Physiological regulation of high transpiration efficiency in winter wheat under drought conditions

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ABSTRACT

Pot experiments were conducted to study the variation and physiological regulation of transpiration efficiency (TE) of four winter wheat (*Triticum aestivum* L.) varieties that are widely grown in different ecological regions in North China. Plants were grown under two soil moisture regimes, normal and drought stress. The results showed that under drought stress condition, both TE at plant level and TE at leaf level (TEl) increased significantly. The transpiration rate (Tr) was reduced more strongly than leaf net CO_2 assimilation rate (Pn). The decline of Tr was mainly affected by stomatal conductance and the decline of Pn was affected by non-stomatal factors, which was confirmed by the decline in net photosynthetic oxygen evolution rate. The leaf soluble sugar content and proline content were significantly increased under drought stress. The stomatal density was increased and the stomatal length was reduced. These results led us to make the following conclusions: (1) Under drought stress, the increase in TEl appears to be regulated in two ways: via the stomata by regulating Tr, and independent of the stomata through regulation of Pn; regulation via the stomata was more sensitive; (2) Osmotic adjustment was closely correlated to the non-stomatal regulation, and stomatal aperture was closely correlated to the stomatal way.

Keywords: winter wheat; drought; transpiration efficiency; stomata; osmotic adjustment; stomatal aperture

Drought is a worldwide issue that impacts seriously on the security of food production. Global climate change makes this even worse (Elisabeth et al. 2009). Winter wheat is almost the most important crop in the world. However, its main growth period is in the winter to spring season when rainfall is inadequate in most parts of the world. To improve wheat biomass and harvest index it is critical for the crop to make better use of the limited availability of water.

Improving the transpiration efficiency (TE) is the inevitable choice. Many studies suggested that TE was affected by the crop genotype and soil mois-ture content (Salvatore et al. 2007, Sankar et al. 2008), and physiological research was significantly contributed to the selection of high TE crops. However, a lack in understanding of the physiological and molecular regulation of TE presents a real constraint for further improvement of TE and selection of new crop varieties (Richards 2006). Recently, most studies on winter wheat focused on the effect of irrigation regime and soil moisture content on yield, TE, the relationship between photosynthetic traits and environmental factors, agronomic traits (Xue et al. 2006, Kahlown et al. 2007). The results show that crop yield was de-

creased or changed under water stress; the water use efficiency was increased. However, the physiological mechanism for this was not clear.

Giorio et al. (1999) reported that stomatal regulation was the major physiological factor in the optimization of water use under drought conditions. Yang et al. (2007) studied the stomatal width of *Leymus chinensis* in different longitudes (with different soil moisture content) in the North of China and came to a similar conclusion. However, DaMatta et al. (2003) concluded that osmotic adjustment under drought conditions was the main factor contributing to maintain higher transpiration efficiency. However, there was no uniform view on the physiological basis on which TE was improved so far.

The objective of this study was to further investigate the regulatory mechanism controlling changes in TE and its physiological components, with the aim to provide a theoretical underpinning for improved water-saving in winter wheat cultivation and for the early selection of high-TE winter wheat varieties.

MATERIAL AND METHODS

Experimental design. Four winter wheat varieties (*Triticum aestivum* L., Shi4185, Kenong199, Jinmai47 and Luohan2) widely grown in different ecological regions in North China, were used in the study. Seeds, surface-sterilized with 0.1% $HgCl_2$, were sowed in 'Zero-evaporation' pots (11.5 × 9 × 12 cm^3), which were made according to a new method, 'a precise method and equipments to measure plant TE' (State patent), containing 1.7 kg loam with 13.2 mg/kg nitrogen, 47.6 mg/kg phosphorus and 35.8 mg/kg potassium. After sowing, the pots were sealed with plastic cloth. The little holes which just allowed wheat coleoptiles through were poked in the plastic cloth above the wheat coleoptiles. Then the wheat coleoptiles were picked out the holes with needle. Two levels of soil moisture were given (CK: 75% maximum capillary water capacity; and drought stress (DS): 45% maximum capillary water capacity) with 6 replications, and the seedling density was 20 for each pot.

Physiological measurements and sampling. Twenty eight days after sowing, all the indexes were measured, and the samples were taken.

The water consumption (Cw_i) was measured by weighing the pots every 2 days. The total water consumption (Cw) for the growth period was calculated as follows: $Cw = \sum Cw_i$. Because the evaporation

could be neglected, the transpiration efficiency at the plant level (TEp) could be calculated with the ratio of plant biomass (BM)/Cw.

Net CO_2 assimilation rate (Pn), transpiration rate (Tr), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of seedling leaves were measured with a Li-6400 (LI-COR, Lincoln, NE, USA) under 25 ± 0.3, 1500 μmol/m²/s intensity of radiation and 380 ± 2.83 μmol/mol CO_2 concentration between 9:30 am and 11:00 am. Net photosynthetic oxygen evolution rate ($Pn(O_2)$) was measured at 25 ± 0.3, with Yaxin-1151 biological oxygen determinator (Yaxinliyi Technology Co., Ltd. Beijing; Zheng 2006). Total chlorophyll content (Chl(a + b)) was measured by the grinding extraction methods in 95% ethanol, using UV-vis spectrophotometer (UV2450, Tokyo, Japan). Soluble sugar and proline content were determined by ninhydrin colorimetric and the authrone colorimetric method, respectively, using UV-vis spectrophotometer (Zheng 2006). A WP4 Dewpoint Potential Meter (Decagon Devices, Inc., Pullman, WA DC., USA) was used to determine leaf water potential (Ψ_w). The second fully-developed leaves were selected and the abaxial epidermis and mesophyll were scraped off to observe the adaxial epidermal stoma to calculate stomatal density under a Zeiss Axioplan 2 imaging MOT microscope (40 × magnification). And the stomatal length was measured under 200 multiple vision with Axio Vision 4.0 software.

Data analysis. The data were analyzed by two-way analysis of variance, followed by LSD test at $P = 0.05$ level to compare the means, and the correlation was analyzed by Pearson Product-moment Correlation Coefficient method using SPSS 13.0 for Windows.

RESULTS AND DISCUSSION

The change of transpiration efficiency at plant and leaf level

Under drought stress, the dry biomass of the four varieties significantly decreased by 15.1% (Shi4185), 26.6% (Kenong199), 10.2% (Jinmai47) and 5.8% (Luohan2) (Figure 1). And the water consumption for plant growth was decreased by more than 42.0%, 48.6%, 53.6% and 53.3%, respectively (Figure 2). However, the transpiration efficiency at plant level was significantly increased by 46.4%, 42.8%, 93.4% and 101.7% (Figure 3). Shi4185 and Kenong199 had significantly higher TEp than Jinmai47 and Luohan2 in CK, but lower TEp in the drought treatment.
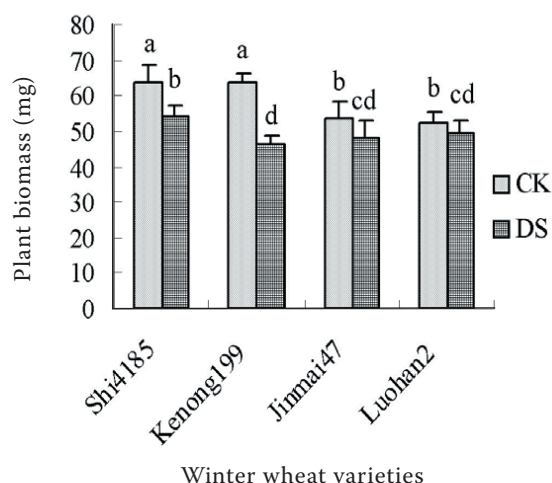
Figure 1. The plant biomass of the 4 varieties in different soil moisture. Determined after sowing 28 days. Different letters above the bars indicated significant difference at $P \leq 0.05$. The little bars represent S.E., and DS means drought stress. The same as below

Transpiration efficiency at leaf level was measured with the ratio of Pn/Tr. The results show that TEl of the 4 varieties significantly increased by 5.1%, 7.9%, 19.0% and 19.9% under drought stress. Shi4185 and Kenong199 had higher TEl than Jinmai47 and Luohan2 in controls, however, the opposite trend was observed for the drought treatment (Figure 4).

Transpiration efficiency at both plant and leaf level was improved. A close linear relationship ($R^2 = 0.9208$) was found between TEp and TEl (Figure 5). However, comparing the changes of TEp and TEl, it could be seen that the increase of TEp was much higher than that of TEl.

It has been demonstrated that there is a TE difference amongst different wheat genotypes (Dong et al. 2008, Blum 2009). In this study, the 4 varieties grown under the same soil moisture conditions had different TE. The TEl and TEp determined using a zero-evaporation pot sample significantly increased when soil water content decreased. This is consist-

ent with other researchers' results at leaf and grain yield level (Xue et al. 2006, Kahlown et al. 2007). The linear regression relationship between TEp and TEl suggests that TEl was the basis of TEp. However, the measurement of TEl was affected by diurnal variation of Pn and Tr and the position on the leaf, so that TEp should be better for showing the characteristics of plant water consumption.

The physiological way to regulate TEl improvement

In the drought treatment, the Pn was reduced by 44.4%, 51.4%, 32.1% and 41.3% for the varieties, while the C_i was increased (Table 1), which indicated that the decline of Pn was possibly mainly affected by non-stomatal factors (CO_2 assimilation ability of mesophyll cells is weaker).

The rate of Pn/g_s (Inner transpiration efficiency, Figure 6) can reflect the status of photosynthesis
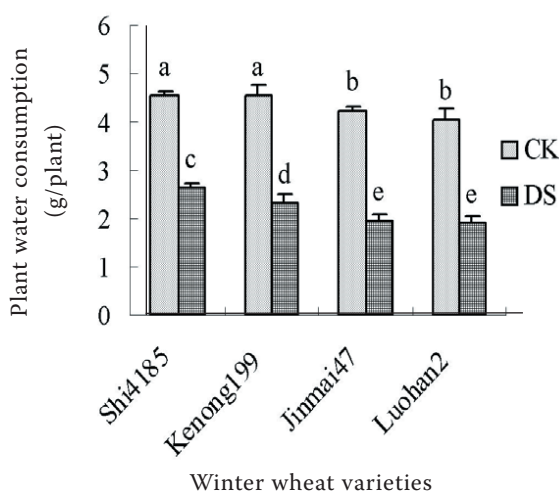

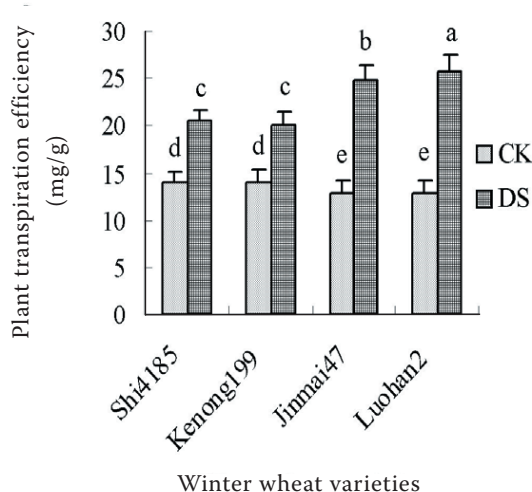Figure 2. The plant water consumption of the 4 varieties in different soil moisture

Figure 3. The transpiration efficiency at plant level of the 4 varieties in different soil moisture
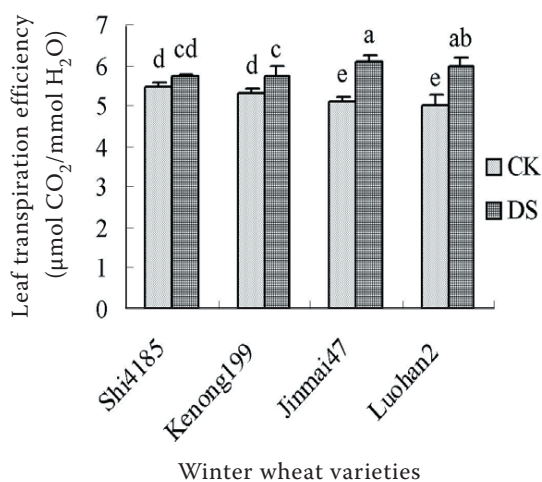
Figure 4. The leaf transpiration efficiency of the 4 varieties in different soil moisture

Figure 5. The relationship between leaf transpiration efficiency and plant transpiration efficiency

(Osmond et al. 1980). The inner transpiration efficiencies of Shi4185 and Kenong199 decreased when soil water content was low, and the inner transpiration of Kenong199 decreased significantly, while the rate of Jinmai47 and Luohan2 were increased. This suggests that a strong non-stomatal limitation of photosynthesis occurred in the case of Shi4185, especially to Kenong199, while Jinmai47 and Luohan2 were relatively less affected by non-stomatal limitation.

In order to further testify the existence of non-stomatal limitation, we determined the net photosynthetic oxygen evolution rate of leaf. In determining $Pn(O_2)$ conditions, photorespiration and CO_2 flow limitation to chloroplasts through stoma (stomatal limitation) are almost eliminated and it is possible to analyze photosynthesis without
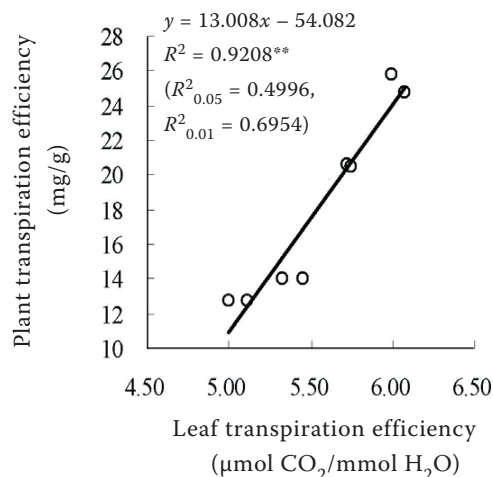
these limitations (Riberio et al. 2003). The result showed that $Pn(O_2)$ was reduced by 46.4%, 51.8%, 32.3% and 42.4% for the four varieties, respectively (Table 1), and a significant relationship ($R = 0.999$) was found between Pn and $Pn(O_2)$ (Figure 7).

g_s was decreased by 42.9%, 43.6%, 34.2% and 53.2% for the four varieties, respectively, and a close linear relationship was found between Tr and g_s ($R^2 = 0.9547$, Figure 8), which suggests that transpiration was regulated by the stomata. This result is similar to that observed by Osmond et al. (1980).

The reasons for the Pn decrease are not beyond the scope of stomata closing and the photosynthetic capability of mesophyll declining. The decrease of Pn was attributed to non-stomatal limitation when g_s decreased and C_i increased simultaneously

Table 1. The photosynthetic traits of the 4 varieties in different soil moisture

		Pn ($\mu molCO_2/m^2/s$)	Tr ($mmol/m^2/s$)	g_s ($mol/m^2/s$)	C_i ($\mu mol/mol$)	$Pn(O_2)$ ($\mu molO_2/m^2/s$)
Shi4185	CK	25.7 ± 2.1[a]	4.73 ± 0.13[a]	0.71 ± 0.023[a]	148.5 ± 6.1[d]	24.8 ± 0.88
	DS	14.3 ± 1.1[c]	2.5 ± 0.2[c]	0.41 ± 0.021[c]	233.2 ± 7.0[b]	13.3 ± 0.30
Kenong 199	CK	25.5 ± 1.8[a]	4.8 ± 0.1[a]	0.73 ± 0.028[a]	154.8 ± 9.0[d]	24.7 ± 0.88
	DS	12.4 ± 1.8[c]	2.15 ± 0.02[d]	0.41 ± 0.080[c]	261.5 ± 7.7[a]	11.9 ± 0.62
Jinmai47	CK	22.4 ± 1.5[b]	4.39 ± 0.72[b]	0.60 ± 0.006[b]	185.1 ± 6.2[c]	21.7 ± 0.52
	DS	15.2 ± 1.0[c]	2.5 ± 0.05[c]	0.40 ± 0.015[c]	194.5 ± 8.7[c]	14.7 ± 0.54
Luohan2	CK	22.3 ± 2.1[b]	4.47 ± 0.05[b]	0.69 ± 0.001[a]	157.3 ± 8.2[d]	21.7 ± 0.59
	DS	13.1 ± 1.4[c]	2.18 ± 0.03[d]	0.32 ± 0.026[d]	187.5 ± 7.1[c]	12.5 ± 0.86

The means followed by different letters are significantly different at $P \leq 0.05$, ± S.E. The parameters were determined at 25 ± 0.3°C. Pn, Tr, g_s, C_i, and $Pn(O_2)$ in the table mean net photosynthetic rate. Transpiratiion rate, stomatal conductance and net photosynthetic oxygen evolution rate, respectively.
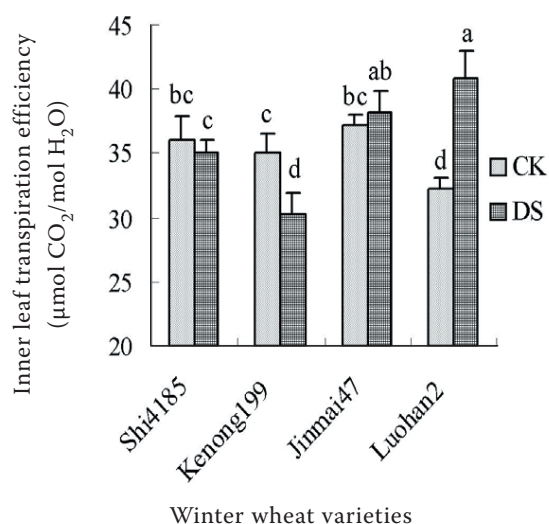
Figure 6. The inner leaf transpiration efficiency of the 4 varieties in different soil moisture

Figure 7. The correlation analysis between net photo-synthetic CO_2 assimilation and net photosynthetic O_2 evolution rate (determined at $25 \pm 0.3°C$)

(Farquhar and Sharkey 1982). We thus concluded that Pn of the four varieties was limited by non-stomatal factors in drought stress. This was consistent with the results of DaMatta et al. (2007) and Rouhi et al. (2007) for coconut and almond plants, respectively. The significant correlation between Pn and $Pn(O_2)$ further confirmed this result. As stomatal conductivity of H_2O was 1.56 times as large as that of CO_2 (Grant et al. 2005), the relationship between Tr and g_s was closer than that between Pn and g_s. And the linear relationship between g_s and Tr indicates that Tr was mainly controlled by stomatal factors.

The results above show that there was a larger decrease scope of Tr than Pn under drought, which
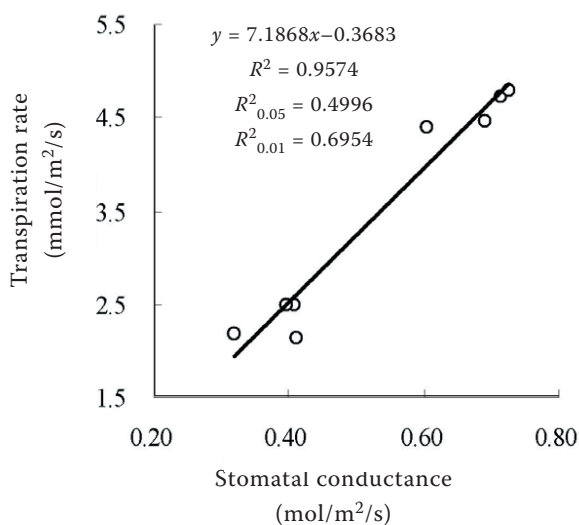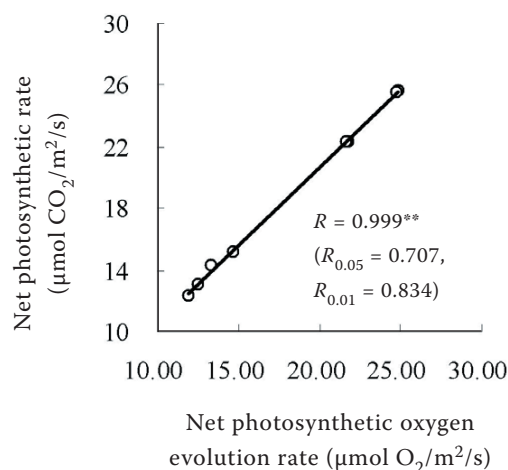
was the main reason why TEl increased. This result was similar to Warren's (2008) conclusion; they pointed out that the increase of TEl was mainly caused by the larger decrease scope of Tr, not by the decrease of Pn.

Our results indicate that TE improvement of the 4 varieties under drought was regulated via the stomata (regulating Tr) and via mechanisms that do not involve the stomata (regulating Pn). The stomatal mechanism is more sensitive than the non-stomatal mechanism because of the larger decline of Tr than Pn.

Physiological activities associated with the regulation way of TE

Activities with non-stomatal way. Soluble sugars and proline are osmolytes induced by adverse conditions. Within a certain range of severity of water stress, many plants can maintain cell function and relative water content by osmotic regulation (Sara et al. 2009). Under drought condition, soluble sugars and proline content of all 4 varieties, Shi4185, Kenong199, Jinmai47 and Luohan2, significantly increased by 52.3%, 60.0%, 103.3%, 92.9%, and by 174.7%, 175.0%, 249.2%, 242.9%, respectively. Proline content was more sensitive (induced to a higher level) to soil water deficit (Figures 9–10).

The main factors limiting Pn under drought stress are the decrease of intracellular free water content, and the destroyed environment of photo-synthesis. Ψ_w was significantly lower after drought treatment in our study (Figure 11).

Figure 8. The relationship between transpiration rate and stomatal conductance (determined at $25 \pm 0.3°C$)
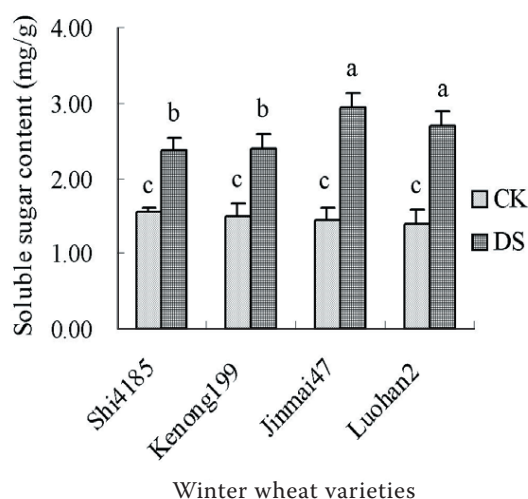
Figure 9. The soluble sugar content of the 4 varieties in different soil moisture
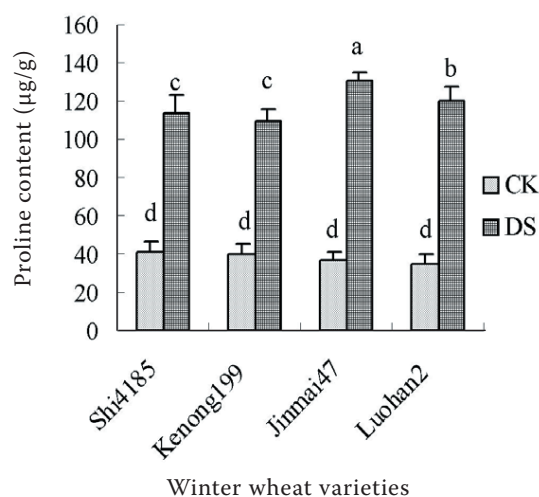

Figure 10. The proline content of the 4 varieties in different soil moisture

The total chlorophyll contents of all 4 varieties significantly decreased under drought condition (Figure 12). Shi4185 and Kenong199 exhibited the characteristics of higher Chl(a + b) in CK, while lower in drought treatment.

TEl is determined by Pn and Tr, so any factor which influences Pn and Tr would have a certain effect on TE. Leaf water potential is the best index which reflects the water status of plant. Low leaf water potential results in TE increase- and adaptation response to water deficit. According to the relation formula of osmotic potential (Lambers et al. 2005): $\Psi_w = \Psi_s + \Psi_p$, it can be achieved that leaf keeps low osmotic potential and high water potential, simultaneously, as soil moisture de-

creased. The increase of osmolytes can improve leaf relative water content, scavenge active oxygen and protect the integrity of membrane. DaMatta et al. (2003) believed that the osmotic adjustment (OA) under drought conditions was the main factor to maintain high transpiration efficiency. In this study, the varieties with higher soluble sugar and proline content had higher Ψ_w, Chl(a + b), Pn and TEl under drought stress (Figures 4 and 10). Hence, we concluded that OA kept leaf water condition well, membrane and photosynthetic system stability was one of the ways by which winter wheat maintained higher Pn. Chl(a + b) was the physiological foundation of photosynthesis. So this suggested the decrease of Chl(a + b) was possibly one of the
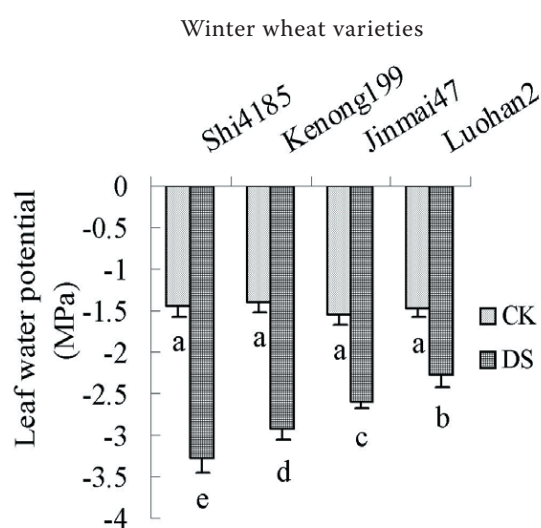

Figure 11. The leaf water potential of the 4 varieties in different soil moisture (determined at 25 ± 0.3°C, 11:00–12:00)
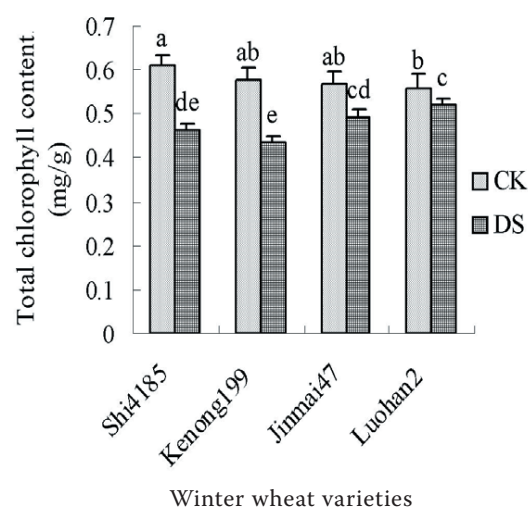

Figure 12. The total chlorophyllcontent of the 4 varieties in different soil moisture
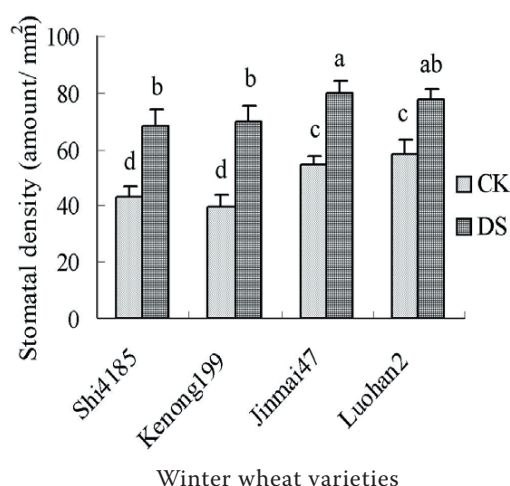
Figure 13. The response of stomatal density of the 4 varieties to soil moisture
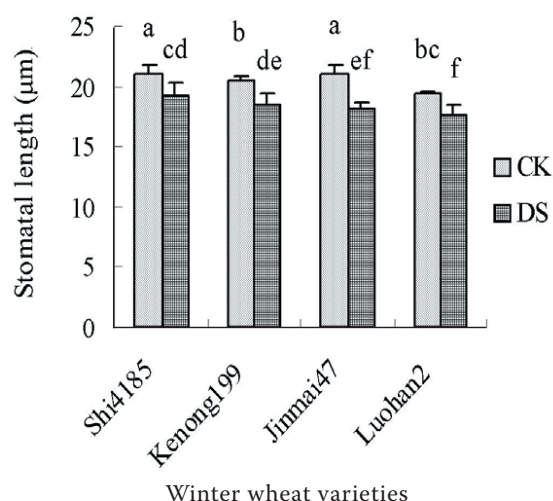

Figure 14. The response of stomatal length of the 4 varieties to soil moisture

reasons why non-stomatal limitation of photosynthesis occurred under drought condition.

Stomatal activities with stomatal way. The increase in stomatal density and the decline of stomatal length are considered as an indication of plant adaptability to drought stress (Maes et al. 2009). The adaxial stomatal density of the four varieties increased by 58.7% (Shi4185), 75.4% (Kenong199), 45.4% (Jinmai47) and 33.2% (Luohan2) under drought, respectively (Figure 13); in the contrary, stomatal length of the four varieties significantly decreased in drought treatment, namely by 9.0%, 9.4%, 13.2% and 9.0%, respectively (Figure 14).

According to the model of stomatal conductance reported by Jarvis (1976), $g_s = g_{max} \times G(x)$; g_{max} means max stomatal conductance, which is determined by stomatal morphology traits and environment factors, $G(x)$ means relative degree of stomatal opening. In our study, the seedlings in different treatments were grown in the same environment. Yet, the g_s was reduced by 42.9%, 43.6%, 34.2% and 68.1%, respectively, which indicated that the g_s decline was mainly controlled by the decline of stomatal aperture.

The scope of stomatal density increased much more than the variation of stomatal length in drought. However, the g_s significantly decreased, and its decline was mainly caused by the closing of stomata. This is consistent with the view of White et al. (2000). Now, a clear threat presents: soil moisture affected stomatal density, stomatal length, especially stomatal aperture, which kept g_s low; g_s determined Tr, and then, lower Tr improved TEl.

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