress as it alleviates water stress by means of antioxidant and osmoregulation defense mechanisms. The proline level was higher in plants exposed to water stress than in well-watered plants (Chołuj et al., 2008; Ahmed et al., 2009; Cha-um et al., 2010b). In rice (cv. Kranti), the proline content of the plants was higher under PEG-induced stress

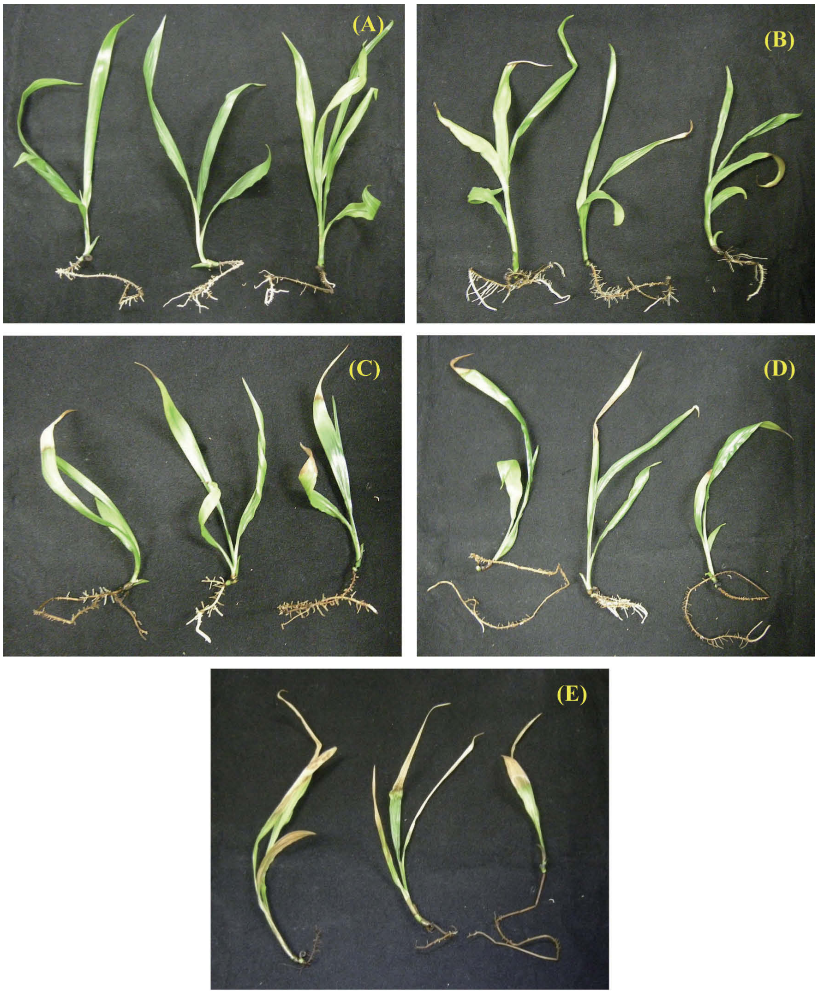

Fig. 4. Morphological characters of oil palm seedlings grown under mannitol- and PEG-induced iso-osmotic stresses at −0.238 MPa-(control) (A), −0.674 MPa-mannitol (B), −0.674 MPa-PEG (C), −0.939 MPa-mannitol (D) and −0.939 MPa-PEG (E).

than under mannitol-induced iso-osmotic stress (Pandey et al., 2004). In contrast, proline accumulation in the leaf tissues of *Sesuvium portulacastrum* under PEG-induced stress was lower than plants grown under mannitol-induced iso-osmotic stress (Slama et al., 2007). Proline accumulation in stress conditions was not only dependent on the type of stressor but also the plant species. In addition, the ornithine-$\delta$-aminotransferase ($\delta$-OAT), glutamate dehydrogenase (GDH), and proline-5-carboxylase reductase (P5CR) in the proline biosynthesis pathway in drought-stressed mulberry (Chaitanya et al., 2009) and *Sesuvium portulacastrum* (Slama et al., 2006) have been investigated. In this study, REL in oil palm seedlings was increased under PEG- and mannitol-induced osmotic stress, as was proline accumulation. REL in different plant species in response to water stress, has been shown to increase, depending on the degree of stress and the type of stressor (Bajji et al., 2001; Beltrano and Ronco, 2008; Cha-um et al., 2010b; Xu and Huang, 2010).

Levels of the photosynthetic pigments, $Chl_a$, $Chl_b$, TC and $C_{x+c}$, in the leaf tissues of oil palm seedlings decreased, leading to diminution of $F_v/F_m$ and $\Phi_{PSII}$ and $P_n$ in response to iso-osmotic stress, especially severe PEG-induced stress ($\Psi_w$=−0.939 MPa). However, in olive plants the $C_{x+c}$ level has been reported to decrease by osmotic stress (Ahmed et al., 2009; Guerfel et al., 2009), while it was maintained in coconut (Gomes et al., 2008). $C_{x+c}$ plays an important role in protection against photo-oxidative damage, as represented by low NPQ (Müller et al., 2001; Omasa and Takayama, 2003). In oil palm, $C_{x+c}$ was reduced significantly under severe water deficit conditions, which might cause NPQ enrichment. In oil palm, the photosynthetic ability dropped significantly under PEG-induced osmotic stress (Cha-um et al., 2010b) and the degree of the degradation of photosynthetic pigments and the $CO_2$ assimilation rate under severe osmotic stress were closely correlated with diminution of $F_v/F_m$ and reduction of $\Phi_{PSII}$ and $P_n$. In addition, $P_n$ in coconut cultivars, Una and Jiqui were reduced by 37.28% and 43.09%, respectively, under water deficit stress (Gomes et al., 2008), to limit the

$CO_2$ assimilation through the stomatal apertures (Cornic, 2000). In the present study, the toxic symptoms in oil palm seedlings grown under PEG-induced osmotic stress were greater than those under mannitol-induced iso-osmotic stress. Similar results have been observed in *Sesuvium portulacastrum* (Slama et al., 2007) and rice (Pandey et al., 2004).

Biomass production in higher plants is achieved by the photosynthetic ability, which is inhibited by osmotic stress. Growth characters, i.e. SH, RL, LA, FW and DW of oil palm seedlings were retarded by water shortage, especially in plants under PEG-induced osmotic stress. Similar results in the overall growth performance have been recorded in maize coleoptiles (Hohl and Schopfer, 1991). Osmotic stress induced by PEG, especially high-molecular weight PEG (MW>1000) has been reported to limit water uptake and $O_2$-disolution (Lawlor, 1970; Janes, 1974), whereas mannitol has been used only as an osmoticum with low absorption rate by the root tissues of plants (<5% radioactive labeling) (Smith and Smith, 1973; Flora and Madore, 1993; Vítová et al., 2002).

In conclusion, water content of the leaf tissues in palm seedlings decreased under osmotic stress, inducing relative electrolyte leakage and damage to photosynthetic pigments. The degradation of pigments and diminution of photosynthetic ability in oil palm seedlings grown under osmotic stress were positively related to the reduction of net photosynthetic rate and overall growth performance. In oil palm seedlings, the toxic effect of PEG-induced osmotic stress was greater than that of mannitol-induced stress.

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## References

Ahmad, M.S.A., Javed, F. and Ashraf, M. 2007. Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. *Plant Growth Regul.* 53: 53-63.

Ahmed, C.B., Rouina, B.B., Sensoy, S., Boukhris, M. and Abdallah, F.B. 2009. Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot.* 67: 345-352.

Ashraf, M. and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59: 206-216.

Bajji, M., Kinet, J.M. and Lutts, S. 2001. The use of the electrolyte leakage method for assessing cell membrane stability as water stress tolerance test in durum wheat. *Plant Growth Regul.* 36: 61-70.

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.

Beltrano, J. and Ronco, M.G. 2008. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: Effect on growth and cell membrane stability. *Braz. J. Plant Physiol.* 20: 29-37.

Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo, A.N., Francia, E., Marè, C., Tondelli, A. and Stanca, A.M. 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crops Res.* 105: 1-14.

Chaitanya, K.V., Rasineni, G.K. and Reddy, A.R. 2009. Biochemical responses to drought stress in mulberry (*Morus alba* L.): evaluation of proline, glycine betaine and abscisic acid accumulation in five cultivars. *Acta Physiol. Plant.* 31: 437-443.

Cha-um, S. and Kirdmanee, C. 2008. Effect of osmotic stress on proline accumulation, photosynthetic abilities and growth of sugarcane plantlets (*Saccharum officinarum* L.). *Pak. J. Bot.* 40: 2541-2552.

Cha-um, S., Mosaleeyanon, K., Kirdmanee, C. and Supaibulwatana, K. 2003. A more efficient transplanting system for Thai neem (*Azadirachta siamensis* Val.) by reducing relative humidity. *SciAsia* 29: 189-196.

Cha-um, S., Thadavong, S. and Kirdmanee, C. 2009. Effects of mannitol-induced osmotic stress on proline accumulation, pigment degradation, photosynthetic abilities and growth characters in $C_3$ rice and $C_4$ sorghum. *Front. Agric. China* 3: 266-273.

Cha-um, S., Ashraf, M. and Kirdmanee, C. 2010a. Screening upland rice (*Oryza sativa* L. ssp. *indica*) genotypes for salt-tolerance using multivariate cluster analysis. *Afri. J. Biotechnol.* 9: 4731-4740.

Cha-um, S., Takabe, T. and Kirdmanee, C. 2010b. Osmotic potential, photosynthetic abilities and growth characters of oil palm (*Elaeis guineensis* Jacq.) seedlings in responses to polyethylene glycol-induced water deficit. *Afri. J. Biotechnol.* 9: 6509-6516.

Chaves, M.M., Perrira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T. and Pinheiro, C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Ann. Bot.* 89: 907-916.

Chaves, M.M. and Oliveira, M.M. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J. Exp. Bot.* 55: 2365-2384.

Chołuj, D., Karwowska, R., Ciszewska, A. and Jasińska, M. 2008. Influence of long-term drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. *Acta Physiol. Plant.* 30: 679-687.

Cochard, B., Amblard, P. and Durand-Gasselin, T. 2005. Oil palm genetic improvement and sustainable development. *OCL* 12: 141-147.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trends Plant Sci.* 5: 187-188.

Dionisio-Sese, M.L. and Tobita, S. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135: 1-9.

Flexas, J., Bota, J., Galmés, J., Medrano, H. and Ribas-Carbó, M. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plant.* 127: 343-352.

Flora, L.L. and Madore, M.A. 1993. Stachyose and mannitol transport in olive (*Olea europaea* L.). *Planta* 189: 484-490.

Fujiwara, K., Kozai, T. and Watanabe, I. 1987. Fundamental studies on environment in plant tissue culture vessels. (3) Measurements of carbon dioxide gas concentration in closed vessels containing tissue cultured plantlets and estimates of net-photosynthetic rates of the plantlets. *J. Agric. Method.* 4: 21-30.

Gomes, F.P. and Prado, C.H.B.A. 2007. Ecophysiology of coconut palm under water stress. *Braz. J. Plant Physiol.* 19: 377-391.

Gomes, F.P., Oliva, M.A., Mielke, M.S., de Almeida, A.F., Leite, H.G. and Aqino, L.A. 2008. Photosynthetic limitations in the leaves of young Brazilian green dwarf coconut (*Cocos nucifera* L. 'nana') palm under well-watered conditions or recovering from drought stress. *Environ. Exp. Bot.* 62: 195-204.

Gomes, E.P., Oliva, M.A., Mielke, M.S., Almeida, A.A.F. and Aquino, L.A. 2010. Osmotic adjustment, proline accumulation and cell membrane stability in leaves of *Cocos nucifera* submitted to drought stress. *Sci. Hort.* 126: 379-384.

Guerfel, M., Baccouri, O., Boujnah, D., Chaïbi, W. and Zarrouk, M. 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Hort.* 119: 257-263.

Henson, I.E. and Dolmat, M.T. 2003. Physiological analysis of an oil palm density trial on a peat soil. *J. Oil Palm Res.* 15: 1-27.

Henson, I.E. and Harun, M.H. 2005. The influence of climatic conditions on gas and energy exchanges above a young oil palm stand in north Kedah, Malaysia. *J. Oil Palm Res.* 17: 73-91.

Hiller, S. and Dexter, A.R. 1997. A comparison of oxygen diffusion rates in sand moistened with water or PEG 20000 solution. *Int. Agrophysic.* 11: 173-176.

Hohl, M. and Schopfer, P. 1991. Water relations of growing maize coleoptiles. Comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting turgor pressure. *Plant Physiol.* 95: 716-722.

Ionenko, I.F. and Anisimov, A.V. 2001. Effect of water deficit and membrane destruction on water diffusion in the tissues of maize seedlings. *Biol. Plant.* 44: 247-252.

Jalani, B.S., Cheah, S.C., Rajanaidu, N. and Darus, A. 1997. Improvement of palm oil through breeding and biotechnology. *J. Amer. Oil Chem. Soc.* 74: 1451-1455.

Janes, B.E. 1974. The effect of molecular size, concentration in nutrient solution, and exposure time on the amount and distribution of polyethylene glycol in pepper plants. *Plant Physiol.* 54: 226-230.

Jones, L.H. 1997. The effect of leaf pruning and other stresses on sex determination in the oil palm and their representation by a computer simulation. *J. Theor. Biol.* 187: 241-260.

Kallarackal, J., Jeyakumar, P. and George, S.J. 2004. Water use of irrigated oil palm at three different arid locations in peninsular India. *J. Oil Palm Res.* 16: 45-53.

Kijne, J.W. 2006. Abiotic stress and water scarcity: Identifying and resolving conflicts from plant level to global level. *Field Crops Res.* 97: 3-18.

Lawlor, D.W. 1970. Absorption of polyethylene glycols by plants and their effects on plant growth. *New Phytol.* 69: 501-513.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* 148: 350-380.

Loggini, B., Scartazza, A., Brugnoli, E. and 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. and Johnson, G.N. 2000. Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* 51: 659-668.

Müller, P., Li, X.P. and Niyogi, K.K. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiol.* 125: 1558-1566.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-479.

Omasa, K. and Takayama, K. 2003. Simultaneous measument of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant Cell Physiol.* 44: 1290-1300.

Pandey, R., Agarwal, R.M., Jeevaratnam, K. and Sharma, G.L. 2004. Osmotic stress-induced alterations in rice (*Oryza sativa* L.) and recovery on stress release. *Plant Growth Regul.* 42: 79-87.

Parry, M.J.A., Androlojc, P.J., Khan, S., Lea, P.J. and Keys, A.J. 2002. Rubisco activity: effects of drought stress. *Ann. Bot.* 89: 833-839.

Passioura, J. 2007. The drought environment: physical, biological and agricultural perspectives. *J. Exp. Bot.* 58: 113-117.

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161: 1189-1202.

Repellin, A., Thi, A.T.P., Tashakorie, A., Sahsah, Y., Daniel, C. and Zuily-Fodil, Y. 1997. Leaf membrane lipids and drought tolerance in young coconut palms (*Cocos nucifera* L.). *Europ. J. Agron.* 6: 25-33.

Shabala, S.N., Shabala, S.I., Martynenko, A.I., Babourina, O. and Newman, I.A. 1998. Salinity effect on bioelectric activity, growth, $Na^+$ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Aust. J. Plant Physiol.* 25: 609-616.

Slama, I., Messedi, D., Ghnaya, T., Savouré, A. and Abdelly, C. 2006. Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum. Environ. Exp. Bot.* 56: 231-238.

Slama, I., Ghnaya, T., Hessini, K., Messedi, D., Savouré, A. and Abdelly, C. 2007. Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum. Environ. Exp. Bot.* 61: 10-17.

Smith, S.E. and Smith, F.A. 1973. Uptake of glucose, thehalose and mannitol by leaf slices of the orchid *Bletilla hyacinthine. New Phytol.* 72: 957-964.

Verslues, P.E., Ober, E.S. and Sharp, R.E. 1998. Root growth and oxygen ralations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiol.* 116: 1403-1412.

Vítová, L., Stodůlková, E., Bartoníčková, A. and Lipavská, H. 2002. Mannitol utilisation by celery (*Apium graveolens*) plants grown under different conditions *in vitro. Plant Sci.* 163: 907-916.

Wahid, M.B., Abdullah, S.N.A. and Henson, I.E. 2005. Oil palm–Achievements and potential. *Plant Prod. Sci.* 8: 288-297.

Weng, J.H. 2000. The role of activity and passive water uptake in maintaining leaf water status and photosynthesis in tomato under water deficit. *Plant Prod. Sci.* 3: 296-298.

Xu, C. and Huang, B. 2010. Differential proteomic responses to water stress induced by PEG in two creeping bentgrass cultivars differing in stress tolerance. *J. Plant Physiol.* 167: 1477-1485.

Yusof, B. and Chan, K.W. 2003. Going back to basics: Producing high oil palm yields sustainably. *Oil Palm Bull.* 46: 1-14.