

**Physiological features and growth characters of oil palm (*Elaeis guineensis* Jacq.) in response to reduced water-deficit and rewatering**Suriyan Cha-um<sup>1\*</sup>, Nana Yamada<sup>1</sup>, Teruhiro Takabe<sup>2,3</sup>, Chalermopol Kirdmanee<sup>1</sup><sup>1</sup>National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand<sup>2</sup>Graduate School of Environmental and Human Sciences, Meijo University, Nagoya 468-8502, Japan<sup>3</sup>Research Institute, Meijo University, 1-501 Shiogamaguchi, Tenpaku-ku, Nagoya 468-8502, Japan

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**Abstract**

Optimum irrigation is very important for nursery stock management of F<sub>1</sub>-hybrid oil palm (*Elaeis guineensis* Jacq.), constituting a major production cost. Generally, over watering of nursery stock can be a cause of loss of productivity. The aim of this study was to investigate the physiological and biochemical responses of oil palm nursery stock to reduced soil water content (SWC) and during the recovery process. Water deficit treatments, full-irrigation (control; 54% SWC), 4-days withholding irrigation (42% SWC), 8-days withholding irrigation (20% SWC), 12-days withholding irrigation (13% SWC) and 16-days withholding irrigation (6% SWC) were applied uniformly to the oil palm plants. Proline content and relative electrolyte leakage (REL) in the leaf tissues increased, relative to a degree of reduced SWC, especially in  $\leq 20\%$  SWC water deficit stress. Oil palm nursery stock was sensitive to severe water deficit (6-13% SWC), which was identified by physiological changes and growth inhibition and those parameters failed to recover in the re-watering process. During recovery, proline content and REL in extreme water deficit conditions were still enriched, resulting in greater photosynthetic degradation, diminution of chlorophyll fluorescence, reduction of net photosynthetic rate (P<sub>n</sub>), and growth retardation. The wilting point of nursery stock oil palm was defined as 20% SWC, below which plants recovered by watering, without toxic symptoms. The study provides useful information for developing irrigation systems for oil palm, especially F<sub>1</sub> hybrid nursery stock.

**Keywords:** chlorophyll fluorescence, net photosynthetic rate, photosynthetic pigments, proline, relative electrolyte leakage, water deficit.

**Abbreviations:** Chl<sub>a</sub>\_chlorophyll a; Chl<sub>b</sub>\_chlorophyll b, DW\_dry weight; FW\_fresh weight; F<sub>v</sub>/F<sub>m</sub>\_maximum quantum yield of PSII; LA\_leaf area; P<sub>n</sub>\_net photosynthetic rate; Φ<sub>PSII</sub>\_quantum efficiency of PSII; REL\_relative electrolyte leakage; SH\_shoot height; SWC\_soil water content; TC\_total chlorophyll.

**Introduction**

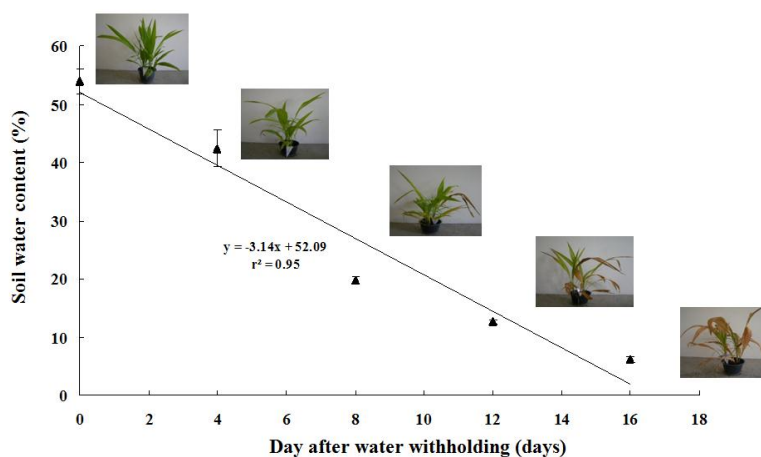
Global warming is a topical issue worldwide that has resulted in extreme environmental conditions, including water deficit (drought) and excess water (flooding), and poses serious risk to mankind (Murry et al., 2012; Sena et al., 2012). It is opined that the area of land affected by extreme drought will increase, due to a high rate of evapotranspiration, from 1% now, to 30% by 2100 (Xu et al., 2010). Drought stress causes growth inhibition, abnormal plant development and loss of crop productivity (Reddy et al., 2004; Blum, 2005; Neumann, 2008; Shao et al., 2008). Photosynthesis, particularly in terms of reduced CO<sub>2</sub> assimilation, decreased stomatal conductance, decreased quantum yield of PSII, impaired ATP production, damaged ATP synthase, inhibited Rubisco enzyme and altered light harvesting complexes, has been reported to be a sensitive parameter to drought stress (Chaves and Oliveira, 2004; Cattivelli et al., 2008; Pinheiro and Chaves, 2011). In higher plants, proline - a well known stress indicator - is a candidate biochemical solute, especially under water-deficit stress (Yoshida et al., 1997). The proline biosynthesis in plants occurs *via* glutamate intermediate, using P5CS (Δ<sup>1</sup>-pyrroline-5-carboxylate synthetase) to P5C (Δ<sup>1</sup>-pyrroline-5-carboxylate), subsequently oxidized to the

final product proline by P5CR (Δ<sup>1</sup>-pyrroline-5-carboxylate reductase). Proline degradation occurs through ProDH (proline dehydrogenase) to P5C (Δ<sup>1</sup>-pyrroline-5-carboxylate) and then to P5CDH (Δ<sup>1</sup>-pyrroline-5-carboxylate dehydrogenase) (Ni et al., 2009; Szabados and Savaouré, 2009; Verslues and Sharma, 2010). Proline has been reported to play a significant role, including signal transduction, osmoregulation and as antioxidant systems, in plant defense responses under water-deficit stress, (Hare et al., 1999; Kishor et al., 2005). Recently, proline enrichment in oil palm has been demonstrated as one of the most evident biochemical indices under water deficit stress (Cha-um et al., 2010; Cao et al., 2011; Cha-um et al., 2011). Oil palm (*Elaeis guineensis* Jacq.) is one of the most important oil crop species in the world, and is widely cultivated in Southeast Asia, including Malaysia, Indonesia and Thailand (Wilcove and Koh, 2010). In general, the fibrous root system of the palm quickly absorbs water from the soil in areas under irrigation (0.7 m in depth), while deep roots (1.1 m) develop in palms growing in rain-fed areas. However, irrigation systems are a new development in oil palm cultivation, and only 0.1% of the total harvested area (97 km<sup>2</sup>) is reported as being under

**Table 1.** Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>), total chlorophyll (TC) contents and Chl<sub>a</sub>:Chl<sub>b</sub> ratio in the leaf tissues of oil palm plants when subjected to water deficit stress and 3-days well watering recovery.

SWC (%)	Chl <sub>a</sub> (µg g <sup>-1</sup> FW)	Chl <sub>b</sub> (µg g <sup>-1</sup> FW)	TC (µg g <sup>-1</sup> FW)	Chl <sub>a</sub> :Chl <sub>b</sub>
54	87.8a	57.4a	145.2a	1.53a
42	60.5b	54.5a	115.0b	1.12b
20	56.5b	54.4a	110.9b	1.04bc
13	36.0c	44.6b	80.6c	0.81c
6	3.6d	3.3c	6.9d	1.10bc
<i>ANOVA</i>	**	**	**	**
54-Re	85.0a	55.2a	140.2a	1.54a
42-Re	58.5b	55.9a	114.4b	1.05bc
20-Re	59.3b	53.1a	112.4b	1.12b
13-Re	38.0c	42.4b	80.4c	0.90c
6-Re	15.1d	22.5c	37.6d	0.68d
<i>ANOVA</i>	**	**	**	**

Means with the different letters are significantly different at  $P \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test.



**Fig 1.** Soil water content (SWC) in the pot culture and morphological characters of oil palm plants when subjected to withholding irrigation for 0, 4, 8, 12 and 16 days. The mortality of leaf was evidently demonstrated i.e. leaf chlorosis and leaf burn relating to a degree of SWC reduction. Error bar represents  $\pm$ SE.

irrigated oil palm (Siebert and Döll, 2010). In mature oil palms, the daily transpiration rate (T) depends on the season, being 3.3–6.5 mm d<sup>-1</sup> in the rainy season and 1.3–2.5 mm d<sup>-1</sup> in the dry season, as well as the cultivation site (Henson and Harun, 2005; Carr, 2011). Also, the level of precipitation (>1500 mm yr<sup>-1</sup>) is one of the most important factors in oil palm cultivation. Water availability in the soil play a key role in palm growth and may also trigger sex representation (Jones, 1997; Adam et al., 2011). In nursery stock, due to the concern over water scarcity, it is pertinent to investigate optimum water supply, (Pereira et al., 2002; Fereres and Soriano, 2007), that has been identified as a major cost of F<sub>1</sub> hybrid oil palm production. In the present study, we evaluated the physiological and biochemical changes in oil palm in response to reduced soil water content (SWC) and rewatering (recovery process).

## Results

### *Proline and relative electrolyte leakage under water deficit stress*

Soil water content (SWC) in pot cultured oil palm plants decreased continuously, relative to the number of days withholding irrigation. The morphological responses of the plants to reduced SWC were demonstrated (Fig. 1). The degree of reduction of SWC was dependent on the number of

days after withholding irrigation, with a strong relationship ( $r^2 = 0.95$ ). At  $\leq 13\%$  SWC, leaf chlorosis, leaf wilting and leaf burn were observed. In the oil palm plants subjected to 20, 13 and 6% SWC, proline content in the leaf tissues was 0.91, 1.67 and 2.09 µmol g<sup>-1</sup> FW, respectively (Fig. 2a). The accumulation of proline under water-deficit suggested it to be a good drought stress indicator. Similarly, REL in the leaf tissues of oil palm plant increased progressively with a reduction of SWC. Electrolyte leakage in the water deficit stressed plants was significantly greater in the case of extreme low SWC ( $\leq 20\%$ ) (Fig. 2b). In the present study, proline content and REL in oil palm plants subjected to low SWC increased, especially under severe water deficit conditions ( $\leq 13\%$  SWC).

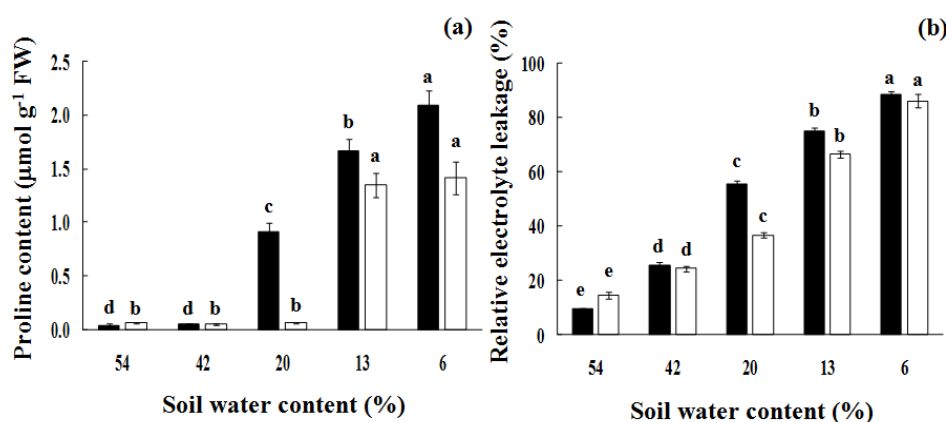
### *Photosynthetic abilities*

The increase in REL in water deficit stressed palms was positively correlated with chlorophyll degradation (Fig. 3a;  $r^2 = 0.79$ ). Chl<sub>a</sub> content in the water deficit stressed plants decreased (significant at  $p \leq 0.01$ ) by 31.1, 35.7, 59.0 and 95.9% at 42, 20, 13 and 6% SWC, respectively. The Chl<sub>a</sub> content was very sensitive to water deficit stress, which caused it to be drastically damaged at 42% (at 6%) SWC. In addition, the degradation of Chl<sub>b</sub> increased significantly at

**Table 2.** Maximum quantum yield of PSII ( $F_v/F_m$ ), quantum efficiency of PSII ( $\Phi_{PSII}$ ) and net photosynthetic rate ( $P_n$ ) of oil palm plants when subjected to water deficit stress and 3-days well watering recovery.

SWC (%)	$F_v/F_m$	$\Phi_{PSII}$	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
54	0.835a	0.724a	3.14a
42	0.788b	0.654b	2.93b
20	0.668c	0.452c	1.50c
13	0.329d	0.175d	0.89d
6	0.183e	0.085e	0.28e
ANOVA	**	**	**
54-Re	0.827a	0.649a	4.22a
42-Re	0.817a	0.645a	3.53b
20-Re	0.769b	0.535b	2.43c
13-Re	0.444c	0.335c	1.35d
6-Re	0.228d	0.164d	0.48e
ANOVA	**	**	**

Means with the different letters are significantly different at  $P \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test.



**Fig 2.** Proline content (a) and relative electrolyte leakage (b) of oil palm exposed to water-deficit stress (dark bars) and 3-days well watering recovery (light bars). Error bars represent  $\pm$ SE.

13% SWC. The trend of changes in total chlorophyll (TC) paralleled that in  $\text{Chl}_a$  and TC was degraded similarly to  $\text{Chl}_a$  (Table 1). The ratio of  $\text{Chl}_a:\text{Chl}_b$  in the water deficit stressed oil palm leaves decreased significantly, relative to the degree of water deficit, except in 6% SWC (Table 1). A positive relationship was observed between  $\text{Chl}_a$  content and maximum quantum yield of PSII ( $F_v/F_m$ ) (Fig. 3b;  $r^2 = 0.89$ ), and between TC and quantum efficiency of PSII ( $\Phi_{PSII}$ ) (Fig. 3c;  $r^2 = 0.81$ ).  $F_v/F_m$  in the water deficit stressed plants diminished (significant at  $p \leq 0.01$ ), by 5.6, 20.0, 60.6 and 78.1% under 42, 20, 13 and 6% SWC, respectively. A similar trend of changes was observed in  $\Phi_{PSII}$  in relation to water deficit stress (Table 2). Further, a positive correlation was determined between  $\Phi_{PSII}$  and net photosynthetic rate ( $P_n$ ) (Fig. 3d;  $r^2 = 0.96$ ).  $P_n$  in the water deficit stressed plants diminished significantly, by 6.7, 52.2, 71.7 and 91.1% at 42, 20, 13 and 6% SWC, respectively (Table 2). Since nearly 50% reduction was noticed in chlorophyll fluorescence ( $F_v/F_m$  and  $\Phi_{PSII}$ ) and photosynthetic ability ( $P_n$ ) in oil palm exposed to 20% SWC, it was considered as the critical point.

#### Growth performance under water deficit stress

The reduction in photosynthetic ability was closely related to growth performance indicators, including, shoot height (SH), fresh weight (FW), dry weight (DW) and leaf area (LA). SH in the water deficit stressed plants decreased significantly, by 11.5, 20.9, 28.4 and 41.9% under 42, 20, 13 and 6% SWC,

respectively (Table 3). Similarly, the FW, DW and LA were significantly reduced, relative to the drop in SWC. The green leaf area may be considered a sensitive parameter, since it dropped by 50% under 20% SWC.

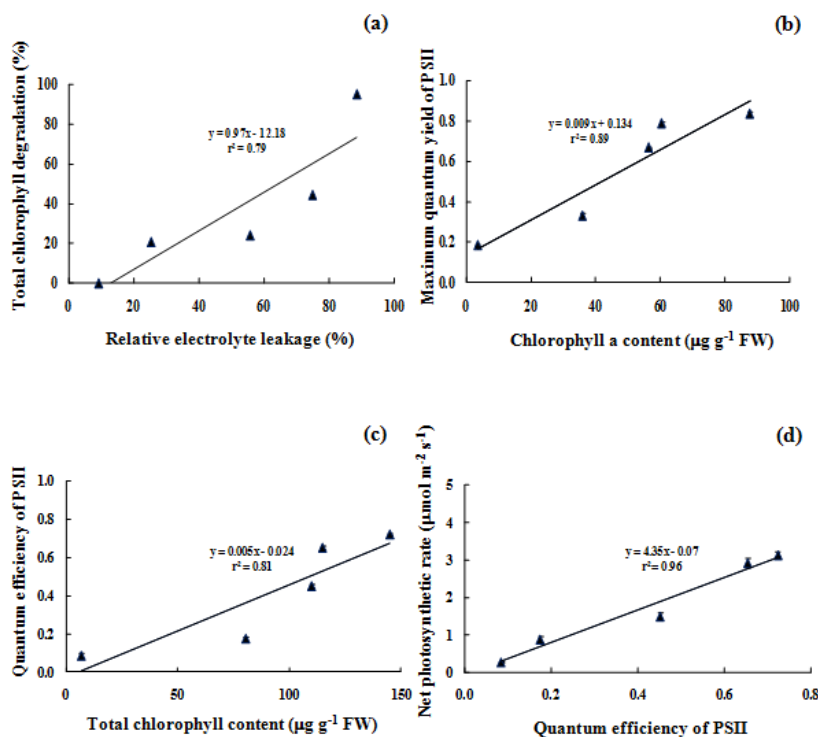
#### Physio-biochemical changes and growth performance in well watering recovery

Upon re-watering (i.e. at  $>20\%$  SWC; recovery), the morphological characters of oil palm recovered, as demonstrated by green leaf area and overall growth performance (Fig. 4). Proline content in the leaf tissues of plants after 3 days of well watering recovery ( $\geq 20\%$  SWC) was reduced to nearly that of the control, whereas in plants still under severe water deficit (6-13% SWC), it was still enriched (Fig. 2a). Similarly, the REL in leaf tissues of plants after 3 days of well watering recovery decreased when compared to plants under water deficit stress, especially in 20% SWC (Fig. 2b). However, a positive relationship between REL and chlorophyll degradation was demonstrated ( $r^2 = 0.95$ ) (Fig 5a).  $\text{Chl}_a$  and TC content in the leaf tissues of water-deficit stressed plants in the recovery step were significantly decreased (about 50% degradation of control) at 13% SWC (Table 1). Moreover, the  $\text{Chl}_b$  content decreased sharply under extreme water deficit stress  $\leq 13\%$  SWC, which was identified as the critical point for recovery by well watering irrigation (Table 1). The  $\text{Chl}_a:\text{Chl}_b$  ratio was reduced in a pattern similar to that of photosynthetic pigment

**Table 3.** Shoot height (SH), fresh weight (FW), dry weight (DW) and leaf area (LA) of oil palm plants when subjected to water deficit stress and 3-days well watering recovery.

SWC (%)	SH (cm)	FW (g)	DW (g)	LA (cm <sup>2</sup> )
54	87.6a	260.9a	120.7a	2635a
42	77.5b	188.2b	99.8b	2002b
20	69.3c	154.8c	82.7c	1335c
13	62.7d	120.9d	66.9d	496d
6	50.9e	59.2e	38.5e	154e
ANOVA	**	**	**	**
54-Re	89.8a	265.2a	126.5a	2663a
42-Re	80.7b	192.8b	105.2b	2159b
20-Re	71.1c	162.5c	88.3c	1353c
13-Re	65.4d	124.5d	68.6d	502d
6-Re	51.7e	68.4e	40.2e	165e
ANOVA	**	**	**	**

Means with the different letters are significantly different at  $P \leq 0.01$  (\*\*) by Duncan's New Multiple Range Test.



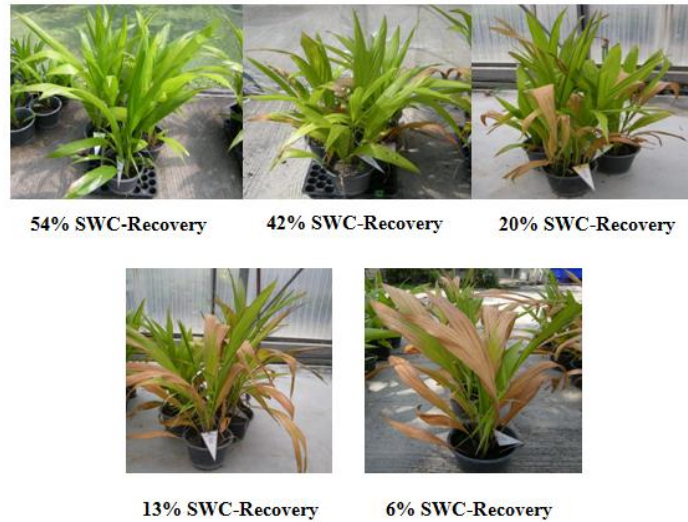
**Fig 3.** Relationship between relative electrolyte leakage and total chlorophyll degradation (a), chlorophyll a content and maximum quantum yield of PSII (b), total chlorophyll content and quantum efficiency of PSII (c), quantum efficiency of PSII and net photosynthetic rate in the leaf tissues of oil palm plants when subjected to water deficit stress. Error bar represents  $\pm$ SE.

levels. A positive relationship between  $Chl_a$  content and  $F_v/F_m$  ( $r^2 = 0.86$ ) (Fig 5b), TC and  $\Phi_{PSII}$  ( $r^2 = 0.93$ ) (Fig 5c),  $\Phi_{PSII}$  and  $P_n$  ( $r^2 = 0.94$ ) (Fig 5d) was observed. The  $F_v/F_m$  and  $\Phi_{PSII}$  of recovered plants were significantly diminished, especially in  $\leq 20\%$  SWC (Table 2). In addition,  $P_n$  was identified not only as a water-deficit sensitive parameter but also in the recovery process (Table 2). Similarly, growth performance parameters, i.e. plant height, fresh weight, dry weight and leaf area, were significantly inhibited in the recovery process, especially in  $\leq 20\%$  SWC (Table 3).

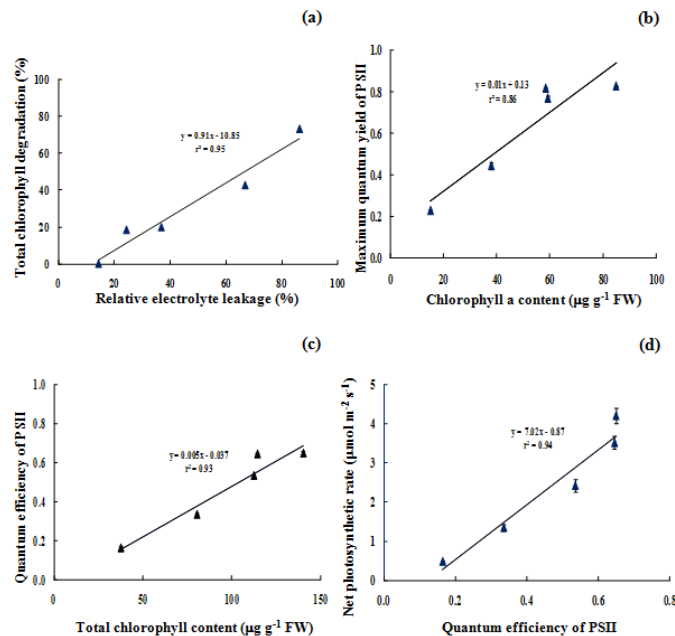
### Discussion

In oil palm, proline content and REL in drought stressed seedlings evidently increased, following the degree of drought stress, especially in plants where watering was withheld for 14 and 21 days (Cao et al., 2011). Similar results

have been reported in oil palm subjected to mannitol-induced water deficit stress (Cha-um et al., 2011) and coconut palm exposed to water stress (Gomes et al., 2010). Previously, the proline level in date palm callus cultured under polyethylene glycol (PEG)-induced drought stress has been reported to increase in relation to increase PEG concentration in the culture medium (Al-Khayri and Al-Bahrany, 2004). REL has, in fact, been regarded as water deficit tolerance criterion in oil palm genotypes (Asemota and Conaire, 2010). Photosynthetic pigment degradation in water deficit stressed palms is a general physiological response in plants subjected to water withholding or reduced SWC. In coconut palm, total chlorophyll ( $Chl_a + Chl_b$ ) and total carotenoids in water stressed plants dropped significantly, causing diminishing of  $F_v/F_m$  and reduced  $P_n$  in both UGD (a hot and humid habitat) and JGD (a hot and dry habitat) genotypes (Gomes et al., 2008). Almost similar observations were made in olive genotypes under water deficit (Boughalleb and Hajlaoui,



**Fig 4.** Morphological characters of water-deficit stressed oil palm in 3-days well watering recovery. Leaf necrosis and chlorosis of oil palm plants grown under 20-42% SWC were quickly recovered in the rewating process whereas leaf burn of those grown under 6-13% SWC was still demonstrated at recovery (well watering).



**Fig 5.** Relationship between relative electrolyte leakage and total chlorophyll degradation (a), chlorophyll a content and maximum quantum yield of PSII (b), total chlorophyll content and quantum efficiency of PSII (c), quantum efficiency of PSII and net photosynthetic rate in the leaf tissues of oil palm plants in 3-days well watering recovery. Error bar represents  $\pm$ SE.

2011). A significant difference in pigment degradation was noticed on the 40<sup>th</sup> day of withholding of watering in olive. Total chlorophyll and total carotenoids concentrations in water deficit stressed olive plants decreased sharply, showing >50% reduction when exposed to extreme water deficit stress for 60 days by withholding watering, especially in the “Chemleli” cultivar, leading to reduced  $F_v/F_m$  and  $P_n$  (Boughalleb and Hajlaoui, 2011). Moreover, the photosynthetic pigments in oil palm subjected to mannitol-induced and PEG-induced water deficit stress decreased drastically, depending on the osmotic potential in the culture medium (Cha-um et al., 2012). The photosynthetic abilities,  $F_v/F_m$ ,  $\Phi_{PSII}$  and  $P_n$ , dropped in the palm plantlets grown under extreme water deficit conditions (Cha-um et al., 2010; Suresh et al., 2010; Cha-um et al., 2011). A positive

relationship between photosynthetic abilities such as  $\text{Chl}_a$  and  $F_v/F_m$ , total chlorophyll and  $\Phi_{PSII}$ ,  $\Phi_{PSII}$  and  $P_n$  has been well established in oil palm grown under mannitol-induced water deficit (Cha-um et al., 2011). In fact, the growth characters of oil palm seedlings have been reported as sensitive parameters. In a previous study, the leaf area was significantly reduced in plants subjected to drought stress (7 days withholding water) whereas plant height did not differ significantly in drought conditions after 7, 14 and 21 days (Cao et al., 2011). In addition, leaf area, root dry mass and shoot dry mass of coconut palm “cv. Brazillian Green Dwarf” were significantly retarded, by 26, 61 and 48%, respectively, in plants exposed to drought stress (Gomes et al., 2010). In previous reports, shoot height, root length, leaf area, fresh weight and dry weight in oil palm seedlings grown under

PEG-induced water deficit stress declined significantly and to a greater extent than those grown under mannitol-induced stress (Cha-um et al., 2010; Suresh et al., 2010; Cha-um et al., 2011). In the recovery process, the morphological and physiological characters of oil palm grown under mild water deficit ( $\geq 20\%$  SWC) were restored. The 20% SWC was identified as critical point or wilting point of oil palm plantlets to be recovered when re-watered. REL decreased in the recovery stage, and the proline content in the water deficit stressed palms dropped to nearly that of plants in the control conditions (54% SWC), leading to maintenance of photosynthetic ability and overall growth performance. The observations made in the present study are in conformity with those of Filippou et al. (2011) who demonstrated a recovery of dropped proline content in drought-stressed *Medicago truncatula* upon rewatering of the plants for 11 days (i.e. during recovery). Additionally, the  $F_v/F_m$  and stomatal conductance in the recovered leaves of *Medicago truncatula* functioned well (Filippou et al., 2011). In oil palm, the  $F_v/F_m$ ,  $\Phi_{PSII}$ , and  $P_n$  abilities in water-stressed plants quickly recovered close to that of well-irrigated plants after 2 days of re-watering (Suresh et al., 2010). Moreover, the total chlorophyll, total carotenoid,  $F_v/F_m$  and  $P_n$  in water deficit coconut palm were restored when plants were subject to full irrigation (Gomes et al., 2008). A full recovery of photosynthetic abilities using re-watering has been reported as a key factor in the further growth and development of drought stressed plants. However, the photosynthetic capabilities in plants grown under extreme drought (6% SWC) could not be recovered, leading to plant death (Xu et al., 2010).

## Materials and methods

### Plant materials

Oil palm fruits were obtained from Suksomboon Palm Co. Ltd., Chonburi province, in eastern 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 then the seeds were 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 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 25±2°C, 60±5% relative humidity (RH) and 60±5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) provided by fluorescent lamps with 16 h d<sup>-1</sup> photoperiod. After two months, the seedlings were transferred aseptically to MS-liquid sugar-free media (photoautotrophic growth) using vermiculite as supporting material for 6 months. Air exchange in the glass vessels was adjusted to 2.32  $\mu\text{mol CO}_2 \text{ h}^{-1}$  by punching a hole in the plastic cap ( $\phi=1$  cm) and covering the hole with gas-permeable microporous polypropylene film (0.22 mm pore size, Nihon Millipore Ltd., Japan). Oil palm seedlings were cultured under 25±2°C, 60±5% RH and 100±5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD provided by fluorescent lamps with 16 h d<sup>-1</sup> photoperiod. Acclimatized plantlets were transferred to plastic pots ( $\phi=15$  cm; height=11 cm) containing clay soil (EC = 2.687 dS m<sup>-1</sup>; pH = 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%) in 50% shaded light intensity. Acclimatized oil palm plants were grown in the greenhouse at Pathumthani province (Latitude 14° 01' 12" N and Longitude 100° 31' 12"

E) and watered daily until they had produced 3-4 full leaves. Water deficit treatments; full-irrigation (control), 4-days withholding irrigation, 8-days withholding irrigation, 12-days withholding irrigation and 16-days withholding irrigation; were applied uniformly to the oil palm plants. Data concerning soil water content (SWC), proline content, relative electrolyte leakage, photosynthetic features and growth performance parameters in the leaf tissues of water-deficit stressed oil palm were collected.

### Soil water content

Soil water content (SWC) was calculated using the weight fraction:  $\text{SWC} (\%) = [(\text{FW}-\text{DW})/\text{DW}] \times 100$ , where FW was the fresh weight of a portion of 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).

### Relative electrolyte leakage (REL) and proline assay

REL was determined according to the method of Dionisio-Sese and Tobita (1998). 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 ( $\text{EC}_0$ ) 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 ( $\text{EC}_1$ ) was measured. REL was determined using the formula:  $\text{REL} = (\text{EC}_0 / \text{EC}_1) \times 100$ . Proline in the leaf tissues was extracted and analyzed according to the method of Bates et al. (1973). Fifty milligrams fresh leaf material 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 H<sub>3</sub>PO<sub>4</sub>) and incubated at 95°C for 1 h. The reaction was stopped by placing the tubes in an ice bath. The reaction mixture was mixed vigorously with 2 mL toluene. After cooling to 25°C, the chromophore was measured on a UV-visible spectrophotometer (model DR/4000, HACH, USA) at 520 nm using L-proline as a standard.

### Photosynthetic features

Chlorophyll a (Chl<sub>a</sub>), chlorophyll b (Chl<sub>b</sub>) and total chlorophyll (TC) content in the leaf tissue were analyzed following the methods of Shabala et al. (1998). One hundred milligrams of leaf material 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<sub>a</sub> and Chl<sub>b</sub> concentrations were measured using a UV-visible spectrophotometer (model DR/4000, HACH, USA) at 662 nm and 644 nm wavelengths. The total carotenoids ( $C_{x+c}$ ) concentration was measured by spectrophotometer at 470 nm. A solution of 95.5% acetone was used as a blank. Chlorophyll fluorescence emission from the adaxial surface on 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 LED light source with typical peak at wavelength 735nm). Original ( $F_0$ ) and maximum ( $F_m$ ) fluorescence yields were measured under weak modulated red light ( $<0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with 1.6 s pulses of saturating light ( $1,540 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) and calculated using FMS software for Windows<sup>®</sup>. The variable fluorescence yield ( $F_v$ ) was calculated by the equation  $F_m - F_0$ . 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 ( $\Phi_{\text{PSII}}$ ) was calculated by  $\Phi_{\text{PSII}} = (F_m' - F)/F_m'$  after 45 s of  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$  actinic light illumination, when steady state was achieved. Net photosynthetic rate ( $P_n$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was measured using a Portable Photosynthesis System with an Infra-red Gas Analyzer (Model LI 6400, LI-CORR Inc., Lincoln, Nebraska, USA) following Cha-um et al. (2007).

### Growth characters

Shoot height (SH), fresh weight (FW), dry weight (DW) and leaf area (LA), of oil palm seedlings were measured. Oil palm seedlings were dried at  $80^\circ\text{C}$  in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of dry weight according to Lutts et al. (1996). The leaf area of oil palm seedlings was measured using a Leaf Area Meter DT-scan (Delta-T Scan<sup>®</sup>, Delta-T Devices Ltd., Cambridge, UK).

### Experiment design and statistical analysis

The experiment was arranged as 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 at  $p \leq 0.01$ .

### Conclusion

The proline content and REL in the leaf tissues of oil palm were enriched, relative to the reduction in SWC, and then dropped during well-watering recovery, especially at 20% SWC. The degradation of photosynthetic pigments, chlorophyll fluorescence diminution and net photosynthetic rate reduction in the leaf tissues were positively related to the increase in REL, leading to growth inhibition in plants subjected to low SWC. In contrast, the physiological parameters and growth performance in 3-day well watering plants recovered, especially at 20% SWC. Also, 20% SWC was considered as the critical point in oil palm plants grown in pot culture as nursery stock. The information related to critical period of water withholding revealed from this investigation may further be applied for the irrigation systems of  $F_1$ -hybrid oil palm production in nursery management.

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