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Physiological responses and effective selection criteria of drought tolerant plants

Authors Info

D.J. Park^{1,2}, H.J. Im³, H.G. Kim²,
W.H. Yang², S.H. Yong²
and M. S. Choi^{1,2*}

¹Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, The Republic of Korea

²Division of Environmental Forest Science, Gyeongsang National University, Jinju 52828, The Republic of Korea

³Plant Propagation and Reproduction Division, Baekdudaegan National Arboretum, Bonghwa 36209, The Republic of Korea

*Corresponding Author Email : mschoi@gnu.ac.kr

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Abstract

Aim : The present study was carried out to establish the method for screening drought tolerant Korean native plants and to analyze the appearances of drought tolerant Korean native plants on drought stress.

Methodology : Twelve Korean native plant species (*Scilla scilloides*, *Commelina communis*, *Hemerocallis fulva*, *Bupleurum latissimum*, *Hosta plantaginea*, *Iris dichotoma*, *Lysimachia barystachys*, *Viola mandshurica*, *Amphicarpaea bracteata*, *Aeschynomene indica*, *Dianthus chinensis* and *Urtica thunbergiana*), which were 16-week-old seedlings, were exposed to drought stress by stopping irrigation to observe phenotypic, physiological and biochemical responses.

Results : Based on the withering rates under drought condition, the plants were classified into three groups; drought tolerant (*S. scilloides*, *C. communis*, *H. fulva*, *B. latissimum*, *H. plantaginea* and *I. dichotoma*), normal (*L. barystachys*, *V. mandshurica* and *A. bracteata*) and sensitive (*A. indica*, *Viola chinensis* and *U. thunbergiana*) group. While chlorophyll contents of the plants were generally decreased in the drought condition, the extents of decrement in the sensitive group were larger than in the tolerant group. RWCs in the tolerant group were higher than in the other groups on the same periods of drought condition. Proline and soluble reducing sugars accumulated in the sensitive group much earlier than in the tolerant group.

Interpretation : The results revealed that *S. scilloides*, *C. communis*, *H. fulva*, *B. latissimum*, *H. plantaginea* and *I. dichotoma* were screened as drought tolerant plants and the established method in the study can be applied to screen drought tolerant plants for Korean natives.

Introduction

Climate changes caused by global warming often provide forest plants with serious drought condition. Rises in temperature increase water loss by evaporation from soils (Helfer *et al.*, 2012). In a previous study, each 1°C increase in soil temperature increased evaporation rate up to 10% (Kidron and Kronenfeld, 2016). Moreover, changes in rainfall mainly cause drought conditions. Decrease in rainfall is the main cause of drought condition in specific areas. *La Nina* and *El Nino*, for example, have been indicated as the reasons of drought in North America and East China, where rainfall has reduced (Dai, 2011). Heavy rains also function for triggering floods and landslides. And then these natural disasters cause drought conditions by removing vegetation. These effects of climate change devastate the earth and accelerate desertification.

In South Korea, areas of devastated lands in forests for developments have increased (Woo and Jeon, 2005; Yim *et al.*, 1999). Landslides are one of the main cause of devastated lands. The areas of landslides in South Korea has increased up to 713 ha in the 2000s from 289 ha in 1970s (Korea Forest Service, 2016). In addition, it has been suggested that land development in South Korea has increased devastated lands in the forest (Kim *et al.*, 2013; Park *et al.*, 2015). Therefore, efficient methods for recovering devastated lands are required (Sim and Kim, 2006). Methods using plants has been suggested to recover devastated lands (Kim *et al.*, 2008; Jeon, 2002). Because of extreme environmental conditions in the devastated lands, the use of environmental stress tolerant plants have been suggested (Atif, 1988). Some introduced plant species such as weeping lovegrass, rudbeckia and tall fescue are usually used for recovering the devastated lands (Kil *et al.*, 2011; Park *et al.*, 2009). However, it is concerned that using few number of plants species inhibits biodiversity. Therefore, various drought tolerant native plants are demanded. Nevertheless, suitable plant species have not been reported even though the devastated lands constrain drought condition.

Most plants undergo water deficiency when supplying water to root system is inhibited or when transpiration in the plants is actively carried out. Several studies have reported plants' physiological and metabolic response to drought stress. Physiological parameters such as relative water content, chlorophyll content have been used as key indicators of drought stress (Soltys-Kalina *et al.*, 2016; Ganji Arjenaki *et al.*, 2012; Mafakheri *et al.*, 2010; Arunyanark *et al.*, 2008). Furthermore, for observing metabolic responses to drought stress, proline and total reducing sugar contents are determined (Wu *et al.*, 2014; Naser *et al.*, 2010). However, these studies were conducted mainly based on model plants and crops.

Drought tolerant plants refer to plants having high adaptability to drought stress, which is considered as an important trait for crops productivity. Several studies have been carried out to screen drought tolerant rice (Kumar *et al.*, 2014;

Sakai *et al.*, 2010; Barba Jr. *et al.*, 2014) and cotton (Longenberger *et al.*, 2006; Ullah *et al.*, 2017). Korean native drought plants need to be screened and planted in forests to enhance the adaptability of forests against climate change and efficiency for recovering devastated lands. In the view of above study was carried out to establish a method for screening Korean native drought tolerant plants and to observe their responses to drought stress.

Materials and Methods

Plant materials and drought stress treatment : To test tolerance to drought stress, twelve Korean native plant species were collected from Gyeongsangnam-do Forest Environment Research Institute in Jinju, Korea (Table 1). All plant species were reconfirmed by using the Korean plant name index (Lee, 2003), and Nature system on Korean National Arboretum (www.nature.go.kr). Collected plants were stabilized grown in a greenhouse in Gyeongsang National University for 30 days. Plant species which showed a similar growth were then selected to avoid experimental errors and used for further experiments. Before the Korean native plants were exposed to drought condition, their growth features such shoot length, leaf number, leaf width and leaf length and thickness were measured (Table 1). The withering rates, as phenotypic response of the plants, were observed for 60 days under drought condition, which was initiated by stopping irrigation.

Chlorophyll content : The chlorophyll content in the leaf of explant was determined with a chlorophyll meter (SPAD-502, Spectrum Technologies Plainfield, IL). Five sample leaves of each species grown in drought condition were measured. The chlorophyll contents were measured at midday (11:00 - 13:30 hr) at a 6 day interval for 4 weeks.

Relative water content and relative water loss : Relative water content (RWC) was determined according to the method of Turner *et al.* (1986). Fresh leaves were weighed immediately to record fresh weight (f.wt.). They were then placed in distilled water for 4 hrs and weighed again to record turgid weight (TW). After that, they were subjected to dry oven at 70°C for 24 hrs to record dry weight (d.wt.). Relative water loss (RWL) was measured as follows : The leaf samples were weighed, wilted for 4 hrs at 35°C, reweighed (W 4 hr) and oven dried for 24 hrs at 72°C to obtain dry weight. The RWC and RWL were measured at 6 days interval for 3 weeks and calculated by the following formula :

$$\text{RWC (\%)} = \frac{[(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100\%}{\dots\dots\dots(1)}$$

$$\text{RWL (\%)} = \frac{[(\text{FM} - \text{W 4hr})/(\text{FW} - \text{DW})] \times 100\%}{\dots\dots\dots(2)}$$

Proline contents analysis : Proline was determined as described by Bates *et al.* (1973). Fresh leaf tissues (0.05 g) were extracted mixed with 5 ml aqueous sulfosalicylic acid (3% w/v). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent and incubated at 100°C for one hr. The reaction mixture was vigorously mixed with 2 ml toluene in an ice bath. The chromophore was measured with a spectrophotometer DR/4000 at 520 nm. The concentration of

proline was calculated from a calibration curve plotted with known concentration of proline as standard.

Estimation of soluble reducing sugar : The soluble reducing sugar was estimated following the DNS (3,5-Dinitrosalicylic acid) method of Miller (1972). Fresh leaf tissues (0.1g) were extracted in 5 ml distilled water at 100°C for 30 min. The extracted solution reacted with 1.0 ml DNS (3,5-Dinitrosalicylic acid) were mixed and then treated in boiling water for 5 min and the absorbance was measured with a spectrometer DR/4000 at 546 nm. Various concentration (50, 100, 150 and 200 µg ml⁻¹) of glucose was measured for calibration curve with “Y=0.2643x-0.254 (R²=0.9893)”.

Statistical analysis : The growth characteristics and chlorophyll content, RWCs, RWLs, proline content, sugar content and the mortality for the twelve plant species were analyzed for significance by one-way ANOVA using the IBM SPSS statistical package and the Duncan multiple range test (Table 1, 2). The correlations among physiological and biochemical factors related to drought tolerance were determined by regression analysis. All of the statistical analysis conducted under 0.05 of significance level.

Results and Discussion

The phenotypic response of each plant material under non-irrigation condition for 60 days was observed to establish the method for screening native Korean drought tolerant plants. The withering rates of each plant species were differently shown although they all increased with duration (Fig. 1). After monitoring them, twelve Korean native plants were divided into three groups: tolerant group, normal group and sensitive group. The tolerant group refers to plant species showing low withering rate than 50% in 30 days with no watering. In the tolerant group, *S. scilloides*, *C. communis*, *H. fulva*, *H. plantaginea* and *I. dichotoma* were

included. The normal group, which showed lower withering rates than 50% in 20 days under drought condition included *L. barystachys*, *V. mandshurica* and *A. bracteata*. Three plant species (*A. indica*, *D. chinensis* and *U. thunbergiana*) in the sensitive group showed higher withering rate than 50% in 20 days. These significant differences in phenotypic response under drought stress suggests that tolerant abilities of Korean native plants vary. Similar results were obtained in the research that 'Katahdin', a drought tolerant genotype of potato, showed higher yields than others under drought condition by stopping watering (Soltys-Kalina *et al.*, 2016). Therefore, the method to treat drought by stopping watering can be used to screen drought tolerant plants. Continuously, it was required to investigate which physiological and metabolic features are shown in the plants of each group at following experiments.

The chlorophyll content on drought stress were determined by measuring SPAD values to monitor how much the native plants maintain their healthiness and to know whether the changes in chlorophyll content of each native plants are related to withering rates (Fig. 2). Even though a decrease in SPAD values were observed in all plants, the change extents in each plant were different (Fig. 2A). The difference among the Korean native plants is shown in twelve days without watering (Fig. 2 B). Among the SPAD values of the plants in the tolerant group, relatively fewer changes were shown than the other groups. There were differences in the tolerance indexes (SPAD value on after dry/SPAD value on before dry) between the tolerant and sensitive groups (Fig. 2 C). While the tolerance indexes of the sensitive group were lower than 70%, those of the tolerant group were higher than 90% except *C. communis* (85.1%). Because the higher SPAD values correspond to higher chlorophyll contents, this result means that the plants in the tolerant group maintained their health on the drought condition. El-Tayeb (2006) reported that the reduction in total chlorophyll content in plantlets of *Vicia faba* was observed under water stress conditions. Arunyanark *et al.* (2008) revealed that there were

Table 1 : The lists of Korean native plant species tested for drought tolerance and their growth features in four weeks

Plant species	Family	Shoot length (cm)	Leaf number (No.)	Leaf width (cm)	Leaf length (cm)	Leaf thickness (mm)
<i>Scilla scilloides</i>	Liliaceae	23.04 ^{e*}	5.80 ^m	0.64 ^h	21.02 ^c	0.34 ^c
<i>Commelina communis</i>	Commelinaceae	28.62 ^e	23.60 ^a	2.06 ^{efg}	8.14 ^e	0.23 ^{gh}
<i>Hemerocallis fulva</i>	Asphodelaceae	29.08 ^b	7.40 ^k	0.62 ^h	21.71 ^b	0.25 ^f
<i>Bupleurum latissimum</i>	Umbelliferae	8.90 ⁿ	3.40 ^o	2.34 ^{def}	3.08 ⁿ	0.24 ^{fg}
<i>Hosta plantaginea</i>	Liliaceae	7.16 ^{op}	3.60 ^o	2.72 ^{cde}	5.24 ^{jk}	0.21 ^h
<i>Iris dichotoma</i>	Iridaceae	26.78 ^d	6.00 ^m	0.54 ^{efg}	24.24 ^a	0.23 ^{gh}
<i>Lysimachia barystachys</i>	Primulaceae	21.32 ^f	6.64 ^l	2.20 ^{efg}	5.20 ^j	0.91 ^a
<i>Viola mandshurica</i>	Violaceae	11.56 ⁱ	11.20 ^g	3.42 ^{bc}	5.20 ^{jk}	0.13 ^m
<i>Amphicarpaea bracteata</i>	Fabaceae	11.84 ⁱ	10.00 ^h	2.90 ^{bcd}	4.34 ^k	0.17 ^k
<i>Aeschynomene indica</i>	Fabaceae	29.00 ^b	18.00 ^d	1.24 ^{gh}	3.88 ⁱ	0.39 ^b
<i>Dianthus chinensis</i>	Caryophyllaceae	5.62 ^q	19.20 ^c	1.45 ^{gh}	4.98 ^j	0.22 ^{gh}
<i>Urtica thunbergiana</i>	Urticaceae	13.42 ^j	17.80 ^d	3.94 ^{ab}	5.44 ⁱ	0.14 ^{lm}

*Different letters indicate significant differences Ducun's multiple range test (DMRT) at p<0.05

differences in reduction of chlorophyll content depending on the genotype of peanuts. Therefore, this result indicates that the plants in the tolerant group could maintain their healthiness on the drought condition. In addition, it can be implied that the drought tolerant plants can keep their photosynthesis even under drought conditions, or they can photosynthesize immediately since their growing conditions are improved, at least.

RWC and RWL are related to drought tolerance and these parameters have also been proposed as more important indicators of water status in plants such as wheat, potato and peanut. (Farshadfar and Ghasem, 2015; Keles et al., 2004; Soltys-Kalina et al., 2016; Arunyanark et al., 2008). These previous studies allow us to measure the water status parameters on drought stress. It was found that drought stress generally affects RWC and RWL in the Korean native plants (Fig. 3). While observing water status for 24 days, all plants in the tolerant group showed higher RWCs than 50% after 18 days of drought stress (Fig. 3 A). On the other hand, RWCs of all plants in the sensitive group and *L. brtstachts*, *V. mandshurica* and *A. bracteata* in the normal group reached 0% in the same period (Fig. 3 B-C). On the

final day, the highest RWC was observed in *H. fulva* (86.52%), followed by *S. Scilloides* (85.44%), *H. plantaginea* (70.63%), *I. dichotoma* (70.54%), *B. latissimum* (55.44%) and *C. communis* (45.00%) in the tolerant group. Only RWCs of *A. acerifolia* among the normal group showed 31.55%. Similar results on water status of the native plants were shown in measurements of RWLs (Fig. 3 D-F). The RWLs of plants in the tolerant group were lower than 50% in the monitoring period. The lowest RWL on 24th day was found in *B. latissimum* (30.52%) followed by *I. dichotoma* (35.50%), *C. communis* (35.20%), *H. plantaginea* (41.39), *S. scilloides* (42.86%) and *H. fulva* (47.14%) in the tolerant group. It means that no plant species totally lost their water on drought stress. However, RWLs of all plants in both normal and sensitive group, except *A. acerifolia*, reached 100% after 24 days of drought stress. The RWL of *A. acerifolia* was 65.15%, which was higher than RWLs of the plant species in the tolerant group. Considering the results of RWC and RWL together, higher RWCs corresponded to lower RWLs. Therefore, the study revealed that the plants in the tolerant group can comparatively maintain their water status under drought stress because high RWC and lower RWL indicates higher potential to hold water in order to survive in drought conditions.

Fig. 1 : Screening drought tolerant Korean native plants. The plants of each species were exposed to drought condition by stopping irrigation and their withering rates were observed for 60 days. Each plant species were grouped into (A) tolerant; (B) normal and (C) sensitive groups, respectively, depending on withering rates

Fig. 2: Changes in chlorophyll content by drought stress treatment. (A) SPAD values of each Korean native plants on drought condition; (B) The SPAD values in 12 days and (C) The tolerant indexes in 12 days. The SPAD values indicate chlorophyll contents. The SPAD values, which indicate the chlorophyll contents, were measured using SPAD device. The SPAD values were obtained from leaves of each plant species every 6 day for 30 days on drought condition

Proline has an osmolytic role for osmotic adjustment to drought stress (Levy, 1983). Irigoyen *et al.* (1992) have reported that the sugar contents in plant leaves can increase under drought conditions. Therefore, proline and soluble sugar contents were determined to understand how much stress by drought are accumulated in each plant species. The proline and soluble reducing sugar contents in all plant species increased with increase in drought condition (Fig. 4). However, differences in the accumulation of proline and soluble reducing sugar was observed among the groups.

The proline content was stable up to 27 days in the tolerant group (Fig. 4 A). The proline content of *H. fulva* increased from 0.958 mg/0.05 g f.wt. on the first day to 1.069 mg/0.05 g f.wt. in 30 days. This change in proline content of *H. fulva* was most stable in the tolerant group. Even though the proline content of *I. dichotoma* increased from 0.973 to 3.584 mg/0.05 g f.wt. in 30 days, the increase was significant in 27 days. In control group, the proline content of most plants was found to accumulate from 18th days, except in *A. bracteata* (Fig. 4 B). The highest increase was found in *V. mandshurica* on the first day. It was found that proline

Fig. 3 : Related water content and related water loss by drought stress treatment. By collecting leaves in every 6 days on drought condition, the RWCs and RLWs were obtained. (A)-(C) RWC values and (D)-(F) RWL values

in the sensitive group began to accumulate earlier compared to the plants in the other groups (Fig. 4 C). Among all plants in the sensitive group, the proline content in *U. thunbergiana* rapidly accumulated between 6th and 9th day. These results indicate that the drought tolerant plants accumulated relatively low proline on drought conditions.

The soluble reducing sugar content in *B. latissimum*, *I. dichotoma* and *C. communis* in the tolerant group were stable (Fig. 4 D). Although increase in the soluble reducing sugar contents was observed in *S. scilloides* (24th day), *H. fulva* (24th day) and *H. plantaginea* (27th day), the days were later than the

other groups. In normal group, the soluble reducing sugar content of *L. brystachys* increased from the 3rd day and was maximum on the 18th day (Fig. 4 E). *V. mandshurica*, the soluble reducing sugar accumulated rapidly from 15th day. Although slight increase was noted, the soluble reducing sugar was accumulated in *A. acerifolia* (12 days), *A. bracteata* (15 days) earlier than in plants of the tolerant group. In the sensitive group, the soluble reducing sugar in *A. indica* began to accumulate from 6th day and its maximum content was shown in 15 days (Fig. 4 F). Even though they increased slightly, the soluble reducing sugar in *U. thunbergiana* (6 days) and *D. chinensis* (15 days) increased earlier than the plants in the other groups. These results indicate

Fig. 4 : Changes in proline and reducing sugars content by drought stress treatment. The contents of proline (A)-(C) and soluble reducing sugar (E)-(F) on drought condition were measured to comparatively examine the extents of stress, which are accumulated in each plant species

that the soluble reducing sugar accumulated in reverse order to drought tolerant abilities of plants.

Similar reports were previously reported in bell pepper, cotton, wheat (Nath *et al.*, 2005; Ronde *et al.*, 2000; Hamada, 2000) and in *Populus* clones (Irigoyen *et al.*, 1992; Arabzadeh, 2012; Gebre *et al.*, 1997). Therefore, these results imply that comparatively lower stress is given to the plants in the tolerant group even though the plants are exposed to same drought conditions. The chlorophyll content, RWCs, RWLs, proline content, sugar content and the mortality of twelve species were examined using repeated measures variance analysis (Table 2). As a result, the plants can be divided into eight groups based on

SPAD values. *L. brystachys*, *S. scilloides* and *I. dichotoma* were grouped high SPAD values species whereas *A. bracteata*, *A. indica* and *D. chinensis* were classified into the lower SPAD values species. In the case of RWCs, eight groups were divided and, the higher RWC species were *L. brystachys*, *S. scilloides* and *I. dichotoma*.

The correlation analysis was performed to discover a correlation between physiological and metabolic factors. RWCs and proline contents were in a negative correlation as showing the Pearson correlation coefficient of -0.625. This result means that plants with higher proline contents show lower RWCs. Between SPAD values and sugar contents, a positive correlation was shown

Table 2 : Chlorophyll content, RWC, RWL, proline contents, sugar contents and mortality of twelve plant species

Species	SPAD values	RWC	RWL	Proline content	Soluble reducing sugar content	Withering rates (%)
<i>B. latissimum</i>	25.41±3.4 ^{fg*}	79.09±22.26 ^{fg}	25.64±4.97 ^{cde}	1.08±0.2 ^h	1.61±0.37 ^{gh}	44.38±45.57 ⁱ
<i>C. communis</i>	31.5±9.85 ^{de}	76.51±24.43 ^{de}	22.92±10.19 ^e	1.03±0.19 ⁱ	1.27±0.53 ^{gh}	22.69±34.4 ⁱ
<i>H. fulva</i>	35.59±3.32 ^{cd}	96.00±5.54 ^{cd}	30.38±10.64 ^b	1.02±0.04 ⁱ	4.16±2.84 ^c	36.24±44.31 ^k
<i>H. plantaginea</i>	24.36±4.42 ^{fg}	84.53±12.41 ^{fg}	27.63±14.34 ^{bc}	1.2±0.37 ^g	3.52±2.75 ^d	48.72±46.42 ^h
<i>I. dichotoma</i>	36.55±4.65 ^c	86.23±10.92 ^c	28.76±5.76 ^{bc}	1.46±0.97 ^e	1.16±0.46 ^h	41.75±48 ^j
<i>S. scilloides</i>	44.44±2.58 ^b	93.84±5.94 ^b	22.68±13.38 ^e	1.26±0.48 ^f	3.95±2.29 ^{cd}	15.08±27.61 ^m
<i>A. acerifolia</i>	27.6±6.27 ^{ef}	72.88±25.78 ^{ef}	38.73±14.65 ^a	1.29±0.18 ^f	2.09±0.67 ^e	57.33±44.47 ^g
<i>A. bracteata</i>	24.06±9.84 ^{fg}	56.01±47.94 ^{fg}	23.87±24.18 ^{de}	1.00±0.04 ⁱ	1.67±0.61 ^{efg}	69.19±41.72 ^d
<i>L. barystachys</i>	58.74±9.44 ^a	47.05±44.51 ^a	26.37±26.63 ^{cd}	2.05±1.27 ^c	6.81±2.42 ^a	68.94±45.53 ^e
<i>V. mandshurica</i>	32.36±8.6 ^{cd}	49.67±45.92 ^{cd}	22.42±19.08 ^e	1.72±0.88 ^d	4.67±3.53 ^b	64.45±43.2 ^f
<i>A. indica</i>	22.89±4.82 ^{gh}	54.69±47.12 ^{gh}	19.22±25.29 ^f	2.77±1.29 ^b	3.81±1.32 ^{cd}	73.07±42.01 ^c
<i>D. chinensis</i>	19.76±5.78 ^h	52.93±45.41 ^h	12.77±11.6 ^g	1.29±0.37 ^f	1.19±0.39 ^h	73.72±36.6 ^b
<i>U. thunbergiana</i>	25.75±6.17 ^{fg}	45.23±42.35 ^g	37.22±35.59 ^a	2.88±1.08 ^a	1.98±0.59 ^{ef}	82.19±34.24 ^a

*Different letters indicate significant differences Ducun's multiple range test(DMRT) at p<0.05

Table 3 : Correlation analysis among physiological factors for determination of dry tolerance

		SPAD values	RWC	RWL	Proline content	Sugar content	Soil moisture content	Withering rate
SPAD values	Pearson correlation coefficient	1						
	P value							
RWCs	Pearson correlation coefficient	.121	1					
	P value	.464						
RWLs	Pearson correlation coefficient	.127	.182	1				
	P value	.440	.268					
Proline contents	Pearson correlation coefficient	.032	-.625**	.130	1			
	P value	.846	.000	.429				
Soluble reducing sugar contents	Pearson correlation coefficient	.673**	-.141	-.011	.293	1		
	P value	.000	.393	.949	.070			
Withering rates	Pearson correlation coefficient	-.300	-.866**	.008	.623**	.073	-.303	1
	P value	.064	.000	.963	.000	.659	.060	

** **Significant differences at p<0.001

Table 4 : Result of regression analysis for identifying selection factors for drought tolerant plant

	Independent variable	Non-standardized coefficients (B)	Standard Error	Standardized coefficients (B)	T	P value	Durbin-Watson	R ²
Withering rates	Constant	5.325E-16	0.050	-	0.000	1.000		
	SPAD values	-0.784	0.073	-0.408	0.00-5.575	0.000		
	RWCs	-0.894	0.069	-0.803	-11.567	0.000		
	RWLs	0.352	0.057	0.120	2.118	0.042	1.749	0.919
	Proline contents	10.633	0.087	0.328	3.751	0.001		
	Soluble reducing sugar Contents	2.089	0.075	0.174	2.333	0.026		

with 0.673 of the Pearson correlation coefficient. This result means that the higher sugar contents were proportioned with the higher chlorophyll levels in drought conditions. A positive correlation between soil moistures and RWCs were observed. The relationship between soil moisture and proline contents was in a negative correlation, and the Pearson correlation coefficient was -0.672. Between mortality and proline contents showed a positive correlation relation with the Pearson correlation coefficient is 0.623.

The regression analyses were performed to identify the most critical factor among physiological and metabolic factors on drought condition (Table 4). As the coefficient of regression analyses, chlorophyll content was -0.784; RWCs was -0.894; RWLs were 0.352; proline contents were 10.633; sugar contents were 2.089; soil moisture contents were 1.083. The orders of the factors affect to the withering rates were RWCs > SPAD values > Soil Moisture > proline contents > RWLs. These results indicated that the correlation between RWCs and proline contents were key factors for screening drought tolerant plants. It corresponds to that "Katahdin", a potato cultivar, showed higher RWC than the others and was most tolerant to drought stress (Soltys-Kalina *et al.*, 2016). RWC, also, was a most effective way for screening drought tolerant sugarcane genotype (Silva *et al.*, 2007). Mwadingeni *et al.* (2016) have suggested measuring proline content to screen drought tolerant wheat genotype base on their observation that the positive correlation between grain yield and proline content under drought stress conditions was shown. Therefore, it can be suggested that measuring RWC and proline content under drought condition is the key ways for selection of drought tolerant plants.

In this study, the method for screening the drought tolerant plants among Korean natives was established. The drought tolerant plants commonly showed higher RWCs, decrease in chlorophyll content and later accumulation of proline and total reducing sugar than the sensitive plants under drought condition. It was shown that RWC and proline content were a key factors to screen drought tolerant plants among physiological and metabolic parameter. Therefore, the method used in this study has been able to be used to screen the drought tolerant plants, especially for wild plants. Six Korean native species (*S. scilloides*, *C. communis*, *H. fulva*, *B. latissimum*, *H. plantaginea* and *I. dichotoma*) were screened. It is expected that these plants can be applied to improve the adaptability of forests against climate change and to enhance efficiency for recovering devastated lands.

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