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Assessment of Drought Tolerance of Some *Triticum* L. Species through Physiological Indices

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Abstract: Wheat is one of the most important crops in the world. Its yield is greatly influenced by global climate change and scarcity of water in the arid and semi-arid areas of the world. So, exploration of gene resources is of importance to wheat breeding in order to improve the crop ability of coping with abiotic stress environment. Wild relatives of wheat are rich repositories of beneficial genes that confer tolerance or resistance not only to drought but also to other environmental stresses. In the present study, the changes in leaf relative water content (RWC), free proline content, and malondialdehyde (MDA) accumulation of five wild wheat species including *T. boeticum* (YS-1L), *T. dicoccum* var. *dicoccoides* (YS-2L), *T. araraticum* (ALLT), and two cultivated varieties of *T. turgidum* ssp. *durum* (MXLK and 87341), with two well-known common wheat cultivars (SH6 and ZY1) possessing strong drought resistance and sensitiveness, respectively, as references were investigated during 3-day water stress and 2-day recovery, in order to assess the drought tolerance of these wild wheat species. The laboratory experiment was conducted under two water regimes (stress and non-stress treatments). Stress was induced to hydroponically grown two weeks old wheat seedlings with 20% PEG 6000. Stress treatment caused a much smaller decrease in the leaf RWC and rise in MDA content in YS-1L compared to the other wheat species. From the data it was obvious that YS-1L was the most drought tolerant among studied species having significantly higher proline and RWC while lower MDA content under water stress conditions. The order of water stress tolerance of these species according to the three parameters is: YS-1L > YS-2L > SH6 > 87341 > ZY1 > MXLK > ALLT. We speculate that the observed drought stress tolerance at a cellular level was associated with the ability to accumulate proline and high water level conservation.

Keywords: drought tolerance; MDA; proline; relative water content; *Triticum* L. species; water stress

Wheat is one of the major crops in the world, and its yield is significantly influenced by global climate change and water resources scarcity in the environment (Al-Ghamdi 2009). Drought is one of the environmental stresses seriously limiting crop production in the majority of agricultural fields of the world (Abedi & Pakniyat 2010) and recent global climate change has made this situation more adverse (Anand *et al.* 2003).

Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition. The extent of these changes is dependent on the time, stage and severity of environmental stress (Cao *et al.* 2011). Water deficit conditions cause water losses within the plant and result in relative water content (RWC) reduction. Therefore, RWC is widely used as one of the most reliable indicators for defining both the sensitivity and the tolerance of plants to water deficit (Rampino *et al.* 2006; Sanchez-Rodriguez *et al.* 2010). Plants generally accumulate some kinds of compatible solutes such as proline, betaine and polyols in the cytosol to raise osmotic pressure and thereby to maintain both turgor and driving gradient for water uptake (Rhodes & Samaras 1994) and to protect membranes and proteins (Delauney & Verma 1993). It has been shown that proline plays an important role in the stabilization of cellular proteins and membranes in the presence of a high osmoticum concentration (Errabii *et al.* 2006). It was indicated that the accumulation of malondialdehyde (MDA), a product of fatty acid peroxidation, in plants due to cellular membrane lipid peroxidation is a measure of oxidative stress induced membrane damage during water stress (Farooq *et al.* 2010).

The search for traits related to drought tolerance is an important step in wheat breeding and production. Field experiments investigating the yields of different cultivars under water deficit conditions are the most reliable way to assess their drought tolerance. However, it takes 2~3 years to obtain results for winter wheat. In addition, a very large area is essential if many cultivars are evaluated at the same time. Therefore, the protocols for assessing drought tolerance of wheat plants in laboratory have been developed, and several methods have been employed to create water stress in plants by the use of certain chemicals including polyethylene glycol (PEG), mannitol etc. (Emmerich & Hardegree 1990). Among these chemicals, PEG 6000 was used most frequently to induce water stress to the hydroponically grown plant seedlings (Lu & Neumann 1998). Plant physiological indices, including RWC, MDA accumulation, free proline content etc., have been extensively used to assess plant drought tolerance under water stress conditions.

Many important agronomical traits including drought tolerance and pathogen resistance present in *Ae. tauschii* ($2n = 2x = 14$, DD), a progenitor of common wheat, have been transferred from related species and genera into wheat (Villareal *et al.* 2003; Mujeeb-Kazi *et al.* 2004). Useful variation in drought tolerance has also been identified in *T. urartu* ($2n = 2x = 14$, AA), *T. boeticum* ($2n = 2x = 14$, AA), *T. dicoccum* var. *dicoccoides* ($2n = 4x = 28$, AABB) (Valkoun 2001) and *Ae. geniculata* ($2n = 4x = 28$, MMUU) (Zaharieva *et al.* 2001). The drought tolerance evaluation of these wild species was a preliminary step before their application to wheat breeding program.

In the present study, three wild species of the genus *Triticum* L. with different ploidy levels and two cultivated varieties of *T. turgidum* ssp. *durum* were used to assess drought tolerance, with two well-known old common wheat cultivars (Shaanhe6 and Zhengying1) possessing strong drought resistance and sensitiveness, respectively, as references. The drought tolerance has been evaluated by three physiological indices, i.e. leaf RWC, MDA accumulation, and proline content, under water stress conditions. The selected drought tolerant species will then be used for proteomic study under drought conditions in later experimentations.

MATERIAL AND METHODS

Plant material and culture. Three wild species of the genus *Triticum* with different ploidy levels, i.e. *Triticum boeticum* ($2n = 14$, A^bA^b), *Triticum dicoccum* var. *dicoccoides* ($2n = 28$, A^uA^uBB), and *Triticum araraticum* ($2n = 42$, A^bA^bGG), and two cultivated varieties of *Triticum turgidum* ssp. *durum* ($2n = 28$, A^uA^uBB) were used to assess drought tolerance, with two well-known old common wheat cultivars (Shaanhe6 and Zhengying1) possessing strong drought resistance and sensitiveness, respectively, as references. The names and abbreviations of the wild and cultivated wheat species of *Triticum* L. used in this investigation are shown in Table 1. All the seeds of these species and the reference cultivars were provided by Wheat Research Centre, Northwest A & F University, Yangling, China.

Seeds of each species were germinated under hydroponic conditions and grown in a greenhouse with a day/night temperature regime of 20–22°C/15–18°C, 65–75% relative humidity and a light period of 16 h/day, regulated with supplementary light, six trays (40–50 plants) for each genotype. The Hoagland solution containing all necessary nutrients for normal plant growth, developed by Hoagland and Snyder (1993), was supplied for wheat growth, and aerated using an air compressor. At the two-leave stage (about 14 days old), plants of each genotype were divided into two groups, each group including three trays. One group normally supplied the Hoagland solution was used as a control, while another group supplied the Hoagland solution containing 20% PEG 6000 for three days and then normally supplied the Hoagland solution again (recovery) was used as a drought treatment.

The leaf samples were randomly taken from the control or drought treated groups of each genotype at the same time point during the stress period, i.e.

Table1. The names and abbreviations of the wild and cultivated wheat species of the genus *Triticum* L. used in this investigation

No.	Species name		Abbreviation	2*n*	Genome
	Latin name	Chinese name			
1	*Triticum boeticum*	Ye Sheng Yi Li	YS-1L	14	A^bA^b
2	*Triticum dicoccum* var. *dicoccoides*	Ye Sheng Er Li	YS-2L	28	A^uA^uBB
3	*Triticum araraticum*	A La La Te	ALLT	28	A^bA^bGG
4	*Triticum turgidum* ssp. *durum*	Mo Xi Li Ka	MXLK	28	A^uA^uBB
5	*Triticum turgidum* ssp. *durum*	87341	87341	28	A^uA^uBB
6	*Triticum aestivum*	Shaanhe6	SH6	42	A^uA^uBBDD
7	*Triticum aestivum*	Zhengyin1	ZY1	42	A^uA^uBBDD

0, 24, 48, and 72 h of drought stress, and recovery period, i.e. 24 and 48 h of recovery (rc24 h, rc48 h, respectively). The experiment was performed in triplicate dependent biological repeat. The collected leaf samples were used for measurements of RWC, MDA, and proline content.

Measurement of leaf RWC, MDA, and proline content. RWC was estimated according to the method of Smirnoff (1993). Proline content was measured by the method of Bates *et al.* (1973). MDA content was measured by the protocol of Hodges *et al.* (1999).

Statistical analysis. Results were based on mean values of at least three replicates from two (independent) repeats of experiment. The mean values were then compared using Duncan's multiple range test at $P = 0.05$. MSTATC computer software was used to carry out statistical analysis (Bricker 1991).

RESULTS

Leaf RWC of different wheat species under water stress

When exposed to water stress for 24 h, all species did not show any significant difference in leaf RWC compared with their control, except for ZY1, ALLT and MXLK which showed a significant decline at this time point (Figure 1a). The leaf RWC of all genotypes was significantly reduced by the water stress prolonged, but ZY1, ALLT, MXLK and 87341 exhibited a larger decrease in leaf RWC under 48 and 72 h water stress compared with SH6, YS-1L and YS-2L (Figure 1a).

Upon recovery from water stress, leaf RWC of all wheat species exhibited a recovery after 24 h recovery from stress. The leaf RWC levels of all

Table 2. Leaf relative water content (RWC in %) of the wheat species and two reference common wheat cultivars under water stress and normal conditions (CK)

Wheat species*	CK	Water stress treatment time (h)**				
		24	48	72	24h-rw	48h-rw
ZY1 (1.993)	96.56^{a} ± 1.51	93.40^{b} ± 1.73	82.47^{c} ± 2.06	74.66^{d} ± 3.45	93.48^{b} ± 1.78	96.27^{a} ± 1.81
SH6 (2.559)	94.80^{a} ± 1.83	92.73^{a} ± 1.68	88.40^{b} ± 1.19	87.30^{b} ± 1.21	89.20^{b} ± 1.24	92.80^{a} ± 1.49
ALLT (1.976)	92.45^{a} ± 1.67	86.00^{b} ± 2.07	81.85^{c} ± 2.73	70.94^{d} ± 3.35	86.77^{b} ± 1.97	91.51^{a} ± 1.71
YS-1L (1.937)	97.24^{a} ± 1.62	95.37^{a} ± 1.89	90.78^{b} ± 2.31	88.31^{c} ± 2.84	91.82^{b} ± 2.01	96.03^{a} ± 1.58
YS-2L (1.762)	97.73^{a} ± 1.49	96.46^{a} ± 1.95	91.85^{b} ± 2.17	87.16^{c} ± 2.91	92.24^{b} ± 2.40	97.51^{a} ± 1.53
MXLK (1.445)	93.35^{a} ± 1.65	91.60^{b} ± 2.38	89.06^{c} ± 3.19	82.05^{d} ± 3.53	88.42^{c} ± 2.98	91.57^{b} ± 2.11
87341 (2.040)	96.77^{a} ± 1.39	96.70^{a} ± 1.83	82.90^{c} ± 2.47	73.52^{d} ± 3.75	92.57^{b} ± 1.88	93.35^{b} ± 1.72

*values given in parenthesis are LSD of respective cultivars; **mean values with the same letters in the same species indicate non-significant difference, and means with different letters show significant difference at $P < 0.05$ level

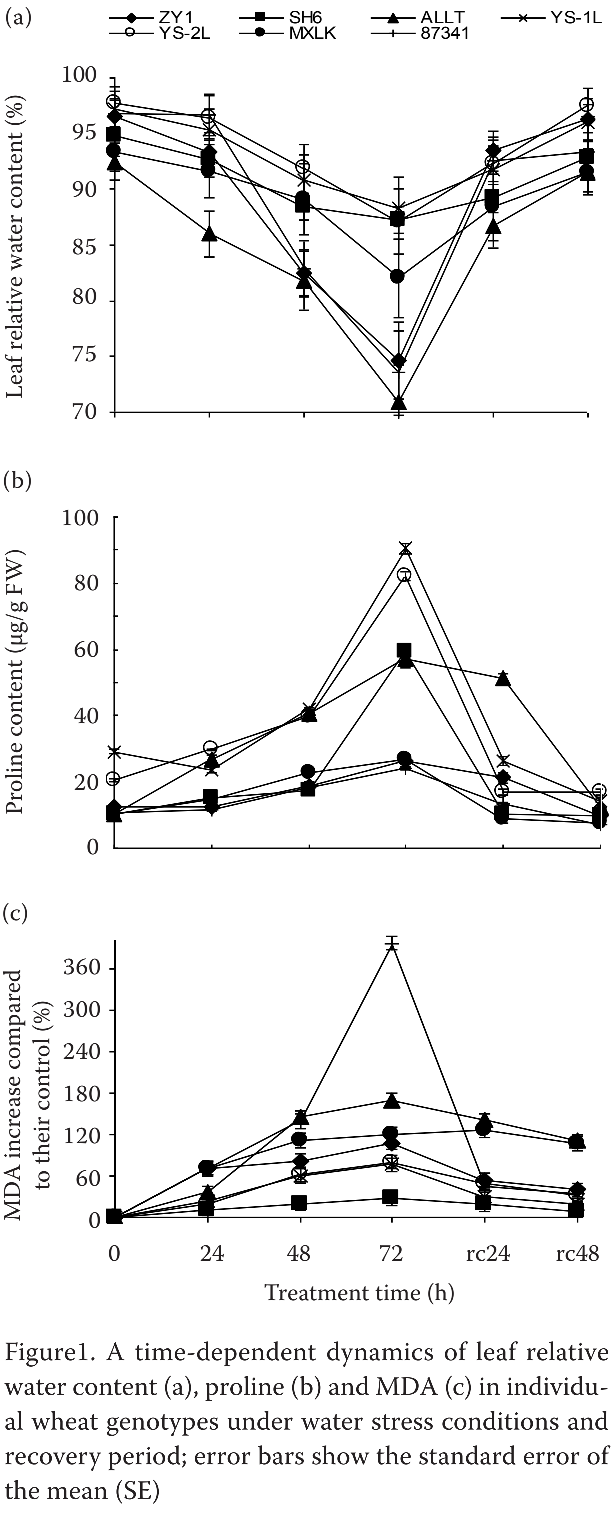

Figure1. A time-dependent dynamics of leaf relative water content (a), proline (b) and MDA (c) in individual wheat genotypes under water stress conditions and recovery period; error bars show the standard error of the mean (SE)

treated groups almost recovered to those of their controls at rc48 h with the exception of MXLK and 87341, which had lower leaf RWC than their control plants (Figure 1a).

Taken together, ALLT and YS-1L are considered as the most drought sensitive and tolerant wheat species, respectively, and the order of drought tolerance of these genotypes under water deficit conditions is ranked as follows:

YS-1L > YS-2L > SH6 > MXLK > ZY1 > 87341 > ALLT

Leaf proline content of the wheat species under water stress

All the species exhibited low free proline content under normal water supply except for YS-1L and YS-2L, which contain a high level of free proline content compared to other genotypes (Figure 1b). ALLT, YS-1L and YS-2L showed a significant increase in free proline content after 24 h of water stress, while SH6 and MXLK showed a lower increase, but ZY1 and 87341 had no significant increase. After 48 h water stress, almost all wheat species showed a significant rise in their free proline contents, but this rise was much higher in ALLT, YS-1L and YS-2L than in the remaining ones. SH6, YS-1L and YS-2L showed a dramatic increase in proline content under 72 h of water stress, while this increase was lower in ZY1, MXLK, 87341 at this time point (Figure 1b).

During the stress recovery period, ALLT, ZY1 and 87341 showed a lower decrease in proline content at rc24 h, whereas all other species showed a dramatic drop, especially SH6, YS-1L and YS-2L. At rc48 h, proline contents of all species decreased close to their control levels except those of YS-1L and YS-2L which were significantly lower than their control values (Figure 1b).

In general, the drought stress extensively enhances the level of proline in the leaves of all wheat species upon prolonged water deficit exposure, and this increase was much higher in the genotypes resistant to water stress than in genotypes sensitive to water stress.

Leaf MDA content of the wheat species under water deficit conditions

A greater increase in MDA was detected in ZY1, ALLT, MXLK and 87341 than in YS-1L and YS-2L under 24 h of water stress, whereas SH6 showed a lower rise in its MDA content (Figure 1c). All species exhibited a significant rise in their MDA content after 48 h of water stress except for SH6 which had no significant difference in MDA content at this time point, whereas this increase was much higher in ALLT, MXLK and 87341. At 72 h of water stress, SH6 showed the smallest rise in MDA, while 87341 showed the largest increase compared to all other species, and the increase of MDA content in YS-1L and YS-2L was not as high as in ZY1, ALLT and 87341 (Figure 1c).

The MDA accumulation levels of almost all species recovered back from stress at rc48 h except for ALLT and MXLK, which still had higher MDA levels compared with their control plants.

On the basis of these consequences, ALLT and YS-1L are considered as the most drought sensitive and tolerant species, respectively. The order of water stress tolerance according to lipid peroxidation membrane damage under water deficit conditions was as follows: SH6 > YS-1L > YS-2L > 87341 > ZY1 > MXLK > ALLT

DISCUSSION

Leaf RWC of all the wheat species declined during the water stress treatment, but YS-1L, YS-2L, and SH6 exhibited relatively higher leaf RWC than ALLT and 87341 as well as ZY1 did at each time point of water stress. This suggested that different species have different threshold levels to retain the leaf water status under water stress and normal water supply conditions. A similar trend under water stress condition was observed by some authors (Pirdashti *et al.* 2009; Bogale *et al.* 2011). YS-1L and YS-2L maintained the highest leaf water status while ALLT exhibited the lowest RWC among all species under water stress conditions. Different plant water levels, under irrigated and water stress conditions, were also reported by Tambussi *et al.* (2000) and Abbad *et al.* (2004).

The maintenance of stable water content is a prerequisite for the normal growth and high yield of plants. Under water stress, plants accumulate certain osmoprotective compounds which include proline, glycine-betaine, mannitol, sorbitol as well as carbohydrates. The proline accumulation observed in the present study provides additional evidence that increased proline levels constitute an adaptive response for plants during water stress. In this research severe drought stress caused a significant increase in proline amount. Our data showed that YS-1L had the highest, whereas 87341 had the lowest proline content compared to other genotypes at prolonged exposure to water stress, suggesting that YS-1L has better ability to maintain the good water status under drought conditions than the other wheat species. Under water deficit conditions, an increase of proline content in wheat and other plants was also reported by Zgallai *el al.* (2005), Vendruscolo *et al.* (2007), Tatar and Gevrek (2008), Johari-Pireivatlou (2010) and Maralian *et al.* (2010).

The cell membrane instability of wheat species under severe water deficit was detected by MDA accumulation levels in plants. In the present study, different species showed different levels of MDA content. ALLT and MXLK did not exhibit much higher MDA accumulation during water deficit conditions, but their MDA level could not recover back to the normal level after 48 h of stress recovery period, indicating that these two species have a lower ability to maintain the cell membrane integrity and to recover the damaged cell membrane. The species 87341 showed the highest MDA accumulation after 72 h of water stress, but it has a great ability to recover back after 48 h of recovery from stress. YS-1L and YS-2L had much lower MDA contents during the water stress period than ZY1, MXLK, ALLT and 87341. A similar trend was observed by many researchers (Fazeli *et al.* 2007; Lu *et al.* 2008; Moussa & Abdel-Aziz 2008; Bandurska & Jozwiak 2010). All of them showed that YS-1Li is the most drought tolerant genotype of wheat species investigated in this study.

CONCLUSION

Three physiological indices including leaf RWC, MDA accumulation and proline content were used to assess the drought tolerance of several *Triticum* L. species. The order of water stress tolerance of these species according to the three parameters studied is the following:
YS-1L > YS-2L > SH6 > 87341 > ZY1 > MXLK > ALLT

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