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1. Introduction

Especially over the last 100 years, our unbridled exploitation of the world’s natural resources has severely damaged its vegetation and has also resulted in worrying accumulations of industrial wastes and greenhouse gases. Together, these have upset natural ecosystem balances and have created many environment and climatic problems, including rising temperatures, increasing desertification, serious soil loss, soil salinization and damaging accumulations of soil nitrogen [39, 31, 37]. In many nations, the recent increased incidences of severe drought and associated desertification are coming into especially sharp focus because of their sudden, long term and devastating consequences for the local human population.

Drought imposes one of the commonest and most significant constraints to agricultural production, seriously affecting crop growth, gene expression, distribution, yield and quality [45, 44, 53]. There are numerous reports on photosynthetic and metabolites characteristics under water stress [22, 52, 25, 5]. Generally, photosynthesis is inhibited by water stress, also affects photosynthetic components and chloroplast stress [54, 52]. Plants have evolved a number of mechanisms to adapt to and survive water stress. Some plant species have evolved mechanisms to cope with the stress, including drought avoidance, dehydration avoidance, or dehydration tolerance. Such adaptive mechanisms are the results of a multitude of morphoanatomical, physiological, biochemical, and molecular changes [1, 2, 6]. But to our knowledge, only a few report about the effects of different level water stress on photosynthetic and metabolites of wheat seedlings.
Wheat is an important crop, with some cultivars tolerant to water stress. The purpose of this study was to investigate the effects of water stress on the growth, chlorophyll fluorescence and accumulations of proline, betaine and carbohydrates of wheat seedlings, by using PEG simulated water stress. It was also desired to elucidate mechanisms of water stress damage and to identify possible adaptive mechanisms to water stress. Understanding how wheat manages water stress is important for the reclamation of drought-prone soils and crop production, and possibly also to discover water-stress resistance genes and hence to develop drought-resistance biotechnology in this crop.

2. Materials and methods

2.1. Design of simulated water stress conditions

Water stress conditions were simulated to polyethylene glycol-6000 (PEG) at one of three concentrations: 0, 5, 15 and 25%. The osmotic potentials of the solutions was measured using a water potential meter (Psypro Wescor Corporation, US) [49]. Table 1 results shows how osmotic potential decreases with increasing PEG-6000 concentration.

<table>
<thead>
<tr>
<th>PEG-6000 concentration</th>
<th>0%</th>
<th>5%</th>
<th>15%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP (MPa)</td>
<td>-0.05</td>
<td>-0.09</td>
<td>-0.34</td>
<td>-0.95</td>
</tr>
</tbody>
</table>

Table 1. The osmotic potential (OP) of solutions of polyethylene glycol (PEG).

2.2. Plant materials and growing conditions

Seeds of wheat (Triticum aestivum) FengYou-68 were sown 20 seeds in germination box. Seedlings were watered daily with 0.5 Hoagland’s nutrient solution [14]. All the boxes were placed in growth chambers [HPG-400, Haerbin, China] with a 16-h photoperiod (Sylvania cool white fluorescent lamps, 200 mmol m\(^{-2}\) s\(^{-1}\), 400–700 nm). The temperature was 25 ± 2°C (day) and 21 ± 1.5°C (night).

After three days, 25 boxes containing uniform seedlings were selected and randomly divided into five sets of five replicates. One set was used to determine the seedling growth parameters just prior to treatment, a second set was used as the untreated control (0% PEG-6000, watered with Hoagland’s nutrient solution), and the three remaining sets were stressed with one or other of the PEG-6000 solutions. Each PEG subtreatment was applied to a set of five boxes, daily for 7 days.

2.3. Measurement of growth

After the seventh day of treatment, the fresh weights (FW) were recorded after removing surface water by blotting and the dry weights (DW) determined after drying for 15 min in an oven at 80°C and then in a vacuum dryer at 40°C to constant weight. The relative growth
rate (RGR) was defined as (ln DW after treatment – ln DW before treatment) / treatment duration. The water content (WC) percentage was calculated as: 100×(FW–DW)/FW [52].

2.4. Measurement of chlorophyll fluorescence and pigments

The maximal photochemical efficiency of PSII (PSII=Fv/Fm), the photosynthetic efficiency of PSII (Y_{II}=Fm'-F/Fm'), non-photochemical quenching (NPQ=Fm-Fm'/Fm'), non-photochemical quenching coefficient (qN=Fm-Fm'/Fm-F0'), photochemical quenching (qP=Fm'-F/Fm'-Fo'), the efficiency of excitation energy capture by open PSII reaction centers (Fv'/Fm') and apparent photosynthetic electron transport rate (ETR) were determined between 09:00 and 11:00 h from fully-expanded leaves using an Imaging-PAM (Walz, Effeltrich, Germany), [12, 48]. The leaves were held in the dark for about 20 min before measurement. The intensities of the actinic and saturating light settings were 185μmol/m$^2$/s and 2500μmol/m$^2$/s PAR, respectively. The contents of carotenoids (Car) and chlorophyll (Chl) a and b were extracted using acetone, and spectrophotometric determination at 440, 645 and 663 nm of each sample was done three times. The calculations were Chl a = 12.7×OD663-2.69×OD645; Chl b = 22.9×OD645-4.86×OD663 and Car = 4.7×OD440-0.27×(20.2×OD645+8.02×OD663).

2.5. Measurement of metabolites and organic acids

Proline was extracted with 3% sulfosalicylic acid for 30 min at 70°C and measured with ninhydrin [55]. Betaine was extracted with 80% methanol for 20 min at 70°C and measured as described by Grieve and Grattan (1983). Total soluble sugars (SS) were extracted for 30 min at 70°C in 70% alcohol, and measured using anthrone.

2.6. Measurement of germination

One hundred wheat seeds were germinated on filter paper in germination boxes. The dry seeds were submerged in 100 mL of each of the PEG-6000 solutions described above (with distilled water as the control). The boxes were maintained at 20°C in the dark for 10 d, five replicates of each PEG treatment were prepared. Percentages of germinated seeds were scored daily, based on the emergence of the radicles. The germinative Energy(Ge), germinative Percentage(Gp), and germination activity Index(Ai) of wheat seeds were modified using Ge = n/Nx100% (n: the number of germination of seeds in 4 days; N: the total number of seeds); Gp = n1/N1x100% (n1: the number of germination of seeds at 10 days; N1: the total number of seeds).

3. Statistical analysis

Statistical analysis included one-way analysis of variance (ANOVA) in SPSS (Version 13.0, SPSS, Chicago, IL, USA) and Duncan’s method to detect differences in physiological parameters in plants under water stress (*P*≤0.05). All measurements represent the means and standard errors (SE) of five replicates.
4. Results

4.1. Growth

The RGR and WC of shoots and roots all decreased with increasing PEG concentration, with the greatest reductions occurring under the highest water stresses (Fig. 1 A - D, $P \leq 0.05$). From the slopes of equations (1) and (2) (Table 2), it was calculated that the RGR for root and shoot increased by 0.229 and 0.231, respectively, per 1% increase in PEG-6000 concentration. Meanwhile, the WC of root and shoot decreased by 24.03 and 21.00, respectively, for each 1% increase in PEG concentration (see equations (3) and (4)) in Table 2).

**Figure 1.** Effects of water stress on shoot (A) and root (B) relative growth rate (RGR) and water content (WC). The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at $P \leq 0.05$ according to Duncan’s method.

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>$R^2$</th>
<th>Decrease in RGR and WC per 1% increment in PEG-6000 concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_R = -0.229x + 1.254$ (1)</td>
<td>0.79</td>
<td>0.229</td>
</tr>
<tr>
<td>$Y_S = -0.231x + 1.389$ (2)</td>
<td>0.80</td>
<td>0.231</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_R = -24.03x + 132.52$ (3)</td>
<td>0.64</td>
<td>24.03</td>
</tr>
<tr>
<td>$Y_S = -21.00x + 125.28$ (4)</td>
<td>0.68</td>
<td>21.00</td>
</tr>
</tbody>
</table>

**Table 2.** A regression analysis between RGR, WC and PEG concentration was performed, where $Y_S$ represented the root RGR and WC, $Y_R$ the shoot, and $x$ was PEG concentration.
4.2. Chlorophyll fluorescence and pigments

The Fv/Fm, Y(II), qP and ETR decreased with increasing PEG concentration, while NPQ and qN contents increased significantly, the effects were much more pronounced under high PEG concentration (Table 3; \( P \leq 0.05 \)). The contents of Chl \( a \) and Chl \( b \) under PEG induced water stress were less than in the control, each parameter decreased gradually with increasing PEG concentration. The Chl \( a/b \) ratio was higher with PEG than in the control (Table 4, \( P \leq 0.05 \)). The content of Car was scarcely changed by water stress (Table 4, \( P \leq 0.05 \)).

<table>
<thead>
<tr>
<th>PEG-6000 concentration</th>
<th>Fv/Fm</th>
<th>Y(II)</th>
<th>NPQ</th>
<th>qN</th>
<th>qP</th>
<th>ETR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.80±0.00(^a)</td>
<td>0.49±0.00(^a)</td>
<td>0.29±0.00(^a)</td>
<td>0.64±0.00(^a)</td>
<td>0.76±0.00(^a)</td>
<td>38.30±0.00(^a)</td>
</tr>
<tr>
<td>5%</td>
<td>0.79±0.00(^a)</td>
<td>0.49±0.00(^a)</td>
<td>0.27±0.00(^a)</td>
<td>0.63±0.00(^a)</td>
<td>0.75±0.00(^a)</td>
<td>38.00±0.00(^a)</td>
</tr>
<tr>
<td>15%</td>
<td>0.76±0.00(^a)</td>
<td>0.27±0.00(^a)</td>
<td>0.48±0.00(^a)</td>
<td>0.78±0.00(^a)</td>
<td>0.51±0.00(^a)</td>
<td>21.20±0.00(^a)</td>
</tr>
<tr>
<td>25%</td>
<td>0.59±0.00(^a)</td>
<td>0.00±0.00 ( ^b )</td>
<td>0.17±0.00(^b)</td>
<td>0.58±0.00(^b)</td>
<td>0.00±0.00 ( ^b )</td>
<td>0.00±0.00 ( ^b )</td>
</tr>
</tbody>
</table>

Table 3. Effects of PEG induced water stress on contents of photosynthetic pigments (g kg\(^{-1}\) FM) in seedlings of wheat. The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at \( P \leq 0.05 \) according to Duncan’s method.

<table>
<thead>
<tr>
<th>PEG-6000 concentration</th>
<th>Chl(a)</th>
<th>Chl(b)</th>
<th>Chl(a)+ Chl(b)</th>
<th>Chl(a)/Chl(b)</th>
<th>Car</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>1.14±0.06(^a)</td>
<td>0.29±0.00(^a)</td>
<td>1.43±0.08(^a)</td>
<td>3.93±0.04</td>
<td>0.31±0.00(^a)</td>
</tr>
<tr>
<td>5%</td>
<td>1.13±0.08(^a)</td>
<td>0.28±0.00(^a)</td>
<td>1.41±0.06(^a)</td>
<td>4.04±0.08</td>
<td>0.34±0.00(^a)</td>
</tr>
<tr>
<td>15%</td>
<td>1.02±0.01(^a)</td>
<td>0.22±0.00(^a)</td>
<td>1.24±0.02(^a)</td>
<td>4.64±0.08</td>
<td>0.32±0.00(^a)</td>
</tr>
<tr>
<td>25%</td>
<td>0.86±0.00(^a)</td>
<td>0.17±0.00(^a)</td>
<td>1.03±0.01(^a)</td>
<td>5.06±0.09</td>
<td>0.28±0.00(^a)</td>
</tr>
</tbody>
</table>

Table 4. Effects of PEG induced water stress on contents of photosynthetic pigments (g kg\(^{-1}\) FM) in seedlings of wheat. The values are the means of five replicates. Means followed by different letters.

4.3. Metabolites

The contents of proline increased with increasing PEG concentration, with that in the shoot being significantly higher than that in the root (Fig. 2, A and B; \( P \leq 0.05 \)). In the roots, increasing water stress had a positive effect on betaine content, causing a significant rise at 25% PEG concentration, however, in the shoots a negative effect (Fig. 2, C and D; \( P \leq 0.05 \)). The impacts of water stress on soluble sugar were similar as proline and betaine, it contents significantly increased under high water stress (Fig. 2 E and F; \( P \leq 0.05 \)).
Figure 2. Effects of PEG induced water stress on the contents of proline, betaine and soluble sugar in roots and shoots of wheat seedling. The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at $P \leq 0.05$ according to Duncan’s method.

5. Organic acids

OA, CA, MA, FA, LA and SA were all detected in both the shoots and roots of the wheat seedlings under water stress (Fig. 3). In response to water stress, the OA, MA and SA content of the roots and shoots decreased with PEG-6000 concentration increased, declined significant reduction above 15% PEG-6000 concentration (Fig. 3 A1, A2, C1, C2, F1 and F2, $P \leq 0.05$). The level of FA increased under low water stress whereas it decreased under high stress in
shoot, but in root it level show completely opposite change (Fig. 3 D1 and D2, \( P \leq 0.05 \)). Water stress had a significant negative impact on CA and LA levels in shoots, but there had no regular impact on the levels of CA and LA in roots (Fig. 3 B and E, \( P \leq 0.05 \)).

**Figure 3.** Effects of water stress on the levels of Oxalic Acid (A1), Citric Acid (B1), Malic Acid (C1), Formic Acid (D1), Lactic Acid (E1) and Succinic Acid (F1) in the root, and Oxalic Acid (A2), Citric Acid (B2), Malic Acid (C2), Formic Acid (D2), Lactic Acid (E2) and Succinic Acid (F2) shoot of wheat seedlings. The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at \( P \leq 0.05 \) according to Duncan’s method.

5.1. Germination

The table 5 shows that the trend in changes in \( G_p \) and \( G_e \) of wheat seeds under water stress conditions was similar; there was a decreased trend with increased PEG-6000 concentration \( (P \leq 0.05) \), the reductions were greater when concentration above 15% \( (P \leq 0.05) \).
Table 5. Effect of water stress on two indices (Gp and Ge) of germination for wheat. Germinative percentage - Gp and germinative Energy - Ge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gp (%)</th>
<th>Ge (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>96.66</td>
<td>96.00</td>
</tr>
<tr>
<td>5% PEG</td>
<td>95.33</td>
<td>91.67</td>
</tr>
<tr>
<td>15% PEG</td>
<td>83.67</td>
<td>80.33</td>
</tr>
<tr>
<td>25% PEG</td>
<td>64.00</td>
<td>63.67</td>
</tr>
</tbody>
</table>

6. Discussion

PEG is an osmotic agent, which play an important role in the regulation of mineral elements, hormone, protein metabolism and effects of signal transduction [50, 41]. The main function of PEG is to slow down the moisture rate of import and export seeds, which benefit to reduce membrane system injury in process of seed imbibition and repair impaired membrane system [27, 16]. PEG has been widely used in seed priming and simulated water stress test, the wheat seedlings were treated by three different PEG concentrations.

7. Impact of water stress treatments on growth

In plants in general, an appropriate growth strategy is key to fitness in a competitive situation, so too in wheat seedlings, their growth strategy is critical to survival [10]. The RGR value of a plant reflects its vigour and is considered a good index of its exposure to stresses of all sorts [26, 52]. The RGR response of wheat seedlings exposed to increasing PEG concentrations (Fig. 1 A, \( P \leq 0.05 \)) revealed a decrease for roots and shoots (Table 2, \( P \leq 0.05 \)). This may reflect the impact of water stress on root cell development, which would likely impair nutrient uptake as well as having detrimental effects on photosynthesis, essential for biomass accumulation and therefore on shoot and root elongation. The change trend for WC was similar to that for RGR but the extension of WC in the root was about 1.14-times that of the shoot (Fig 1. B; Table 2; \( P \leq 0.05 \)). Water stress therefore appears to reduce the absorption and utilization of water to such an extent that the tolerance mechanisms employed by these plants in a drought are insufficient to maintain normal growth.

7.1. Impact of water stress treatments on chlorophyll fluorescence and pigments

The chlorophyll fluorescence kinetics react to the “intrinsic” characteristic of photosynthesis and can rapidly and sensitively reflect a plant’s physiological status and its relationship with the environment [Huang et al., 2009]. In this study, PSII values decreased with increasing PEG concentration but these began to decline significantly in 15% PEG concentration. The results indicate that photoinhibition occurs under water stress as a result of damage to the reaction center of photosystem II (Table 3, \( P \leq 0.05 \)). The values of Y(II), qP and ETR de-
creased, while those of NPQ and qN increased with increasing PEG concentrations. These results indicate that electron transport activity and the photosynthetic apparatus of wheat seedlings with certain drought-resistance are damaged.

Chl and Car are the main photosynthetic pigments of plants, so these are good indicators of the photosynthesis capability of a plant. Under water stress, with the exception of Car which barely changed, the contents of Chl a and b decreased slightly at first but then decreased more sharply at the 15% PEG concentration (Table 4; \( P \leq 0.05 \)). This may be linked with the observation that under -0.34MPa water potential conditions Chl synthesis was severely inhibited with the result that the functioning of the photosynthetic apparatus became seriously impaired [25, 5]. Compared with the control, the water stress effects on Chl \( a/b \) were high and this appears to be closely related to the metabolic regulation of Chl; this possibility is worth further investigation.

7.2. Impact of water stress treatments on metabolites

Proline and betaine are also known to play important roles in osmotic adjustment with their accumulation under water stress being observed in many species [46, 38]. Here, the results show that, along with a decrease in osmotic potential, the accumulation of free proline and betaine increased significantly both in the roots and the shoots. This increase would lower the osmotic potential [i.e. make it more strongly negative] in the cells which would help to maintain turgor and thus sustain the normal physiological and biochemical processes in the face of drought (Fig. 2, \( P \leq 0.05 \)).

Soluble sugars are the main osmotic adjustment substances and so are important indicators of drought tolerance. The results show that the soluble sugars contents of wheat seedlings increases under high PEG concentration. This indicates that they may help to regulate and maintain the activity of physiological processes within the plant in a high water-stress environment by raising the osmotic potential of the cells [14].

7.3. Impact of water stress treatments on organic acids

The accumulation of organic acids is a physiological response of plants to stress, when plants are suffered by water stress, they can through cells apperceive and transmit drought signal [42]. There nearly no impact on the content of organic acids under blew 15% water stress, it decreased significantly under high stress, but in shoot FA completely opposite change (Fig. 3, \( P \leq 0.05 \)). The results confirmed that the organic acids metabolic regulation was closely related to the plant water stress resistance. The change of organic acid may be adaptive mechanism by which wheat seedlings maintain their intracellular osmotic balance under water stress [47, 55].

7.4. Impact of water stress treatments on germination

Germination is one of the most critical periods in the life cycle of plants. Under water stress, low water potential is a determining factor inhibiting seed germination [51, 43]. The inhibit-
ing action of water stress on the wheat germination was increased with PEG-6000 concentration increasing (Table 5).

8. Conclusion

In summary, the growth of wheat seedlings was inhibited by water stress, especially in roots. The function of water regulation occurs outside root, or in apoplast of root, or both outside root and in apoplast of root. Therefore, we propose that the water-potential adjustment of the roots may be a key physiological mechanism for wheat resisting water stress. Proline, betaine and soluble sugar content increase to a greater extent in response to water stress, these data suggest that wheat seedlings may initially sense high drought environments, the harmful effects of water stress on the distribution and accumulation of carbohydrates, it was reflecting the specific detrimental effects of a drought environment. With the extension of PEG-6000 concentrations, wheat seedlings photosynthetic electron transport and photosynthetic primary reaction inhibited, heat disseminate which possess photoprotective effect increased. It implies that there was a closed relationship between the effects of water stress on chlorophyll fluorescence parameters of wheat seedlings. These results provide useful data that will facilitate the development of strategies for the creation of engineered wheat varieties that are more tolerant towards water stress.

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tion and distribution of 2,4-diaminoobutyric acid and other free amino acids in tis-

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