# Effects of Water Stress on Germination and Growth of Wheat, Photosynthetic Efficiency and Accumulation of Metabolites

Rui Guo, Wei Ping Hao, Dao Zhi Gong, Xiu Li Zhong and Feng Xue Gu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51205

# 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.

PEG-6000 concentration	0%	5%	15%	25%
OP (MPa)	-0.05	-0.09	-0.34	-0.95

**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 per 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<sup>-2</sup> s<sup>-1</sup>, 400–700 nm). The temperature was  $25 \pm 2^{\circ}$ C (day) and  $21 \pm 1.5$ °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 (In DW after treatment - In 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<sub>(II)</sub>=Fm'-F/Fm'), non-photochemical quenching (NPQ=Fm-Fm'/Fm'), non-photochemical quenching coefficient (qN=Fm-Fm'/Fm-Fo'), 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/m²s and 2500µmol/m²s PAR, respectively. The contents of carotenoids (Car) and chlorophyll (Chl) a and b were extracted using acetone, and spectrophotometeric 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 =  $22.9 \times OD645 - 4.86 \times OD663$  and Car =  $4.7 \times OD440 - 0.27 \times (20.2 \times OD645 + 8.02 \times OD663)$ .

# 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 = nl/Nlx100% (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 ( $P \le 0.05$ ). 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,  $P \le 0.05$ ). 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).

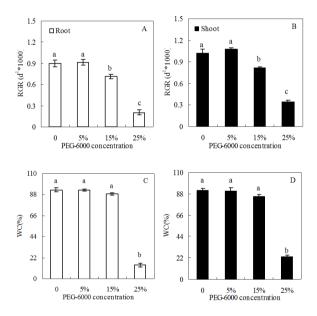


Figure 1. Effects of water stress on shoot (A) and root (B) relative growth rate (RGR) and water content (WC). The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at  $P \le 0.05$  according to Duncan's method.

	Regression equation		Decrease in RGR and WC per 1% increment in PEG-6000 concentration	
	Y <sub>R</sub> =-0.229x+1.254 (1)	0.79	0.229	
RGR	Y <sub>s</sub> =-0.231x+1.389 (2)	0.80	0.231	
WC	Y <sub>R</sub> =-24.03x+132.52 (3)	0.64	24.03	
VVC	Y <sub>s</sub> =-21.00x+125.28(4)	0.68	21.00	

**Table 2.** A regression analysis between RGR, WC and PEG concentration was performed, where  $Y_R$  represented the root RGR and WC,  $Y_S$  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≤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,  $P \le 0.05$ ). The content of Car was scarcely changed by water stress (Table 4,  $P \le 0.05$ ).

PEG-6000 concentration	Fv/Fm	Y(II)	NPQ	qN	qP	ETR
0%	0.80±0.00a	0.49±0.00a	0.29±0.00b	0.64±0.00b	0.76±0.00a	38.30±0.00 <sup>a</sup>
5%	0.79±0.00°	$0.49\pm0.00^{a}$	0.27±0.00 <sup>b</sup>	0.63±0.00b	0.75±0.00a	38.00±0.00a
15%	0.76±0.00°	0.27±0.00 <sup>b</sup>	0.48±0.00a	0.78±0.00a	0.51±0.00 <sup>b</sup>	21.20±0.00 <sup>b</sup>
25%	0.59±0.00 <sup>b</sup>	0.00±0.00	0.17±0.00 <sup>c</sup>	0.58±0.00°	0.00±0.00	0.00±0.00

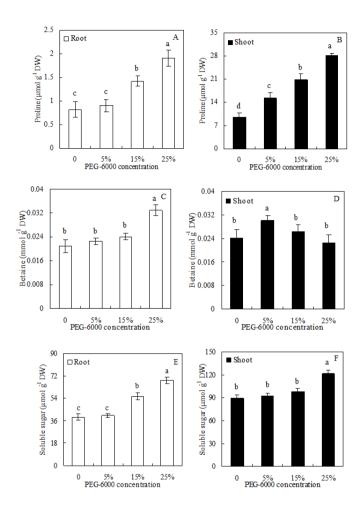
Table 3. Effects of PEG induced water stress on contents of photosynthetic pigments (g kg-1FM) in seedlings of wheat. The values are the means of five replicates. Means followed by different letters in the same stress type are significantly different at  $P \le 0.05$  according to Duncan's method.

PEG-6000 concentration	Chla	Chl <i>b</i>	Chla+ Chlb	Chla/Chl <i>b</i>	Car
0%	1.14±0.06ª	0.29±0.00 <sup>a</sup>	1.43±0.08ª	3.93±0.04	0.31±0.00 <sup>a</sup>
5%	1.13±0.08 <sup>a</sup>	0.28±0.00 <sup>a</sup>	1.41±0.06ª	4.04±0.08	0.34±0.00ª
15%	1.02±0.01 <sup>a</sup>	0.22±0.00b	1.24±0.02 <sup>ab</sup>	4.64±0.08	0.32±0.00 <sup>a</sup>
25%	0.86±0.00b	0.17±0.00°	1.03±0.01 <sup>b</sup>	5.06±0.09	0.28±0.00 <sup>b</sup>

Table 4. Effects of PEG induced water stress on contents of photosynthetic pigments (g kg<sup>-1</sup>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;  $P \le 0.05$ ). 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≤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≤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  $P \le 0.05$  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,  $P \le 0.05$ ). 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,  $P \le 0.05$ ). 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,  $P \le 0.05$ ).

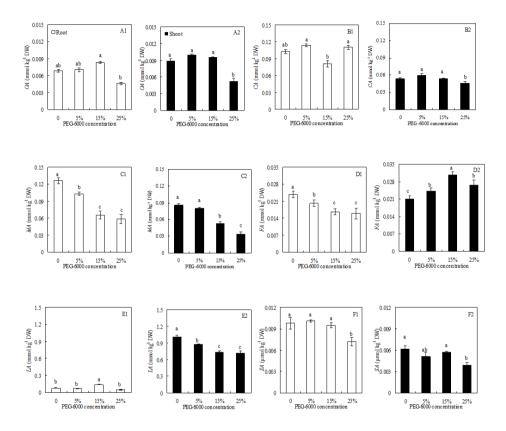


Figure 3. Effects of water stress on the levels of Oxalic Acid (A1), Citric Acid (B1), Malic Acid (C1), Formic Acid (D1), Lactic Acid (E1) and Succinic Acid (F1) in the root, and Oxalic Acid (A2), Citric Acid (B2), Malic Acid (C2), Formic Acid (D2), Lactic Acid (E2) and Succinic Acid (F2) shoot of wheat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

## 5.1. Germination

The table 5 shows that the trend in changes in Gp and Ge of wheat seeds under water stress conditions was similar; there was a decreased trend with increased PEG-6000 concentration ( $P \le 0.05$ ), the reductions were greater when concentration above 15% ( $P \le 0.05$ ).

Treatment	Gp (%)	Ge (%)
CK	96.66	96.00
5% PEG	95.33	91.67
15% PEG	83.67	80.33
25% PEG	64.00	63.67

Table 5. Effect of water stress on two indices (Gp and Ge) of germination for wheat. Germinative percentage - Gp and germinative Energy - Gp.

#### 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,  $P \le 0.05$ ), revealed a decrease for roots and shoots (Table 2,  $P \le 0.05$ ). 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≤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,  $P \le 0.05$ ). The values of Y(II), qP and ETR decreased, 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;  $P \le 0.05$ ). 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,  $P \le 0.05$ ).

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≤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 inhibiting 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.

# Acknowledgements

This work supported by grants from the Project of the National Natural Science Foundation of China (No. 31170303, 30870238, 30871447, 50709040, 31070398). The basic research special fund operations (1610122012001), the international scientific and technological cooperation projects (No. 2010DFB30550).

# Author details

Rui Guo\*, Wei Ping Hao, Dao Zhi Gong, Xiu Li Zhong and Feng Xue Gu

\*Address all correspondence to: guor219@yahoo.com

Institute of Environment and Sustainable Development in Agriculture(IEDA), Chinese Academy of Agricultural Sciences (CAAS)/Key Laboratory of Dry land Agriculture, MOA, Beijing 100081, China

# References

- [1] Abdalla, M. M., & El -Khoshiban, N. H. (2007). The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two Triticium aestivum cultivars. J. Appl. Sci., 3, 2062-2074.
- [2] Ali, Q., & Ashraf, M. (2011). Induction of Drought Tolerance in Maize (Zea mays L.) due to Exogenous Application of Trehalose: Growth, Photosynthesis, Water Relations and Oxidative Defence Mechanism. Journal of Agronomy and Crop Science, 197, 258-271.
- [3] Almansouri, M., Kinet, J. M., & Lutts, S. (1999). Compared effects of sudden and progressive impositions of salt stress in three durum wheat (Triticum durum Desf.) cultivars. J. Plant Physiol., 154, 743-752.
- [4] Armstrong, D. P., & Westoby, M. (1993). Seedlings from large seeds tolerate defoliation better: a test using phylogenetically independent contrasts. Ecology, 74, 1092-1100.
- [5] Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. Biotechnology Advances, 28, 169-183.
- [6] Ashraf, M., Ahmad, M. S. A., Öztürk, M., & Aksoy, A. (2012). Crop Improvement Through Different Means: Challenges and Prospects. Crop Production for Agricultural Improvement, 1-15.
- [7] Bjorkman, O., & Demmi, G. B. (1987). Photon yield of O2 evolution and chlorophyll fluorescence at 77K among vascular plants of diverse origins. Planta, 170, 489-504.
- [8] Bush, J. K., & Van Auken, O. W. (1991). Importance of time of germination and soil depth on growth of Prosopis glandulosa (Leguminosae) seedlings in the presence of a C4 grass. Am. J. Bot., 78, 1732-1739.
- [9] Chen, Y. P., Chen, Y. N., Li, W. H., & Xu, C. X. (2009). Effect of high temperature on photosynthesis in populus euphratica under drought condition. Journal of desert research, 29(3), 474-479.
- [10] Du, Y., & Huang, Z. L. (2008). Effects of seed mass and emergence time on seedling performance in Castanopsis chinensis. Forest Ecology and Management, 255, 2495-2501.
- [11] Gale, K. R. (2005). Diagnostic DNA markers for quality traits in wheat. Journal of Cereal Science, 41, 181-192.
- [12] Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of Photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimiea et Bio Physica Aeta, 990, 87-92.
- [13] Harms, K. E., & Dalling, J. W. (1997). Damage and herbivory tolerance through resprouting as an advantage of large seed size in tropical trees and lianas. J. Trop. Ecol., 13, 617-621.

- [14] He, H., Sun, C. H., Du, W., & Li, Y. (2006). Effect and evalution of Entomophthora spp. on controlling Aphis glycines. Chinese journal of oil crop science, 28, 76-78.
- [15] Huang, H. Y., Dou, X. Y., Deng, B., Wu, G. J., & Peng, C. L. (2009). Responses of different secondary provenances of Jatropha curcas to heat stress. Scientia silvae sinicae, 45(7), 150-155.
- [16] Jiao, S. Y., Li, Y. Q., Shayila, S. H., & Chen, X. L. (2009). Seeds Germination and Seedling Growth about 3 Pennisetum Ornamental Grasses under Drought Stress. Acta Bot.Borea l.-Occident. Sin., 29(2), 0308-0313.
- [17] Jing, J. H., & Ding, Z. R. (1981). Determining organic acid content.]- In: Boqinnoke, X.H. (ed.): Analysis Method of Plant Biochemistry. 264-267.
- [18] Johnson, G. N., Youn, G. A. J., Scholes, J. D., et al. (1993). The dissipation of excess excitation energy in British plant species. Plant, cell and Environment, 16, 673-679.
- [19] Jurado, E., & Westoby, M. (1992). Seedling growth in relation to seed size among species of Arid Australia. J. Ecol., 80, 407-416.
- [20] Kerepesi, I., Galiba, G., & Ba'nyai, E. (1998). Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedlings. J. Agric. Food Chem, 46, 5347-5354.
- [21] Kerepesi, I., & Galiba, G. (2000). Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. Crop Sci., 40, 482-487.
- [22] Krause, G. H., & Weis, E. (1991). Chlorophyll floresence and photosynthesis. Ann. Rew. Plant Physiol., 142, 313-349.
- [23] Leishman, M. R., & Westoby, M. (1994). The role of seed size in seedling establishment in dry soil conditions-experimental evidence from semiarid species. J. Ecol., 82, 249-258.
- [24] Li, Z. J., Luo, Q. H., Wu, W. M., & Han, L. (2009). The effects of drought stress on photosynthetic and chlorophyll fluorescence characteristics of Populus euphratica and P.pruinosa. Arid zone research., 24(4), 5-9.
- [25] Liu, H. Y., Li, J. Y., Zhao, Y., & Huang, K. K. (2007). Influence of drought stress on gas exchange and water use efficiency of Salix psammophila growing in five places. Arid zone research., 24(6), 815-820.
- [26] Lutts, S., Almansouri, M., & Kinet, J. M. (2004). Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Science, 167, 9-18.
- [27] Ma, H. Y., & Liang, Z. W. (2005). Research progress on improving germination rate of Leymus chinensis. *Grassland of China*, 27(4), 64-68.
- [28] Martin, M., Miceli, F., Morgan, J. A., Scalet, M., & Zerbi, G. (1993). Synthesis of Osmotically Active Substrates in Winter Wheat Leaves as Related to Drought Resistance of Different Genotypes. J. Agric. Crop Sci, 171, 176-184.

- [29] Paz, H., & Martinez-Ramos, M. (2003). Seed mass and seedling performance within eight species of Psychotria (Rubiaceae). Ecology, 84, 439-450.
- [30] Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence, a practical guide. J. Exp., 51, 659-668.
- [31] Naumann, J. C., Young, D. R., & Anderson, J. E. (2008). Leaf chlorophyll fluorescence, reflectance, and physiological response to freshwater and saltwater flooding in the evergreen shrub, Myrica cerifera. Environ. Exp. Bot., 63, 402-409.
- [32] Pawl, B. (1998). Cities and economic development: from the dawn of history to the present. Chicago: Chicago University Press.
- [33] Pilon-Smits, E. A. H., Ebskamp, M. J. M., Paul, M. J., Jeuken, M. J. W., Weisbeek, P. J., & Smeekens, S. C. M. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol., 107, 125-130.
- [34] Qu, Y., Y.,, P., Mu,, X. Q., Li, Y. X., Tian,, F., Wen,, H. L., Zhang, , & Li, Z. C. (2008). QTL mapping and correlations between leaf water potential and drought resistance in rice under upland and lowland environments. Acta agronomica sinica, 34(2), 198-206.
- [35] Ralph, P. J., & Burchett, M. D. (1998). Photosynthetic response of Halophila ovalis to heavy metal stress. Environ Pollut., 103, 91-101.
- [36] Rau, S., Miersch, J., Neumann, D., Weber, E., & Krauss, G. J. (2007). Biochemical responses of the aquatic moss Fontinalis antipyretica to Cd, Cu, Pb and Zn determined by chlorophyll fluorescence and protein levels. Environmental and Experimental Botany, 59, 299-306.
- [37] Reich, P. B., Tjoelker, M. G., Walters, M. B., Vanderklein, D. W., & Buschena, C. (1998). Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. Funct. Ecol., 12, 327-338.
- [38] Rengasamy, P. (2002). Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Aust. J. Exp. Agric., 42, 351-361.
- [39] Rhodes, D., & Hanson, A. D. (1993). Quaternary ammonium and tertiary sulphonium compounds in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 44, 357-383.
- [40] Richards, J. F. (1990). Land transformation. Turner II, B.L., et al. (Eds.), The Earth as transformed by human action. New York: Cambridge University Press, 163-178.
- [41] Ross, M. A., & Harper, J. L. (1972). Occupation of biological space during seedling establishment. J. Ecol., 60, 70-88.
- [42] Shao, H. B., Liang, Z. S., Shao, M. G., & Wang, B. C. (2005). Impacts of P EG-6000 pretreatment for barley ( Hordeum vulgare L. ) seeds on the effect of their mature embryo in vitro culture and primary investigation on its physiological mechanism. Colloids and Surfaces B: Biointerfaces, 41, 73-77.

- [43] Shen, L. M., David, M., & Joyee, G. F. (1990). Influence of drought on the concent ration and distribution of 2,4- diaminaobutyric acid and other free amino acids in tissues of flat pea (Lathyrus sylvestris L.). *Enviro Expt Bot.*, 30, 497-504.
- [44] Shi, G. Y., Liao, W. X., Qin, L. F., & Lu, L. L. (2009). PEG simulated water stress effects on physiological and biochemistry indexes of germination of Toona sinensis seeds. *Journal of Forestry Sci and Tech.*, 4, 142-145.
- [45] Shi, Z., Shi, S. Q., Xiao, W. F., & Qi, L. W. (2008). Influence of dehydration on characteristics of chlorophyll fluorescence of detached leaves in Haloxylon ammodendron and Populus euphratica. *Forest Research*, 21(4), 566-570.
- [46] Stendle, E., & Peterson, C. A. (1998). How does water get through roots? J Exp Bot. , 49, 775-788.
- [47] Stewart, G. R., & Lee, J. A. (1974). The role of proline accumulation in haloplytes. Planta, 120, 279-289.
- [48] Sun, L., Liu, S. H., Shi, X. D., Xiao, M., Tang, Z. Y., Zhu, H. W., & , J. Z. (2006). Chen-Salt-tolerant physiological and biochemical properties of ten Species of chenopodiaceae halophytes growing in deserts, Xi njiang. ARI D ZONE RESEARCH, 23, 209-313.
- [49] Van , K. O., & Snel, J. F. H. (1990). The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research*, 25, 147-150.
- [50] Verslues, P. E., Ober, E. S., & Sharp, R. E. (1998). Root growth and oxygen relation at low water potentials impact of oxygen availability in polyethylene glycol solution. Plant Physio1.:, 116, 1403-1412.
- [51] Wang, J. G., Chen, G. C., & Zhang, C. L. (2002). The effect s of water stress on soluble protein content, the activity of SOD, POD and CAT of two eco types of reeds (Phragmi tescommunis). ActaBot. Boreal. Occident. Sin., 22(3), 561-565.
- [52] Xu, S. G., Wang, J. H., & Bao, L. J. (2006). Effect of Water Stress on Seed Germination and Seedling Growth of Wheat. *Journal of Anhui Agri. Sci.*, 34, 5784-5787.
- [53] Yang, X. Q., Zhang, S. Q., Liang, Z. S., & Shan, Y. (2004). Effects of water stress on chlorophyll fluorescence parameters of different drought resistance waiter wheat cultivars seedlings. Acta Bot. Boreal. Occident. Sin., 24, 812-816.
- [54] Zhao, Y. J., Weng, B. Q., Wang, Y. X., & Xu, G. Z. (2009). Plant physio-ecological responses to drought stress and its research progress. *Fujian science and technology of rice and wheat.*, 27(2), 45-50.
- [55] Zhang, Y. Q., Mao, X. S., & Sun, H. Y. (2002). Effects of drought stress on chlorophyll fluorescence of winter wheat. *Chinese Journal of Eco-Agriculture*, 10, 13-15.
- [56] Zhou, J. L., Tang, X. Q., & Wang, K. C. (2009). Effect of water stress on content of four organic acids in different cultivated populations of Isatis indigotica. China. *Journal of Chinese Materia Medica*, 34, 127-131.