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Comparisons of Chlorophyll Fluorescence and Physiological Characteristics of Wheat Seedlings Influenced by Iso-Osmotic Stresses from Polyethylene Glycol and Sodium Chloride

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Abstract: Wheat (*Triticum aestivum*) cultivar Taichung SEL2 (TCS2) is a salt-tolerance variety, but the mechanism involved remains unclear. This study aims to distinguish between the non-ionic osmotic and salt-mediated physiological effects on TCS2. Osmotic agents polyethylene glycol (PEG) and sodium chloride (NaCl) were applied at three iso-osmotic levels, level 1 containing 24% (*w/v*) PEG and 200 mM NaCl, level 2 containing 26.5% (*w/v*) PEG and 250 mM NaCl, and level 3 containing 29% (*w/v*) PEG and 300 mM NaCl, respectively. According to the investigation of chlorophyll fluorescence in the better NaCl-treated seedlings, maximal quantum yield of photosystem II (PSII) (F_v/F_m) and significant higher effective quantum yield of PSII (Φ_{PSII}) at level 3 were observed. Meanwhile, the non-photochemical quenching of PSII (NPQ) and the quantum yield of regulated energy dissipation of PSII [$Y(NPQ)$] were significantly higher in the NaCl-treated seedlings, and the quantum yield of non-regulated energy dissipation of PSII [$Y(NO)$] in the NaCl-treated seedlings was lower than the PEG-treated ones at level 2 and level 3. Furthermore, the less extensive degradation of photosynthetic pigments, the better ascorbate peroxidase (APX) activity and the less accumulation of malondialdehyde (MDA) were also observed in NaCl-treated seedlings. In the morphological traits, shoot elongation in NaCl-treated seedlings was also preserved. These results suggest that TCS2 is more resistant to NaCl-induced osmotic stress than to the PEG-induced stress. This study contributes to plant breeder interest in drought- and/or salt-tolerant wheat varieties.

Keywords: wheat; *Triticum aestivum*; salt stress; osmotic stress; APX activity

1. Introduction

Salinity in increasing frequency and intensity impacts wheat (*Triticum aestivum*) production around the world. It is estimated that more than 69% of wheat production is seriously influenced by high salinity [1]. Therefore, it is crucial to understand the mechanisms of salt stress tolerance in wheat and/or how to adapt to salt stress, and to discover how to prevent the impacts of salinity on wheat production. Salt stress would lead to osmotic and/or ionic stress over different time scales [2–4]. This osmotic and/or ionic stress would trigger signal sensing and induce overexpression of transcription factors to

regulate downstream genes in plant sequentially. These regulated downstream genes would lead to the promotion of a biosynthetic pathway of osmolytes, such as proline, glycine betaine, trehalose, etc., the overexpression of transporter genes to stabilize both osmotic and ionic (Na^+/K^+) homeostasis, the increase of activities of antioxidative enzymes against stress-induced free radicals, and accumulation of polyamines [5]. A recent study also reported that aquaporins, which are a group of proteins regulating water transport, are also involved in the response to osmotic stress [6]. Furthermore, the moderate salt stress might induce salt adaptation in plant [7].

Salt stress might trigger a series of reactions that are similar to the physiological response to drought stress in a plant [8]. Almansouri et al. [9] reported that the iso-osmotic potential of NaCl has been imitated by polyethylene glycol (PEG), which is widely applied to stimulate osmotic stress. PEG is a non-ionic, neutral, and water-soluble polymer that does not penetrate roots [10]. The study of Marcińska et al. [11] reported that PEG-induced osmotic stress stimulated the higher accumulation of proline in drought-resistant wheat than in drought-sensitive one. Previous studies have found that PEG was more disadvantageous compared to NaCl treatments in the germination stage of durum wheat [9], and the seedling stage of wheat [3,10], *Hordeum* species [12], and *Haloxylon aphyllum* [13]. However, Muranaka et al. [14,15] found just the opposite in the seedling stage of wheat. Greenway and Munns [16] and Sharma et al. [17] evaluated salt-sensitive and -tolerant varieties and suggested that salt-tolerance varieties were more susceptible to PEG-induced water deficits compared to ionic stress, while there was an opposite response in sensitive types. Salinity resistance in plants also varies depending on the growth stage [18,19]. Sayar et al. [10] demonstrated that the seedling stage under iso-osmotic potential treatments was more susceptible to NaCl than the germination stage.

Each plant species/variety displays dynamic responses to salinity during different growth stages [18,19]. Plant species/varieties during specific growth stages lack a genetically related ability to reduce salt uptake [20,21], compartmentalize salt in cell vacuoles, prevent salt from reaching toxic levels in leaves [4], and display more susceptibility to ion-induced stress. Furthermore, plant species/varieties during specific growth stages display a tolerance to ion-induced stress [22]. Both Na^+ and Cl^- act as environmental signals that trigger rapid osmotic adjustment and stabilize shoot turgor [23,24]. NaCl-induced osmoregulation is much faster and less energy- and carbon-demanding than PEG-induced osmotic adjustment [25]. Recent research reported that wheat seedlings responded with different metabolic regulations to osmotic stress induced by NaCl and PEG [26].

To evaluate the relative influence of salt-induced osmotic and ionic stress, biochemical, physiological responses, and morphological traits can be investigated for the estimation of salt-tolerance mechanisms [27]. Under salt stresses, the enzymatic and non-enzymatic antioxidant systems, photosynthesis systems, and growth and development of a plant will be inhibited, but lipid peroxidation products will increase [28]. Recent research revealed that severe salt stress caused the accumulation of H_2O_2 and malondialdehyde (MDA), which is an indicator of lipid peroxidation products, and induced enzymatic antioxidant activities, while low salinity stimulated growth, photosynthesis, and promoted the activity of ascorbate peroxidase (APX) in plant [7].

Biochemical information responds to plant status under salt stress, but destructive biochemical assays will not allow real-time monitoring, whereas chlorophyll fluorescence (ChlF) analysis effectively estimates physiological changes and is a rapid and real-time screening system [29,30]. Recent studies reported that chlorophyll a fluorescence, also called prompt chlorophyll a fluorescence, and its parameters, which were calculated based on OJIP transient curves, effectively responded to shade [31], salt [32], light, heavy metal and other stress [33,34] in plant. Delayed chlorophyll a fluorescence, which is also reemitted from PSII antenna complex and displays multiple phase during emission time, was also recommended for monitoring of photosynthetic response under salt stresses [35]. Dąbrowski et al. [36] revealed moderately integrating fluorescence parameters and gas exchange measurements would be applied for exploring the physiological response of plant to drought stress. However, Kalaji et al. [37] indicated that it was difficult to distinguish between the effects of drought and salinity by chlorophyll

fluorescence parameters. Thus, the investigated ChlF parameters were integrated with growth analysis and biochemical assays for evaluating the effects of osmotic and ionic stress from salinity in this study.

Previously, we reported that wheat (*Triticum aestivum*) cultivar Taichung SEL. 2 (TCS2), one of the most widely cultivated cultivars in Taiwan, appears to be a salt-tolerant cultivar [38]. However, the salt-tolerant mechanisms of TCS2 are unclear. Therefore, the purpose of the present study is to evaluate the biochemical and physiological responses of TCS2 under iso-osmotic potentials induced by PEG and NaCl separately, followed by determining the salt-tolerance ability of TCS2 and its mechanisms. We hypothesized that TCS2 was more susceptible to the PEG-induced osmotic stress than the NaCl-induced ionic toxic effect under different iso-osmotic levels stimulated with applied PEG and NaCl, respectively.

2. Materials and Methods

2.1. Plant and Growth Conditions

Seeds of wheat (*T. aestivum*) cultivar TCS2 were collected from the Department of Agronomy, National Taiwan University, Taipei, Taiwan. Seeds were sterilized with 1% hydrogen peroxide for 5 min, washed with distilled water, and then germinated in Petri dishes with wetted filter paper at 25 °C in the dark. After 24 h dark incubation, uniformly germinated seeds were selected and cultivated in 150 mL beakers with complete Hoagland's nutrient solution (PhytoTech, Lenexa, KS, USA). Nutrient solutions were replaced every three days. Hydroponic wheat seedlings were raised in growth chambers under a 12/12 h day/night photoperiod at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) with 25/20 °C day/night temperature.

2.2. Experimental Treatments

Hydroponic seedlings that reached stage Z1.0 [39] on day 6 were treated with the osmotic agents polyethylene glycol (PEG-6000, Sigma-Aldrich) and NaCl separately according to the treatment design for seven days. According to the treatments from Almansouri et al. [9], these osmotic agents were used to prepare three iso-osmotic levels respectively, level 1 containing 24% (*w/v*) PEG or 200 mM NaCl, level 2 containing 26.5% (*w/v*) PEG or 250 mM NaCl, and level 3 containing 29% (*w/v*) PEG or 300 mM NaCl. Treatment without PEG and NaCl application was considered as control (CT). The experiment was independently performed three times for a randomized design of growth conditions.

2.3. Growth Analysis

Shoot height and root length of hydroponic seedlings were determined with a ruler manually before measuring chlorophyll fluorescence (ChlF) and sample collection.

2.4. Measurements of ChlF

The parameters of ChlF in seedling leaves were determined after PEG and salt treatments. ChlF was measured in the middle portion of the first leaf of each seedling taken at ambient temperature with Chl fluorometer imaging-PAM (Walz, Effeltrich, Germany). Plant materials were dark adapted for 20 min before ChlF measurements. Actinic light and saturating light intensities were set to 185 and 7200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR), respectively. According to previously described methods [31,40,41] the maximum quantum yield of PSII (F_v/F_m), effective quantum yield of PSII (Φ_{PSII}), non-photochemical quenching of PSII (NPQ), quantum yield of regulated energy dissipation of PSII [$Y(\text{NPQ})$], and quantum yield of non-regulated energy dissipation of PSII [$Y(\text{NO})$] were determined.

2.5. Measurement of APX Activity

APX activity was measured based on the method of Nakano and Asada [42]. Briefly, 0.1 g of the last newly expanded leaf was ground and extracted with 2 mL of sodium phosphate buffer (50 mM,

pH 6.8) in an ice bath, and then centrifuged at 4 °C and 12,000 rpm for 20 min. The supernatant (0.1 mL) was collected, and mixed with 2.7 mL of potassium phosphate buffer (150 mM, pH 7.0), 0.4 mL of ethylenediaminetetraacetic acid (EDTA, 0.75 mM), 0.5 mL of H₂O₂ (6 mM), 0.5 mL of H₂O, and 0.5 mL of ascorbate (1.5 mM) sequentially. The APX activity of sample solution was determined by a spectrophotometer (Hitachi U3010, Tokyo, Japan) at 290 nm. A blank containing the same mixture without sample extract was also determined.

2.6. Determination of Photosynthetic Pigment Concentration

The concentrations of photosynthetic pigments were determined according to the method of Yang et al. [43]. Briefly, 0.01 g of lyophilized sample powder was extracted with 12 mL 80% acetone solution, and then centrifuged at 4500 rpm for 5 min. The supernatant of the sample extract was collected. The concentrations of Chl *a*, Chl *b*, and carotenoids (Car) in sample solution was determined by a spectrophotometer (Hitachi U3010, Tokyo, Japan) at 663.6, 646.6, and 440.5 nm, respectively. A blank containing the same solvent without sample extract was also determined.

2.7. Determination of MDA Concentration

MDA was determined according to the method of Heath and Packer [44]. Briefly, 0.03 g of lyophilized sample powder was ground and extracted with 1 mL of 5% TCA, and then centrifuged at 10,000 rpm and 20 °C for 5 min. The supernatant (250 µL) was collected and mixed with 1 mL of 0.5% thiobarbituric acid (TBA), which was made up with 20% TCA. The mixture was incubated at 95 °C for 30 min with a water bath, and then immediately cooled in an ice bath. The reaction mixture was centrifuged at 3000 rpm and 20 °C for 10 min. The MDA concentration of sample mixtures was determined by a spectrophotometer (Hitachi U3010, Tokyo, Japan) at 532 and 600 nm. A blank containing the same reaction mixture without sample extract was also determined.

2.8. Statistical Analyses

All determined data were subjected to statistical analysis by using analysis of variance (ANOVA) followed by a least significant difference (LSD) test and *t*-test at *p* < 0.05. All statistical analyses were conducted using R i386 3.5.1 software (<https://cran.r-project.org/bin/windows>).

3. Results

3.1. Growth Analysis

The shoot heights and root lengths of NaCl- and PEG-treated seedlings are presented in Figure 1. The average shoot height of non-treated (CT) seedlings was 24.3 cm. Shoot heights of the PEG-treated seedlings declined dramatically to 12.6 cm at osmotic level 1 [24% (*w/v*) PEG] and then decreased gradually to 11.6 cm at osmotic level 3 [29% (*w/v*) PEG]. A similar declining trend was observed in the NaCl-treated seedlings. However, the shoot heights of NaCl-treated seedlings were significantly higher than in PEG-treated seedlings under the iso-osmotic potential treatments. The trend in root length under PEG and NaCl treatment was similar to the trend in shoot height.

Figure 1. Means $\pm$ SD ($n = 5$) of shoot height (A) and root length (cm) (B) from seedlings grown under three iso-osmotic potential levels under separate polyethylene glycol (PEG) and sodium chloride (NaCl) treatments. Level 1: 24% (w/v) PEG and 200 mM NaCl; Level 2: 26.5% (w/v) PEG and 250 mM NaCl; Level 3: 29% (w/v) PEG and 300 mM NaCl. Non-treated control (CT) shown twice was easy for the identification of NaCl and PEG treatment. Means with different lowercase letters significantly differ in a least significant difference (LSD) test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a t -test between PEG and NaCl concentration at the same osmotic potential.

3.2. *ChlF*

F_v/F_m and Φ_{PSII} in leaves are the indexes of the maximum and effective quantum yield of PSII, respectively. These two indexes have to be determined after dark adaption and under illumination, and are widely used to estimate the status of plants under stress [45]. Figure 2 shows that the value of F_v/F_m in the leaves of non-treated (CT) seedlings was 0.781, and the F_v/F_m of the seedlings under PEG treatment declined dramatically to 0.019. However, the F_v/F_m of seedlings under NaCl treatment was stable until osmotic level 3 (300 mM NaCl). Furthermore, the F_v/F_m values of NaCl-treated seedlings were significantly higher than for PEG-treated seedlings under iso-osmotic potential treatments. Φ_{PSII} in the leaves of seedlings with PEG treatment declined from 0.526 to 0.013 with increasing PEG concentrations. The Φ_{PSII} in the leaves of seedlings with NaCl treatment showed a similar trend to the PEG-treated seedlings. However, the Φ_{PSII} values of NaCl-treated seedlings were significantly higher than for PEG-treated seedlings at osmotic level 3 (Figure 3A).

NPQ represents the non-photochemical quenching of PSII, while $Y(NPQ)$ and $Y(NO)$ are important fluorescence parameters of photo-protection and photo-damage, respectively [41]. NPQ in the leaves of

the PEG-treated seedlings declined dramatically from 0.22 to 0.00 with increasing PEG concentrations. On the other hand, the NPQ of NaCl-treated seedlings increased suddenly at osmotic level 2, and then gradually decreased to the level of the CT seedlings. The NPQ values in the leaves of NaCl-treated seedlings were always significantly higher than in PEG-treated seedlings under the same osmotic potential treatments (Figure 3B). A similar dynamic was observed in $Y(NPQ)$ in the leaves of PEG and NaCl-treated seedlings (Figure 3C). Both PEG and NaCl applications increased $Y(NO)$ values, while the PEG-treated seedlings exhibited significantly higher $Y(NO)$ values than the NaCl-treated seedlings (Figure 3D).

Figure 2. Images and the mean $\pm$ SD ($n = 3$) of the maximum quantum yield of photosynthetic system II (F_v/F_m) of leaves collected from seedlings grown under three iso-osmotic potential levels under separate PEG and NaCl treatments. Level 1: 24% (w/v) PEG and 200 mM NaCl; Level 2: 26.5% (w/v) PEG and 250 mM NaCl; Level 3: 29% (w/v) PEG and 300 mM NaCl. Means with different lowercase letters significantly differ in an LSD test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a t -test between PEG and NaCl concentration at the same osmotic potential level. The false color code depicted on the top of the images ranges from 0.0 (black) to 1.0 (purple).

Figure 3. Means $\pm$ SD ($n = 3$) of the effective quantum yield of photosystem II under illumination (Φ_{PSII}) (A) non-photochemical quenching (NPQ); (B) quantum yield of regulated energy dissipation of photosystem II [Y(NPQ)]; (C) quantum yield of non-regulated energy dissipation of photosystem II [Y(NO)]; (D) of leaves collected from seedlings grown under three iso-osmotic potential levels with separate PEG and NaCl treatments. Level 1: 24% (w/v) PEG and 200 mM NaCl; Level 2: 26.5% (w/v) PEG and 250 mM NaCl; Level 3: 29% (w/v) PEG and 300 mM NaCl. Means with different lowercase letters significantly differ in an LSD test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a t -test between PEG and NaCl concentration at the same osmotic potential level.

3.3. APX Activity

The results of the APX activity measured in the wheat seedlings in this study are presented in Figure 4. The APX activity of PEG- and NaCl-treated seedlings both showed descending trends. Furthermore, APX activity in NaCl-treated seedlings was significantly higher than in PEG-treated seedlings under the same iso-osmotic potential.

Figure 4. Means $\pm$ SD ($n = 3$) of ascorbate peroxidase (APX) activity of leaves collected from seedlings grown under three iso-osmotic potential levels under separate PEG and NaCl treatments. Level 1: 24% (w/v) PEG and 200 mM NaCl; Level 2: 26.5% (w/v) PEG and 250 mM NaCl; Level 3: 29% (w/v) PEG and 300 mM NaCl. Means with different lowercase letters significantly differ in an LSD test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a t -test between PEG and NaCl concentration at the same osmotic potential level.

3.4. Photosynthetic Pigments

The Chl a , b , and total Chl concentrations of PEG and NaCl-treated seedlings all showed descending trends, and the concentrations of these pigments in NaCl-treated seedlings were significantly higher than in PEG-treated seedlings under the same iso-osmotic potential (Table 1). The Car concentration in the non-treated seedlings was $1.40 \text{ mg g}^{-1} \text{ DW}$. In the PEG-treated seedlings, Car concentrations decreased significantly but stayed at a stable level ($0.81\text{--}0.84 \text{ mg g}^{-1} \text{ DW}$). A similar trend was also observed in the Car concentrations of the NaCl-treated seedlings, and Car concentrations in NaCl-treated seedlings were always significantly higher ($0.87\text{--}0.92 \text{ mg g}^{-1} \text{ DW}$) ($p < 0.05$) than in PEG-treated seedlings under iso-osmotic potential treatment.

Table 1. Means $\pm$ SD of chlorophyll (Chl a , Chl b , total Chl) and carotenoids (Car) in leaves collected from seedlings growing under three iso-osmotic potential levels under separate PEG and NaCl treatments.

Osmotic Potential Level	Osmoticum	Chl a [mg g^{-1} (DW)]	Chl b [mg g^{-1} (DW)]	Chl $a + b$ [mg g^{-1} (DW)]	Car [mg g^{-1} (DW)]
CT	PEG	6.92 ± 0.16 a	3.19 ± 0.09 a	10.1 ± 0.3 a	1.40 ± 0.04 a
	NaCl	6.92 ± 0.16 a	3.19 ± 0.09 a	10.1 ± 0.3 a	1.40 ± 0.04 a
Level 1	PEG	3.77 ± 0.19 b B	1.68 ± 0.09 b B	5.45 ± 0.17 b B	0.84 ± 0.02 b B
	NaCl	4.52 ± 0.26 b A	2.04 ± 0.09 b A	6.56 ± 0.35 b A	0.92 ± 0.04 b A
Level 2	PEG	3.10 ± 0.16 c B	1.48 ± 0.05 c B	4.58 ± 0.18 c B	0.81 ± 0.05 b B
	NaCl	4.43 ± 0.00 b A	1.82 ± 0.05 c A	6.25 ± 0.05 b A	0.90 ± 0.02 b A
Level 3	PEG	2.29 ± 0.04 d B	1.04 ± 0.03 d B	3.33 ± 0.07 d B	0.84 ± 0.03 b B
	NaCl	3.25 ± 0.07 c A	1.43 ± 0.03 d A	4.68 ± 0.10 c A	0.87 ± 0.01 b A

Values are the mean $\pm$ SD ($n = 3$). Level 1: 24% (w/v) PEG and 200 mM NaCl; Level 2: 26.5% (w/v) PEG and 250 mM NaCl; Level 3: 29% (w/v) PEG and 300 mM NaCl. Means with different lowercase letters significantly differ in an LSD test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a t -test between PEG and NaCl concentration at the same osmotic potential level. CT, Control. Chl, chlorophyll. Car, carotenoids.

3.5. MDA Concentration

Figure 5 reveals that the MDA concentration of non-treated seedlings was $13.5 \text{ nmol g}^{-1} \text{ DW}$. The MDA concentrations of seedlings with PEG treatments increased dramatically to $116.7 \text{ nmol g}^{-1} \text{ DW}$ at osmotic level 3 (29% (*w/v*) PEG), while the MDA concentration in NaCl-treated seedlings increased slightly to $33.2 \text{ nmol g}^{-1} \text{ DW}$ at the same osmotic potential (300 mM NaCl). Furthermore, the differences in MDA concentrations of NaCl-treated seedlings were insignificant among all NaCl treatments. The MDA concentrations of PEG-treated seedlings were significantly higher than in NaCl-treated seedlings under iso-osmotic potential treatment.

Figure 5. Means $\pm$ SD ($n = 3$) of the malondialdehyde (MDA) content of leaves collected from seedlings grown under three iso-osmotic potential levels under separate PEG and NaCl treatments. Level 1: 24% (*w/v*) PEG and 200 mM NaCl; Level 2: 26.5% (*w/v*) PEG and 250 mM NaCl; Level 3: 29% (*w/v*) PEG and 300 mM NaCl. Means with different lowercase letters significantly differ in an LSD test under different PEG or NaCl concentrations, respectively ($p < 0.05$). Means with different capital letters significantly differ in a *t*-test between PEG and NaCl concentration at the same osmotic potential.

4. Discussion

In order to investigate the osmotic and ionic effects from salt stress, the growth and physiological status of PEG- and/or NaCl-treated seedlings grown under three iso-osmotic levels were evaluated in this study. The treatment concentrations of PEG and NaCl are based on the morphological symptoms of osmotic and ion-toxic effects on wheat leaves. The obvious symptoms of water deficits are leaf wilting and rolling because of stomatal closure in PEG-induced osmotic stress [3], while ion-excess symptoms in NaCl-induced stress are chlorotic or senescent leaves [4]. The durations of osmotic treatments based on PEG- and/or NaCl-induced symptoms are observable and obvious, and lead to irreversible damage to wheat [3]. The ChlF of PSII can then be determined for early monitoring.

The F_v/F_m , which is one of the parameters of prompt chlorophyll fluorescence, was determined after dark adapt for 20 min. Previous study revealed that F_v/F_m in plant effectively responded to NaCl, PEG, light, and heavy metal stress [34]. In this study, F_v/F_m values in the PEG-treated seedlings declined significantly with raising osmotic level, while the decrease of F_v/F_m in the NaCl-treated group was only observed at level 3 (300 mM NaCl) (Figure 2). The investigation of F_v/F_m in the study [37] also revealed that the changes of F_v/F_m in *Tilia cordata* under salinity and drought stress showed different dynamics. In addition, the dynamic of F_v/F_m was correlated to photosynthetic rate in sugarcane under osmotic stress [46]. However, Dąbrowski et al. [31] indicated that only F_v/F_m did not reliably reflect photosynthetic rate in shade-treated *Lolium perenne*. Recent studies surveyed multiple parameters of prompt and delayed ChlF and observed several parameters highly correlated to photosynthetic rate in *Lolium perenne* [35,36].

Recent studies integrated multiple ChlF parameters for evaluating the PSII function under salt [32,35] and drought stress [36]. Other ChlF parameters such as Φ_{PSII} , NPQ, Y(NPQ), and Y(NO) were calculated from ChlF signals that were determined with continuous actin light [41]. The photons absorbed by PSII would be divided into three complementary quantum yields, Φ_{PSII} (also known as Y(II)), Y(NPQ), and Y(NO). In this study, all Φ_{PSII} in osmotic treatments were significantly decreased (Figure 3A), and this result suggested that the osmotic treatment groups performed lower degree photons, absorbed by PSII used in photochemistry, than control group. Furthermore, the Φ_{PSII} difference between PEG- and NaCl-treated seedlings was insignificant except at osmotic level 3 (Figure 3A), indicating that the PSII capability in the photochemical reaction was damaged by both PEG and NaCl. Muranaka et al. [14] and Flagella et al. [47] also report that Φ_{PSII} was influenced under extreme water deficit. The study of Suriyan and Chalermopol [46] also supported osmotic stress reduced Φ_{PSII} in plant.

The values of NPQ and Y(NPQ) in the NaCl-treated seedlings were significantly higher but slightly declined with raising NaCl concentration, whereas the values of Y(NO) in the PEG-treated seedlings were significantly higher at osmotic level 2 and 3 (Figure 3B,D). These results suggested that the capacity of PSII for processing photochemistry in seedlings might be inhibited under NaCl stress, while the excessive energy would be dissipated through regulated NPQ pathway, such as xanthophyll cycle [41]. On the other hand, PEG-induced stress also inhibited PSII capacity for processing photochemistry, but this osmotic stress did not induce the NPQ-mechanism. The dynamics of these parameters reflected the various effects of osmotic and ionic stress on ChlF in wheat TCS2 seedling (Figure 3). Suriyan and Chalermopol [46] revealed NPQ and proline in leaves of plant responded to the iso-osmotic levels, and different dynamics of ChlF were also observed. ChlF parameters were well applied to monitoring plant response to various abiotic stresses [33,34], but it was still difficult to distinguish between drought and salinity stress by ChlF [37].

Photosynthetic pigments, including Chl *a*, Chl *b*, and Car, provide the light-harvesting function that is essential for photosynthesis [29]. In addition, Car also absorbs excessive light to protect chlorophyll from excessive light-loading and further damage. Car-provided protection is known as photoprotection [48]. In this study, the accumulation of Chl sharply dropped in all of the treated seedlings with increasing treatment concentration (Table 1). However, the concentration of Chl *a*, Chl *b*, and their sum were influenced more by PEG-induced osmotic stress than NaCl treatment at all levels of iso-osmotic potential. These results suggest that the osmotic stress-induced damage was more severe in PEG-treated seedlings. In this study, PEG and NaCl treatments also strongly inhibited Car accumulation, and PEG treatments reduced Car concentrations in wheat seedlings more significantly than NaCl treatments. Car accumulation results indicate that Car provided photoprotection in NaCl-treated seedlings better than in PEG-treated seedlings (Table 1). These results indicate that the mechanisms involved are highly species dependent. Nxele et al. [8] published that both drought and salinity inhibited the accumulation of Chl in sorghum, but a more severe decrease in Chl was observed under salinity. The study on sugarcane also revealed NaCl-induced stress led to more degradation of Chl and Car in plant, and the Chl content was highly correlated to photosynthetic rate [46]. The recent study on sugar beet indicated that photosynthesis capability and the accumulation of Chl were induced at moderate salinity (75–100 mM NaCl), but were inhibited under severe salt stress [7]. The photosynthetic pigment results in this study were consistent with the results of ChlF. Siringam et al. [29] revealed that Chl *a* and total Chl were positively related to ChlF values.

There are many enzymes, such as APX, superoxide dismutase, glutathione peroxidase, and glutathione reductases involved in the enzymatic antioxidant pathway [5]. However, enzymes that require K^+ as a cofactor are more sensitive to high salinity [20]. APX participates in the ascorbate-glutathione pathway [49], and it has a K^+ binding site essential for APX activity [50]. K^+ will be displaced by Na^+ at the essential binding site of APX, resulting in inhibited APX activity with increasing Na^+ concentrations when a plant is under NaCl-induced stress. A recent study of Tahjib-UI-Arif [7] indicated that activities of APX, catalase, and peroxidase were significantly induced under salt salinity, but APX activity declined with increasing salinity level. A familiar result was

also observed in this study (Figure 4). However, APX activity in seedlings with PEG treatments were inhibited more than from NaCl treatment at all iso-osmotic potential levels. This phenomenon indicated that ionic stress also served as environmental signal factor for induction of the mechanism against osmotic stress.

MDA, a product of lipid peroxidation, has been regarded as a good indicator of oxidative stress [51]. Many studies reported that salinity and drought significantly stimulated the accumulation of MDA coupled with an increase of proline in plant [7,8,11]. The results of the MDA determination in this study indicated that PEG treatments strongly induce lipid peroxidation and injure PEG-treated seedlings with higher PEG concentrations (Figure 5). However, stable MDA levels in NaCl-treated seedlings were observed among all osmotic potentials. The higher NPQ and Y(NPQ) values (Figure 3B,C) were correlated to the stable MDA levels in the NaCl-treated seedlings. These results suggest that wheat TCS2 seedlings needed the ionic stress as environmental signal to activate the mechanism to stabilize membrane system even under 250–300 mM NaCl.

Under osmotic stress, osmotic adjustment is accomplished by synthesizing osmolytes such as proline, glycine betaine, fructans, etc. [5,10]. Nxele et al. [8] observed a similar level of MDA in stressed plant under salinity and drought, but salinity stimulated more proline content. Recent study on wheat seedling also reported that NaCl stress stimulated proline accumulation dramatically, PEG induced accumulation of sugar metabolites (glucose, fructose) and glycine betaine, while polyamines' synthetic pathway was also altered by various osmolytes [26]. Furthermore, under salt stress, turgor would be maintained by transporters such as Na^+/H^+ antiporter, H^+ -pyrophosphatase, high efficiency potassium transport [5], and aquaporin, which is specific transporter for water [6].

Shoot height in the PEG-treated seedlings was influenced more than in NaCl-treated seedlings at all iso-osmotic levels, and root length in treated seedlings also showed a similar trend at osmotic level 1. Cell division and elongation are highly affected by water and/or osmotic stress, and are important for plant growth [52]. PEG treatments constrained the balance of turgor and resulted in poor shoot height performance. On the other hand, a higher shoot height in NaCl-treated seedlings was observed because NaCl treatments provided Na^+ and Cl^- as osmotics to reduce the osmotic stress in NaCl-treated seedlings [12]. Root length is a vital trait reflecting the depth of the plant root in soil, and indicates how a plant functions under osmotic stress [53]. NaCl-treated seedlings performed better than PEG-treated seedlings at osmotic level 1, while there were no differences between treatments at osmotic level 2 and 3. This should be attributed to roots having a higher ability to adapt to osmotic stress [23]; therefore, root growth rate can be stably maintained under various levels of PEG-induced osmotic stress [54]. Moreover, recent study revealed that the biosynthetic pathway of polyamines, including spermine, spermidine and putrescine, which participate in cell division and differentiation [55], also mediated under osmotic stress, and PEG led to increase of spermine, spermidine and putrescine [26].

TCS2 grown under all PEG treatments had visual signs of wilting, indicating disabled water absorption. However, TCS2 remained vigorous under NaCl treatments, indicating it had the ability to cope with excess ion stress caused by NaCl application. This phenomenon was supported by the ChlF, photosynthetic pigments, APX activity, MDA concentration, and shoot lengths found in this study. Overall, NaCl-induced stress, as ionic signal, stimulated the wheat TCS2 seedlings to reduce lipid peroxidation for stabilization membrane system and photosynthetic functions, such as F_v/F_m and photosynthetic pigments. These results help us to understand the mechanisms in salt-tolerant wheat cultivar TCS2 to adapt to osmotic stress and also revealed the physiological traits of TCS2 for further plant breeding.

5. Conclusions

The results of this study supported our hypothesis that TCS2 is more susceptible to osmotic stress than to ionic stress at three iso-osmotic levels of PEG and NaCl treatments. As a salt-tolerant wheat cultivar, the NaCl-treated TCS2 seedlings showed a higher ability to induce regulated non-photochemistry quenching in PSII (NPQ and Y(NPQ)) and APX activity to prevent lipid

peroxidation and degradation of photosynthetic pigments. Therefore, these stabilized physiological functions led to the better performance of seedling growth.

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