Phenotypic and Genotypic Analyses of Drought Tolerance in Korean and Tunisian Wheat Cultivars

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ABSTRACT Common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. subsp. *Durum*) are major staple food crops in the world. However, their production are limited by environmental stress such as drought. In order to evaluate wheat's response to drought, a total of 77 common wheat and durum wheat consisted of 19 Korean common wheat, 30 Tunisian common wheat and 28 Tunisian durum wheat were used in this study. Drought stress was applied for 21 days by suspending water application starting at the third leaf-expansion stage, followed by watering for the recovery of wheat until harvesting. Phenotypic parameters such as plant height, leaf length, tiller number, chlorophyll content, days to flowering and dry weight were scored during and after the treatment. Drought tolerance trait index (DTTI) and drought tolerance index (DTI) were calculated and used as criteria for selection of drought tolerance. At the end of treatment, most of the parameters except tiller numbers significantly decreased. Even after re-watering, plant height, leaf length, and dry weight continuously decreased. However, leaf chlorophyll content, and days to flowering of both stressed and non-stressed plants showed no significant differences. A total of 17 drought tolerance related simple sequence repeats (SSR) markers were used to identify genetic distance between Korean and Tunisian cultivars and elucidate possible use of marker systems for drought resistance. The common wheat and durum wheat cultivars formed different clusters for drought tolerance (resistance, moderate resistance, susceptible) using the SSR data. The results obtained in this study could help to increase genetic resources and breeding program for drought tolerance.

Keywords Common wheat, Drought tolerance, Durum wheat, SSR marker

INTRODUCTION

There is a current concern for future global food production using crops amidst an increasing world population (Brown & Funk 2008). Environmental problems such as drought, floods, and salinity have been reported to be more severe in some areas. In semi-arid areas, it is predicted that the grain yield of main cereal crops such as wheat and rice would decrease in the following 20 years (Lobell *et al.* 2008). To overcome these problems, crop improvements not only to increase yield but also to enhance tolerance to environmental stress are needed to improve global food security (Takeda & Matsuoka 2008).

Wheat (*Triticum* spp.) is a major staple food crop around the world (Munns *et al.* 2006). Common wheat (*T. aestivum*

L.) and durum wheat (*T. turgidum* L. subsp. *Durum*) are major species of cultivated wheat. Drought stress is one of the global problems that limit crop production (Pan *et al.* 2002). About 32% of the wheat cultivating regions in developing countries experience drought stress during the growing season (Rajaram 2001).

Microsatellites or simple sequence repeats (SSR) have been widely utilized in plants as molecular markers for specific phenotypic traits (Gupta & Varshney 2000). Marker-assisted selection is useful for improving drought tolerance in wheat (Quarrie *et al.* 2003). SSR markers can show the genetic diversity for drought stress in wheat (El Siddig *et al.* 2013).

Recent studies involving *in vitro* selection using polyethylene glycol (PEG) or mannitol applied to generate

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water stress in plants were performed to develop drought-tolerant plants (Rai *et al.* 2011). However, some results of *in vivo* and *in vitro* studies are not necessarily associated and had to be considered individually (Farshadfar *et al.* 2012). Further, plant responses to PEG treatment could not indicate the drought tolerance for the entire plant growth period. Drought stress is a limiting factor to plant growth and dry matter (Jaleel *et al.* 2009); therefore, morphological traits can be used as a criteria for drought tolerance.

Korea is located in East Asia. The winter climate is cold and dry, whereas it is hot and humid during summer. In Korea, wheat is cultivated as a winter crop. Accordingly, wheat is subjected to drought stress during its early stages of growth in Korea.

Tunisia is located in North Africa; it is bordered by the Mediterranean Sea on the north and east, whereas the Sahara desert is positioned to its south. The climate in the north is warm with dry summer climate (Mediterranean climate), whereas the climate in the mid-south is semi-arid, where soil moisture barely meets wheat seedling growth

and rainfall is low. Thus, in Tunisia, wheat experiences drought stress during its entire growing period.

The main objective of this study was to screen Korean common wheat, Tunisian common wheat, and Tunisian durum wheat cultivars for drought tolerance by using morphological traits and SSR markers to utilize them for future breeding programs.

MATERIALS AND METHODS

Plant materials and experimental conditions

A total of 77 cultivars (49 common wheat lines and 28 durum wheat lines) were used in this study. Of these, 19 lines were Korean common wheat, and 58 lines were Tunisian wheat. Seeds of Korean wheat cultivars were provided by the National Institute of Crop Science (NICS), Rural Development Administration (RDA) of Korea. The Tunisian seeds were obtained from the National Agrobiodiversity Center (NAC), RDA and the National Plant Germplasm

Table 1. The list of wheat cultivars used in this study.

Entry No.	Name	Species	Location	Supplier	Accession Number
1	Chunggyemil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-4 ²⁾
2	Olgeurumil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-9 ²⁾
3	Alchanmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-10 ²⁾
4	Gobunmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-11 ²⁾
5	Keumgangmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-12 ²⁾
6	Seodunmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-14 ²⁾
7	Saealmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-13 ²⁾
8	Jinpummil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-15 ²⁾
9	Joeunmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-17 ²⁾
10	Anbaekmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-18 ²⁾
11	Sinmichalmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-20 ²⁾
12	Jonongmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-22 ²⁾
13	Jokyoungmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-23 ²⁾
14	Younbaekmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-24 ²⁾
15	Sukangmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-29 ²⁾
16	Hanbaekmil	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-30 ²⁾
17	Soan	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-33 ²⁾
18	Dajung	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-36 ²⁾
19	Goso	<i>T. aestivum</i> L.	Korea	NICS, RDA	01-0006-35 ²⁾
20	ZAAFRANE	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205894 ³⁾
21	U14014	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205910 ³⁾
22	U14018	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205912 ³⁾
23	U14021	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205915 ³⁾
24	U14038	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205922 ³⁾
25	U14040	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205923 ³⁾

Table 1. Continued.

Entry No.	Name	Species	Location	Supplier	Accession Number
26	U14045	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205926 ^{y)}
27	U14046	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205927 ^{y)}
28	U14054	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205933 ^{y)}
29	U14057	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205935 ^{y)}
30	U14076	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 205938 ^{y)}
31	FRIGUI 2	<i>T. aestivum</i> L.	Tunisia	NAC, RDA	IT 229475 ^{y)}
32	U14010	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205908 ^{y)}
33	U14031	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205917 ^{y)}
34	U14050	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205929 ^{y)}
35	U14053	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205932 ^{y)}
36	U14055	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205934 ^{y)}
37	U14078	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205940 ^{y)}
38	SINLIKAT	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205941 ^{y)}
39	AGIM SINLIKA	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205943 ^{y)}
40	MELANGE	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205944 ^{y)}
41	BEDI	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NAC, RDA	IT 205947 ^{y)}
42	Allorca	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 41032 ^{x)}
43	Florence Aurore	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 150608 ^{x)}
44	Florence Aurore	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 150609 ^{x)}
45	Koudiat A2	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 174665 ^{x)}
46	Florence 135	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 185357 ^{x)}
47	Mahon 73	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 185372 ^{x)}
48	Mahon 124	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 185373 ^{x)}
49	Roussla	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 189775 ^{x)}
50	Cailloux	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 191446 ^{x)}
51	Mahon 73	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 191734 ^{x)}
52	Irakie 231	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 191738 ^{x)}
53	Mahon 124	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 191929 ^{x)}
54	Mahon 73	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 192024 ^{x)}
55	Cailloux	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 304915 ^{x)}
56	Baroota 52	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 410889 ^{x)}
57	Soltane	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 428416 ^{x)}
58	Ariana 66	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 519955 ^{x)}
59	BT 2288	<i>T. aestivum</i> L.	Tunisia	NPGS, USDA	PI 559656 ^{x)}
60	Huguenot Bariole 360	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 174646 ^{x)}
61	Jenah Retifah 24	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 184537 ^{x)}
62	Mekki 13	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 185194 ^{x)}
63	Sbei 7	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 185195 ^{x)}
64	Ble Dur 116	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 189772 ^{x)}
65	Ble Dur 870	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 189773 ^{x)}
66	Jenah Retifah 1	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 192516 ^{x)}
67	Jenah Retifah 24	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 192517 ^{x)}
68	Sbei 272	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 278383 ^{x)}
69	Mahmoudi	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 306573 ^{x)}
70	Inrat 69	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 324939 ^{x)}
71	Maghrebi	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 422313 ^{x)}
72	Roussia	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 428434 ^{x)}
73	Maghrebi 72	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 428462 ^{x)}
74	Amal 72	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 433749 ^{x)}
75	Badri	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 433751 ^{x)}
76	Maghrebi 72	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 433758 ^{x)}
77	Maghrebi 72	<i>T. turgidum</i> L. subsp. <i>Durum</i>	Tunisia	NPGS, USDA	PI 520062 ^{x)}

^{z)}The registration number of cultivar in Korea Seed & Variety Service (KSVS) (<http://www.seed.go.kr>).

^{y)}The IT accession number in the RDA-Genebank Information Center (<http://www.genebank.go.kr/>)

^{x)}The PI accession number in the ARS-GRIN (<http://www.ars-grin.gov/>).

System (NPGS), United States Department of Agriculture (USDA). Information about the Tunisian common wheat and durum wheat accessions was obtained from the RDA-Genebank Information Center (<http://www.genebank.go.kr/>) and the Agricultural Research Service-Germplasm Resources Information Network (ARS-GRIN) (<http://www.ars-grin.gov/>).

The experiment was conducted during the 2013 growing season at the greenhouse of the Research Farm of Korea University (Namyangju-si, Gyeonggi-do, Korea). Two seeds were planted per pot (5' 5' 16 cm) filled with soil (Sunshine mix #1). Immediately after emergence, seedlings were thinned out to let each plant grow in each pot. Five plants per line from each control and treatment groups were subjected to the test.

All plants were grown with sufficient watering until the drought treatment was initiated. Drought stress was imposed at the fully expanded third leaf stage by suspending water application, whereas plants in the non-stressed condition received sufficient water every two days. The treatment was applied for three weeks. After the treatment, all plants were provided with sufficient water and grown until spike harvest.

Morphological traits measurement

Phenotypic parameters such as plant height, average leaf length, number of tillers per plant, and leaf chlorophyll content were measured at three time points (the initiation of treatment, the end of treatment, and 28 days after the end of treatment). The first measurement was used for normalization, and the others were used in calculating the degree of recovery after drought stress. Days to flowering and shoot dry weight were also scored.

Leaf chlorophyll content was recorded by using a portable chlorophyll meter (SPAD-502, Minolta, Japan). SPAD-502 can measure leaf chlorophyll content in a fast and nondestructive way (Yıldırım *et al.* 2001). Drought tolerance trait index (DTTI) and drought tolerance index (DTI) were calculated using the formula of Shahzad *et al.* (2012) and Ali *et al.* (2007). If the mean DTTI for a trait was >100, it indicates that the trait would increase during drought stress. Thus, the reciprocal of DTTI was used to calculate DTI.

$$DTTI = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under non - stress condition}} \times 100$$

DTI = The average of DTTIs

Table 2. The list of SSR markers used in this study.

Name	F Primer	R Primer	Annealing Temperature (°C)
<i>Xbarc108</i>	GCGGGTCGTTTCCTGGAAATTCATCTAA	GCGAAATGATTGGCGTTACACCTGTTG	50
<i>Xbarc121</i>	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	50
<i>Xgwm573</i>	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACACTAC	50
<i>Xgwm130</i>	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	60
<i>Xgwm332</i>	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	60
<i>Xgwm635</i>	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	60
<i>Xwmc233</i>	GACGTCAAGAATCTTCGTCGGA	ATCTGCTGAGCAGATCGTGGTT	61
<i>Xwmc9</i>	AACTAGTCAAATAGTCGTGTCCG	GTCAAGTCATCTGACTTAACCCG	61
<i>Xwmc596</i>	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	61
<i>Xwmc603</i>	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	61
<i>Xwmc695</i>	GAGGGCACCTCGTAAGTTGG	GGCAGGAGCCCCCTACAAGAT	61
<i>Xwmc65</i>	TGGATGGGAAGGAGAATAAGTG	ATCCAACCGGAACCTACCGTCAG	61
<i>Xgwm260</i>	GCCCCCTGCACAAATC	CGCAGCTACAGGAGGCC	55
<i>Xgwm350</i>	ACCTCATCCACATGTTCTACG	GCATGGATAGGACGCCC	55
<i>Xgwm276</i>	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	55
<i>Xwmc17</i>	ACCTGCAAGAAATTAGGAACTC	CTAGTGTTTCAAATATGTCGGA	51
<i>Xwmc182</i>	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	51

DTI was calculated as the mean of DTTIs for morphological traits showing the tendency to drought stress and recovery. Based on DTI, drought tolerance of 77 common wheat and durum wheat cultivars was determined as resistant lines, moderate lines, and susceptible lines.

SSR marker analysis

A total of 17 SSR primers were used in the analysis (Table 2). Information on the markers was obtained from the Agricultural Research Service-GrainGenes 2.0 (<http://wheat.pw.usda.gov>). Polymerase chain reactions (PCRs)

were performed as described by Ciucă & Petcu (2009), with minor modification. PCR products were separated on agarose gel, and visualized and recorded using a gel documentation system.

The analysis was performed using the NTSYS-pc version 2.1 software using qualitative data for the similarity analysis, Sequential Agglomerative Hierarchical Nesting (SAHN) based on Unweighted Pair-Group Method with Arithmetic average (UPGMA) for the cluster analysis, and tree plot for the graphic visualization of dendrograms.

Fig. 1. Average plant height (A) and average leaf length (B) of Korean common wheat cultivars (a), Tunisian common wheat cultivars (b), and Tunisian durum wheat cultivars (c). Control (closed symbols) and treatment (open symbols).

RESULTS

Morphological traits measurement

Agronomic traits of both control (non-stressed) and stressed plants were measured at three time points: at the initiation of the treatment, end of treatment (21 days without watering), and 28 days after the end of treatment. Compared to the non-stressed plant, plant height, leaf length, leaf chlorophyll content, and shoot dry weight of the treated plants decreased (Fig. 1A, B, and 2). Despite re-watering, the gap of those parameters between control and treatment increased.

DTTI analysis

The magnitude of DTTI at the end of the treatment indicates the response of the plants to drought stress. At the end of the treatment, the mean DTTI for plant height was 88.3 ± 1.57 . Tunisian durum wheat cultivars showed the highest plant height ($88.7 \pm 2.18\%$), followed by the Korean common wheat cultivars and Tunisian common wheat cultivars (Table 3). The mean DTTI for average leaf length was $93.2 \pm 1.88\%$. Korean common wheat cultivars showed the highest leaf length ($94.7 \pm 5.49\%$) followed by the Tunisian common wheat cultivars and Tunisian durum wheat cultivars (Table 3). The mean DTTI for the number

Fig. 2. Average leaf chlorophyll content of Korean common wheat cultivars (a), Tunisian common wheat cultivars (b), and Tunisian durum wheat cultivars (c), and shoot dry weight of each cultivar (d).

Table 3. Drought tolerance trait indices (DTTIs) at 21 days after drought treatment.

Group	Plant Height (%)	Average Leaf Length (%)	No. of Tillers (%)	Leaf Chlorophyll Content (%)
Korean common wheat	88.5 ± 3.70	94.7 ± 5.49	109.1 ± 4.33	82.7 ± 4.57
Tunisian common wheat	87.9 ± 2.67	93.6 ± 2.50	98.3 ± 6.63	76.8 ± 2.17
Tunisian durum wheat	88.7 ± 2.18	91.9 ± 2.56	91.6 ± 4.39	68.6 ± 2.74
Total	88.3 ± 1.57	93.2 ± 1.88	98.5 ± 3.27	75.3 ± 1.82

of tillers was $98.5 \pm 3.27\%$. Korean common wheat cultivars showed the highest number of tillers ($109.1 \pm 4.33\%$) followed by the Tunisian common wheat cultivars and Tunisian durum wheat cultivars (Table 3). The mean DTTI for leaf chlorophyll content was $75.3 \pm 1.82\%$. Korean common wheat cultivars showed the highest leaf chlorophyll content ($82.7 \pm 4.57\%$), followed by the Tunisian common wheat cultivars and the Tunisian durum wheat cultivars (Table 3). Overall, DTTIs in most genotypes were $<100\%$, except for the number of tillers in Korean common wheat cultivars.

The magnitude of DTTI at 30 days after the end of the treatment indicates the ability of plants to recover from the drought stress. At 28 days after the end of the treatment, the mean DTTI for plant height was $84.3 \pm 2.04\%$. Tunisian common wheat cultivars showed the greatest plant height ($86.9 \pm 3.59\%$), followed by the Tunisian durum wheat cultivars and the Korean common wheat cultivars (Table 4). The mean DTTI for average leaf length was $75.5.2 \pm 2.60\%$. Tunisian common wheat cultivars showed the highest leaf length ($79.3 \pm 4.03\%$), followed by the Korean common wheat and the Tunisian durum wheat cultivars (Table 4). The mean DTTI for the number of tillers was $132.5 \pm 6.72\%$. Korean common wheat cultivars showed the highest number of tillers ($154 \pm 15.02\%$), followed by the Tunisian common wheat and the Tunisian durum wheat cultivars (Table 4). The mean DTTI for leaf chlorophyll

content was $100.2 \pm 1.24\%$. Tunisian durum wheat cultivars showed the highest leaf chlorophyll content (101.9 ± 2.14), followed by the Korean common wheat and Tunisian common wheat cultivars (Table 4). Compared to measurement at the end of the treatment, the mean DTTIs for plant height and average leaf length decreased, whereas those of the number of tillers per plant and leaf chlorophyll content increased.

For shoot dry weight, the mean DTTI was $84.2 \pm 2.22\%$; the Tunisian durum wheat cultivars showed the highest shoot dry weights, followed by the Korean common wheat cultivars and Tunisian common wheat cultivars (Table 5). For days to flowering, the mean DTTI was $101.0 \pm 0.35\%$ and the Tunisian durum wheat cultivars showed the highest values, followed by the Tunisian common wheat cultivars and Korean common wheat cultivars (Table 5). Most of the shoot dry weight in the treatment was lower than in the control, whereas the days to flowering in both the treatment and the control were almost same.

Calculation of DTI and determination of drought tolerance

The magnitude of DTI indicates a combined plant response using four parameters (plant height, average leaf length, leaf chlorophyll content, and dry shoot weight) and two stages (21 days after treatment and 28 days after the end of the treatment). The average DTI of 77 wheat cultivars

Table 4. Drought tolerance trait indices (DTTIs) at 28 days after re-watering.

Group	Plant Height (%)	Average Leaf Length (%)	No. of Tillers (%)	Leaf Chlorophyll Content (%)
Korean common wheat	82.1 ± 4.21	76.1 ± 5.8	154 ± 15.02	100.3 ± 2.83
Tunisian common wheat	86.9 ± 3.59	79.3 ± 4.03	105.6 ± 8.24	98.5 ± 1.74
Tunisian durum wheat	83.1 ± 2.99	70.9 ± 4.11	146.8 ± 11.03	101.9 ± 2.14
Total	84.3 ± 2.04	75.5 ± 2.6	132.5 ± 6.72	100.2 ± 1.24

Table 5. Drought tolerance trait indices (DTTIs) for shoot dry weight and days to flowering.

Group	Shoot Dry Weight (%)	Days to Flowering (%)
Korean common wheat	83.2 ± 6.09	100.5 ± 1.00
Tunisian common wheat	76.8 ± 3.04	100.9 ± 0.41
Tunisian durum wheat	92.7 ± 2.39	101.5 ± 0.51
Total	84.2 ± 2.22	101.0 ± 0.35

was 85.5%. Based on the magnitude of DTI, drought tolerance of the 77 wheat cultivars was determined as resistant lines (above 90%), moderate lines (81–90%), and susceptible lines (below 80%). From the 77 cultivars, 23 lines were resistant, 33 lines were moderate, and 21 lines were susceptible (Table 6). Four cultivars that showed >100% DTI were selected as the most resistant lines (Table 7).

SSR analysis

SSR analysis was performed to identify markers for drought tolerance. A dendrogram was constructed from the cluster analysis of similarity coefficients of SSR markers (Fig. 3). A total of 77 cultivars were classified into two groups: group A (comprised of Korean cultivars) and group

B (composed of Tunisian cultivars). Groups A and B were further categorized into three clusters each. Cluster A-1 is comprised of moderate resistant and resistant lines and had average DTI of $90.9 \pm 4.45\%$. The average DTI of cluster A-2 was $72.4 \pm 5.52\%$, which is comprised of mostly susceptible lines and a few moderate lines. The average DTI of cluster A-3 was $92.5 \pm 2.88\%$, which is comprised of mostly resistant lines and a few moderate lines. The average DTI of cluster B-1 was $92.5 \pm 1.96\%$, composed mostly of resistant lines and a few of moderate lines. The average DTI of cluster B-2 was $89.7 \pm 1.38\%$, consisted of resistant lines and moderate lines. The average DTI of cluster B-3 was $80.1 \pm 1.15\%$, consisted of of susceptible lines and moderate lines.

Table 6. Drought tolerance grouping of wheat cultivars based on drought tolerance index (DTI).

Drought Tolerance Category	Range of DTI	No. of Cultivars	Entry No. (Name)
Resistant	>90%	23	1 (Chunggyemil), 4 (Gobunmil), 5 (Keumgangmil), 14 (Younbaekmil), 16 (Hanbaekmil), 17 (Sooan), 18 (Dajung), 19 (Goso), 22 (U14018), 23 (U14021), 26 (U14045), 27 (U14046), 31 (FRIGUI 2), 32 (U14010), 34 (U14050), 35 (U14053), 36 (U14055), 37 (U14078), 39 (AGIM SINLIKA), 40 (MELANGE), 41 (BEDI), 42 (Allorca), 45 (Koudiat A2)
Moderate	80–90%	33	3 (Alchanmil), 8 (Jinpummil), 10 (Anbaekmil), 11 (Sinmichalmil), 12 (Jonongmil), 13 (Jokyoungmil), 15 (Sukangmil), 20 (ZAAFRANE), 21 (U14014), 24 (U14038), 25 (U14040), 30 (U14076), 33 (U14031), 38 (SINLIKAT), 47 (Mahon 73), 48 (Mahon 124), 49 (Roussla), 50 (Cailloux), 54 (Mahon 73), 55 (Cailloux), 56 (Baroota 52), 57 (Soltane), 60 (Huguenot Bariole 360), 61 (Jenah Retifah 24), 62 (Mekki 13), 63 (Sbei 7), 64 (Ble Dur 116), 65 (Ble Dur 870), 66 (Jenah Retifah 1), 68 (Sbei 272), 71 (Maghrebi), 75 (Badri), 76 (Maghrebi 72)
Susceptible	<80%	21	2 (Olgeurumil), 6 (Seodunmil), 7 (Saealmil), 9 (Joeunmil), 28 (U14054), 29 (U14057), 43 (Florence Aurore), 44 (Florence Aurore), 46 (Florence 135), 51 (Mahon 73), 52 (Irakie 231), 53 (Mahon 124), 58 (Ariana 66), 59 (BT 2288), 67 (Jenah Retifah 24), 69 (Mahmoudi), 70 (Inrat 69), 72 (Roussia), 73 (Maghrebi 72), 74 (Amal 72), 77 (Maghrebi 72)

Table 7. The most resistant cultivars to drought stress.

Entry No.	Name	Group	DTI (%)
14	Younbaekmil	Korean common wheat	104.7
34	U14050	Tunisian durum wheat	102.6
42	Allorca	Tunisian common wheat	113.6
45	Koudiat A2	Tunisian common wheat	106.8

DISCUSSION

This study demonstrates that wheat cultivars are sensitive to drought treatment. Plant height, average leaf length, and leaf chlorophyll content decreased, whereas the number of tillers did not change. After re-watering, plant height and average leaf length continued to decrease, whereas leaf chlorophyll content showed similar values to that of the control and the number of tillers increased. Shoot dry weight also decreased, whereas days to flowering did not

change. SSR analysis showed the genetic differences between the Korean and Tunisian cultivars based on drought tolerance.

Based on morphological trait measurement, the Korean common wheat and Tunisian common and durum wheat cultivars were affected by drought stress. Plant height and average leaf length did not recover fully, decreasing at the end of treatment and at 28 days after the end of treatment. Further, drought stress resulted in a reduced shoot dry weight. Thus, drought stress may result in the inhibition of

Fig. 3. SSR-based genetic relationship among 77 wheat cultivars.

growth. According to Zhu (2001), water stress inhibits plant growth by inhibiting cell division. However, the leaf chlorophyll content of the drought-stressed plants decreased at the end of treatment, whereas it increased to levels similar to that of the control at 28 days after the end of the treatment. This indicates that the chlorophyll content of the newly produced leaves after re-watering in the treatment condition was similar to that in the control condition.

In terms of the number of tillers, Korean common wheat cultivars increased at both the end of treatment and 28 days after the end of treatment, whereas that of the Tunisian common wheat and durum wheat cultivars decreased at the end of treatment. According to Veesar *et al.* (2007), Indus-66, which is a more tolerant wheat genotype to water stress than other genotypes, formed a lower number of tillers. In addition, Castillo *et al.* (2007) reported that the number of non-productive tillers, which developed after restoration, increased with osmotic stress in rice. There might be a negative relationship between the number of non-productive tillers and drought tolerance.

Further, there was no clear difference between the control and treatment in terms of days to flowering, indicating that the number of tillers and days to flowering were not affected by drought stress. According to Foulkes *et al.* (2002), flowering date was typically neutral to drought stress.

Anjum *et al.* (2011) reported that plant height, stem diameter, and leaf area decreased under drought stress

condition. These factors are related to average leaf length, and shoot dry weight. Further, there was a positive correlation between chlorophyll content (SPAD) and high transpiration efficiency, wherein SPAD could be utilized as an indicator of drought tolerance (Fotovat *et al.* 2007). Therefore, the calculation of DTI with morphological traits such as plant height, average leaf length, dry weight, and leaf chlorophyll weight can reflect drought tolerance.

Korean wheat and Tunisian wheat showed different DTIs at the end of treatment and 28 days after the end of treatment (Fig. 4). The DTI of Korean wheat cultivars was higher at the end of treatment than at 28 days after the end of treatment. However, the DTI of Tunisian common wheat and durum wheat cultivars were lower at the end of treatment than at 28 days after the end of treatment. This indicates that the tolerance to drought stress and the ability to restore must be considered separately. Costa *et al.* (1997) reported that the stress index between continuous stress and transient stress was different. Therefore, separate strategies should be used for these cultivars to improve drought tolerance.

The dendrogram showed distant relationship between the Korean and Tunisian cultivars (Fig. 3). Environmental conditions could affect genetic diversity (Li *et al.* 2012). Consequently, the genetic distance of Korean common wheat and Tunisian common wheat and durum wheat reflects the environmental differences between Korea and Tunisia.

Fig. 4. Drought tolerance index (DTI) at the end of treatment and at 28 days after the end of treatment.

In Tunisian cultivars, common wheat and durum wheat were completely mixed within the clusters, indicating that the SSR markers used in this study can be applied to both common wheat and durum wheat. In addition, both clusters A and B were categorized into some groups with drought tolerance. This suggests that the cluster analysis using SSR markers can be useful in evaluating drought tolerance in common wheat and durum wheat.

In conclusion, morphological traits and SSR markers could be used in evaluating drought tolerance in Korean and Tunisian wheat cultivars. It is necessary to establish an accurate standard to effectively evaluate tolerance to drought stress for wheat improvement. The results obtained in this study could help increase genetic resources and improve breeding programs for drought tolerance in wheat.

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