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Efficient Selection Method for Drought Tolerant Plants Using Osmotic Agents

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Abstract

An efficient method to select drought tolerant Korean native plants using *in vitro* culture system was established in this study. While the plant growths and root inductions of each plant were proportionately affected by concentrations of mannitol on *in vitro* culturing seven plant species to test tolerance to osmotic stress, growth index (GI) and number of root induction of *Chrysanthemi zawadskii* var. *latilobum* and *Dianthus chinensis var. semperflorens* plantlets were higher than the others in 125mM mannitol. In test with polyethylene glycol (PEG), plantlets of *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* showed higher GI and number of root induction than the others in 33.3mM. On testing whether the well grown plants under osmotic stress are tolerant to virtual drought stress, there were significant differences in the withering rates of *C. zawadskii* var. *latilobum* and *D. chinensis* and those of were *Aster yomena* and *Centaurea cyanus* after 12 days without watering. It was found that significantly lower stomata numbers were shown in both drought tolerant plants than the sensitive plants. Averages of the stomata circumferences and the stomata area in the plantlets of the tolerant species were larger than those of the sensitive plants *D. chinensis var. semperflorens* showed the lowest transpiration level per unit area. The highest stomatal area per unit area was found in *C. zawadskii*, followed by *D. chinensis var. semperflorens*, *Aster yomena* and *C. cyanus*. In conclusion, *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* were more tolerant to drought than other two species. Furthermore in vitro selection was successfully used to screen drought tolerance species of native plant species.

Key Words: drought tolerance; *In vitro* selection; Korean native plants; osmotic stress agents

Introduction

Environmental stresses such as drought, high salinity, and low temperature are major environmental factors that significantly limit agricultural plant productivity (Boyer 1982). Especially, drought and/or water stress are major abiotic stress inducers which limit plant growths and productivities. Drought stress is characterized by a reduction of water content, diminish of water potential and sugar in leaves, closure of stomata, and decrease in cell enlargement and growth (Jaleel et al. 2009).

As a result of rapid economic growth and land develop-

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ments in South Korea, devastated lands have increased every year (Woo and Jeon 2005). These lands fundamentally accompanied drought stress conditions, which disadvantageously work on plant growths. Inhibitions on plant growths have often resulted in expansions of land devastation. An effective afforestation method using drought tolerant plants is required for reforestation in devastated lands. However, selection of drought tolerant plants by the traditional methods is a time-consuming process, which is inefficient (Dorffling et al. 1993). Thus, a rapid breeding method for screening drought tolerant plants has been emphasized.

In Korea, there are countless native plants, and most of them remain their characters in questions. Especially, it is not known which plants are tolerant to drought stress. Of Korean native wild plant, 4 species including *Chrysanthemum. zawadskii*, *Aster yomena*, *Dianthus chinensis var. semperflorens*, and *Indigofera pseudotinctoria* never have been studied about their drought tolerance. Therefore, we assessed whether these plants are tolerant to drought stress by establishing *in vitro* selection system. The other plants including *Brassica napus*, *Centaurea cyanus*, and *Chrysanthemum burbankii* were tested to compare their drought tolerance with those of the native plants in that these exotic plants have been used wildly for revegetation process (Ahn et al. 2007; Kil et al. 2015; Park et al. 2014).

It is usual that drought-tolerant plants are able to withstand the conditions of poor water conditions. It has been reported that their resistant ability against arid conditions can be measured by physiological, molecular, and biochemical factors (Chaves et al. 2003). Hassanein and Dorion (2006) reported a simple method to evaluate and select water stress-tolerant *Pelargonium* xhortorum genotypes using few morphological parameters such as plant growths and leaf development on drought stress. The *in vitro* culture techniques minimize environmental variation due to defined media, controlled conditions, homogeneity of stress application, and simplicity of such manipulation (Smith et al. 1985; Hassanein 2010). Therefore, *in vitro* selection can be suggested to screen drought-tolerant plants. In this study, mannitol and PEG were used to provide drought conditions *in vitro* cultures. PEG, which is a non-penetrating osmotic agent and functions to lower the water potential as similar to soil drying, has been applied to simulate drought stress in plants. It was previously used to establish an efficient *in vitro* experimental method to evaluate drought tolerance in *Pelargonium* (Hassanein 2010). Sugar alcohols such as mannitol and sorbitol have often been used as metabolic inert osmotic in plant cell culture (Thompson et al. 1986). Mannitol has been used to control the osmotic potential of the nutrient solutions in order to induce water deficit conditions, especially in the root zone (Zang and Komatsu 2007). Therefore, this study was conducted to establish the *in vitro* selection system of drought-tolerant plant species in Korea using the osmotic stress agents.

Material and Methods

Plant materials

Four Korean native plant species (D. *zawadskii* var. *latilobum* K., *Aster yomena* H., *Dianthus chinensis var. semperflorens* M., and *Indigofera pseudotinctoria* M.) and three introduced species (*Brassica napus* L, *Centaurea cyanus* L., *Chrysanthemum burbankii* M.) , which have been usually applied in ecological restoration, were utilized to search drought-resistant plants. These plants were grown by germinating seeds obtained from Germplasm Bank of Korea National Arboretum.

In vitro selection of drought tolerant plants by treating osmotic agents

To obtain *in vitro* cultivated plantlets, surface sterilizations seed of seven species were performed with 70% (v/v) ethanol for one minute and tween-20 for three minutes. The seeds were further disinfected using 3% (v/v) sodium hypochlorite for 15 min and rinsed with sterile distilled water. The sterilized seeds were then placed in test tubes containing MS medium supplemented 3% sucrose (w/v) and 0.4% gelrite (w/v). The pH of the basal medium was adjusted to 5.7 before autoclaving at 121°C for 15min. The plant cultures were maintained under 16h per a day of photoperiod of fluorescent light at 25±1°C. The shoot segments from *in vitro* plantlets of each species were exposed to osmotic pressure by being sub-cultured in the MS media containing osmotic agents. To provide the plantlets with osmotic stress, various concertation of mannitol (125, 250, 500 and 1000mM) were included in the MS media. PEG,

Table 1. The responses of *in vitro* plants of seven kinds with 5 different concentrations of mannitol

	Species	Mannitol (mM)				
		0	125 (2.2%)	250 (4.6%)	500 (9.1%)	1000 (18.2%)
Growth index (mm)	*C. zawadskii*	2.07±0.35ab*	1.81±0.05a	1.40±0.08a	0.91±0.32a	0.50±0.03a
	A.yomena	3.29±0.21a	1.03±0.07b	0.51±0.02b	0.41±0.04ab	0.20±0.01c
	D. chinensis var. semperflorens	1.80±0.03bc	1.79±0.08a	1.43±0.07a	0.94±0.01a	0.61±0.02a
	B.napus	2.79±0.33ab	0.36±0.08cd	0.20±0.00c	0.20±0.01b	0.20±0.03c
	I.pseudotinctoria	0.63±0.10c	0.16±0.02d	0.16±0.01c	0.10±0.01b	0.10±0.01c
	C.cyanus	2.20±0.20ab	0.31±0.06cd	0.28±0.02c	0.13±0.01b	0.10±0.02c
	C.burbankii	1.44±0.28bc	0.62±0.07c	0.54±0.03b	0.48±0.04ab	0.35±0.03b
Root numbers	*C. zawadskii*	4.0±0.25bc	3.3±0.14a	2.0±0.25b	0.0±0.00b	0.0±0.00
	A.yomena	1.0±0.25cd	0.0±0.00c	0.0±0.00c	0.0±0.00b	0.0±0.00
	D. chinensis var. semperflorens	6.0±0.50ab	3.7±0.14a	3.3±0.14a	5.0±0.25a	0.0±0.00
	B.napus	4.0±0.43bc	0.0±0.00c	0.0±0.00c	0.0±0.00b	0.0±0.00
	I.pseudotinctoria	0.0±0.00d	0.0±0.00c	0.0±0.00c	0.0±0.00b	0.0±0.00
	C.cyanus	3.0±0.25bcd	0.0±0.00c	0.0±0.00c	0.0±0.00b	0.0±0.00
	C.burbankii	8.0±1.09a	1.7±0.38b	0.3±0.14c	0.0±0.00b	0.0±0.00

*Different letters indicate Duncan's multiple range tests(Significant at $p \leq 0.05$).

also, was supplemented to MS media with different concentrations (33.3, 50.0, 58.3 and 66.6mM).

After five weeks of culture on osmotic stress media, growth parameters including shoot length and the number of green and wilted leaves per plant were determined to understand how much growth of each plant was inhibited by the osmotic stress inducers. The data were collected after washing up the media residues with sterile water in all experiments. The plant withering rates were calculated after five weeks of osmotic stress treatment.

Ex vitro tests on drought tolerance of the selected plants

The seeds of seven plant species were sowed in the greenhouse of Gyeongsang National University. All seedling pots were watered every day to obtain uniform seed-

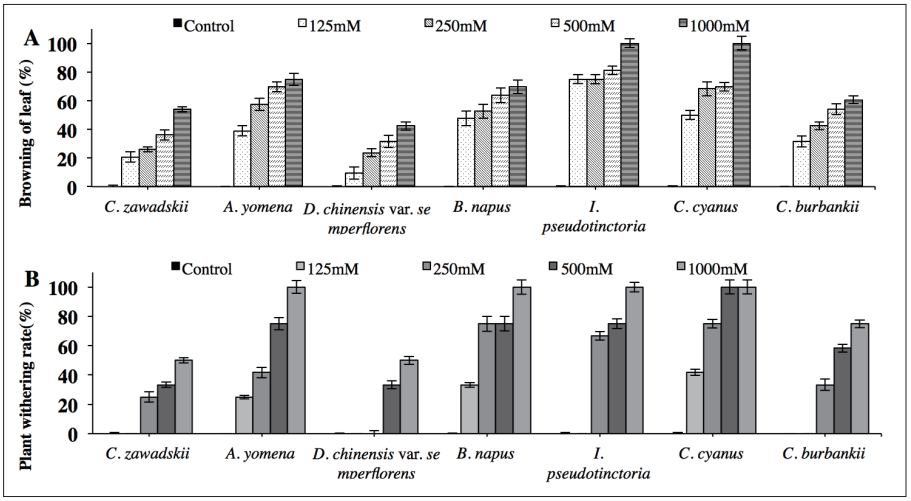

Fig. 1. *In vitro* morphological features of seven plant species on osmotic stress. (A) Leaf browning rates (%) on MS media containing various concentrations of mannitol, (B) Plant withering rate (%) on MS media containing various concentrations of mannitol. A-week-old plantlets of each plant species were exposed to osmotic stress by various concentrations of mannitol for 5 weeks.

lings until four weeks after sowing. Supply of water was stopped after four weeks to give drought condition. Plant withering rates under the drought stress were obtained by applying the following formula (1).

Formula (1) ··· Plant withering rates (%) = (The number of withered plants / all number of plants) ×100

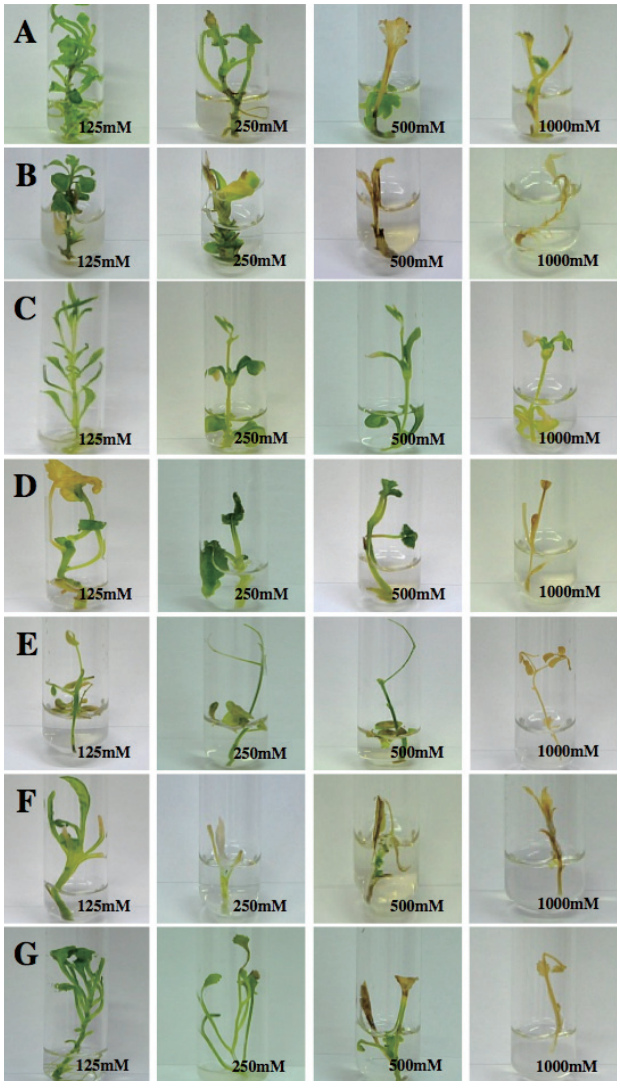

Fig. 2. *In vitro* phenotypic features of seven plant species on osmotic stress. (A) *C. zawadskii*, (B) *A. yomena*, (C) *D. chinensis var. senperflorens*, (D) B. napus, (E) *I. pseudotinctoria*, (F) *C. cyanus*, (G) *C. burbankii*. Phenotypic features of a-week-old plantlets of each plant species were observed since they were exposed to osmotic stress by various concentrations of mannitol for 5 weeks.

Growth characteristics were including shoot length (mm) , leaf length (mm) , leaf width (mm) , root length (mm) and plant fresh weight (g). Plant dry weight (g) was recorded after drying at 80°C for 48h. Physiological characteristics were observed by examining leaf area, transpiration per unit and transpiration per hour. Leaf area was measured using a leaf shape analysis program (WinFOLIA, Regent Instruments Inc.). Transpiration per hour (ml/hr) and transpiration per unit (ml/cm^2·hr) was calculated by weight change amount. The measurement of transpiration status was carried out by measuring the changed amount water contents of experimental plants in an hour after putting root parts into a beaker containing water. Histological factors were obtained by measuring stomata number per leaf area (25mm^2) , the circumference of stomata, and area of stomata. For observing stomata, top 2-4 leaves were collected. Samples of stomata were prepared by peeling the back side of leaves using corrosion solution. Then, stomata of the plants were observed using an optical microscope (BH-2, OLYMPUS) under a hundred magnification. Stomata number per leaf area (25mm^2) , the circumference of stomata and area of stomata were measured using Dinocapture 2.0 program.

Statistical analyses

Data are expressed as an average of five separate experiments. The bars indicate standard deviation from the mean of each replicate treatment. Data were subjected to statistical analysis by using the SPSS software. One-way analysis of variance (ANOVA) was conducted, and means were compared using Duncan's multiple-range test (DMRT) at 0.05 level of probability.

Results and Discussion

Responses of in vitro plants against mannitol treatments

It has been clearly shown that mannitol treatments influenced the shoot growths and root numbers (Table 1). In results of growth index (GI) , all plant growths were being less as the concentrations of treated mannitol increase. Comparative fewer inhibitions in growth were observed in C. zawadski and *D. chinensis var. semperflorens var. semperflorens* whereas GIs of *A. yomena* and *C. cyanus* were

Table 2. The responses of *in vitro* plants of seven kinds with 5 different concentrations of PEG

	Species	Polyethylene glycol (mM)				
		0	33.3 (20.0%)	50.0 (30.0%)	58.3 (35.0%)	66.6 (40.0%)
Growth index (mm)	*C. zawadskii*	2.07±0.35ab*	0.39±0.06ab	0.22±0.03ab	0.19±0.04a	0.00±0.00
	A.yomena	3.29±0.21a	0.28±0.01b	0.17±0.01ab	0.14±0.03ab	0.00±0.00
	D. chinensis var. senperflorens	1.80±0.03bc	0.74±0.17a	0.25±0.01a	0.24±0.04a	0.00±0.00
	B.napus	2.79±0.33ab	0.10±0.02b	0.10±0.03bc	0.10±0.00ab	0.00±0.00
	I.pseudotinctoria	0.63±0.10c	0.00±0.00b	0.00±0.00c	0.00±0.00b	0.00±0.00
	C.cyanus	2.20±0.20ab	0.32±0.02b	0.18±0.01ab	0.11±0.00ab	0.00±0.00
	C.burbankii	1.44±0.28bc	0.34±0.03b	0.23±0.03a	0.13±0.03ab	0.00±0.00
Root numbers	*C. zawadskii*	4.0±0.33bc	3.0±0.67a	2.0±0.33ab	0.0±0.00	0.0±0.00
	A.yomena	1.0±0.33cd	0.0±0.00c	0.0±0.00b	0.0±0.00	0.0±0.00
	D. chinensis var. senperflorens	6.0±0.67ab	1.7±0.19bc	3.0±0.33a	0.0±0.00	0.0±0.00
	B.napus	4.0±0.58bc	0.0±0.00c	0.0±0.00b	0.0±0.00	0.0±0.00
	I.pseudotinctoria	0.0±0.00d	0.0±0.00c	0.0±0.00b	0.0±0.00	0.0±0.00
	C.cyanus	3.0±0.33bcd	0.0±0.00c	0.0±0.00b	0.0±0.00	0.0±0.00
	C.burbankii	8.0±1.45a	1.3±0.19ab	3.0±0.88a	0.0±0.00	0.0±0.00

*Different letters indicate Duncan's multiple range tests (Significant at $p \leq 0.05$).

highly inhibited. A similar result was observed in the root inductions. No roots of *A. yomena*, B. napus, I. pseudotinctoria and *C. cyanus* were induced even in 125mM mannitol treatments. In contrast, the root of *D. chinensis var. semperflorens* was successfully induced at 500mM mannitol, and C. zawadski also was able to induce roots in 250mM. Although root inductions of C. *burbankii* were observed at 250mM mannitol, the numbers were significantly less in higher mannitol concentration.

It was observed that effects on the morphology of tested plants were also found by treating mannitol (Fig. 1 and 2). The green leaves were changed to brown with the increase of mannitol concentration and no green leaves were observed in *C. cyanus* at 1000mM mannitol treatment. It was a significant change of leaf color in *A. yomena* with mannitol treatment. Comparative less effect of mannitol was observed in C. zawadski and *D. chinensis var. semperflorens* even though it was dependent on the concentra-

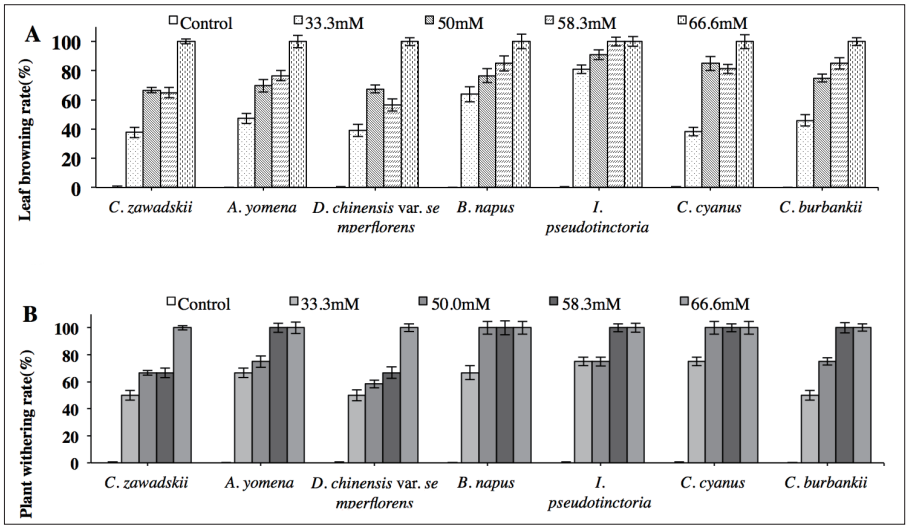

Fig. 3. *In vitro* morphological features of seven plant species on PEG. (A) Leaf browning rates (%) on MS media containing various concentrations of PEG, (B) Plant withering rate (%) on MS media containing various concentrations of PEG. A-week-old plantlets of each plant species were exposed to osmotic stress by various concentrations of PEG for 5 weeks.

Table 3. Growth appearance of *in vivo* plants after 4 weeks germination

Plant species	Shoot length (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm2)	Root length (mm)	Dry wt. (mg)	Moisture (%)
C. zawadskii	2.95±0.55a*	1.15±0.02b	1.01±0.05a	0.89±0.12b	44.53±1.25b	5.8±0.09a	91.2±0.07b
A. yomena	4.54±0.39a	1.99±0.02a	0.42±0.02b	0.93±0.02b	50.02±0.35a	2.8±0.08b	90.7±0.13bc
D. chinensis var. senperflorens	6.09±0.86a	1.30±0.02b	0.34±0.01b	1.74±0.04a	16.74±0.11c	2.1±0.07b	90.2±0.05c
C. cyanus	6.18±0.52a	0.43±0.03c	0.28±0.01b	0.73±0.05b	17.31±0.13c	2.8±0.32b	96.0±0.17a

*Different letters indicate Duncan's multiple range tests (Significant at $p \leq 0.05$).

tions. Similarly, it was observed that all plantlets of *C. cyanus* were withered in 500mM mannitol and of *A. yomena*, *B. napus* and *I. pseudotinctoria* were withered in 1000mM (Fig. 1, B). However, plantlets of *C. zawadski*, *D. chinensis var. semperflorens* and *C. burbanskii* were partially withered even though they were exposed to 1000mM mannitol. The withering rate of *D. chinensis var. semperflorens* and *C. zawadskii* var. *latilobum* was less than 50% in 1000mM mannitol.

Responses of in vitro plants against PEG treatments

Growth variations of each shoot segment of seven plants were shown in PEG treatment (Table 2). Similar to mannitol treatment, the growths of the plant were proportionally inhibited by PEG. In 33.3mM PEG treatment, shoot of *D. chinensis var. semperflorens* was highly tolerant than the other species, and *C. zawadskii* var. *latilobum* was comparatively more tolerant against PEG. Moreover, roots of C. *zawadskii*, *D. chinensis var. semperflorens* and *C. burbanskii* were induced until they were exposed in 50.0mM PEG whereas no roots were induced in *A. yomena*, B. napus and *C. cyanus* even in 33.3mM PEG.

After 4 weeks culture on PEG treatments, differences in morphology were shown among seven plants (Fig. 3). It was observed that all the plants of *C. cyanus* and B. napus were withered in 33.3mM PEG (Fig. 3, A). Similarly, *A. ymena, I. pseudotinctoria* and C. *burbankii* were withered in 50.0mM PEG treatment. However, plants of *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* were partially endured in 50mM PEG.

Ex vitro test for drought tolerance of selected plants species and those characteristics of stomata

In the plant kingdom, it has been known that drought stress factors are strictly related to osmotic stress. It means that osmotic stress tolerant plants have abilities to resist

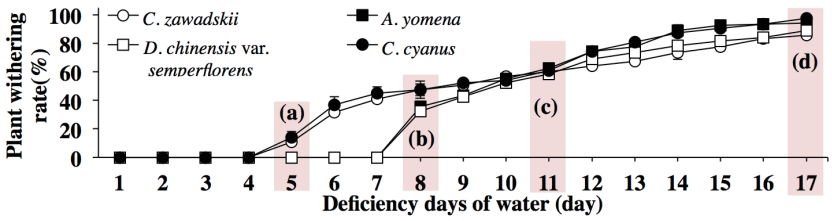

Fig. 4. *In vivo* morphological features of drought tolerant and sensitive plants. Four-week-old plantlets, which were grown in the greenhouse, of each species were exposed to drought stress by stopping watering to measure the plant withering rates at (a) 5 days, (b) 8 days, (c) 12 days, and (d) 17 days.

against arid conditions. This fact allowed us to test whether the plants tested in the in-vitro-system earlier are tolerant to drought stress. Two osmotic stress tolerant plants (*C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens*) and two osmotic stress sensitive plants (*A. yomena* and *C. cyanus*) were chosen to confirm whether their osmotic stress tolerance/sensitivity function as drought tolerance in dry conditions or not. Water supplying was stopped to give drought condition to the plantlets, which were grown for 4 weeks in the greenhouse. In 12 days of drought stress, there were differences between the osmotic stress tolerant and sensitive plant species (Fig. 4). The withering rates of C. zawadaskii and *D. chinensis var. semperflorens* were 64.2% and 69.2%, respectively whereas relative higher withering rates were observed in the drought-stressed plantlets of *A. yomena* (74.2%) and *C. cyanus* (74.2%). While the plant withering rates were increased in all species on 17 days of drought stress, the withering rates of C. zawadaskii (85.8%) and *D. chinensis var. semperflorens* (89.2%) were lower than those of *A. yomena* (94.2%) and *C. cyanus* (97.5%). Therefore, these results indicated that the osmotic stress tolerant plants are more tolerant to drought stress than the osmotic stress sensitive plants.

Growth features of each plant species, grown in the greenhouse for 4 weeks, were shown in Table 3. It was considered that there were no common growth features between tolerant species. *D. chinensis var. semperflorens* showed the longest shoot length and the broadest leaf, however, *C. zawadskii* var. *latilobum* showed the lowest growth than the sensitive plants. The root length of *D. chinensis var. semperflorens* was 16.74mm while root length of *C. zawadskii* var. *latilobum* was 44.54mm. Differences in

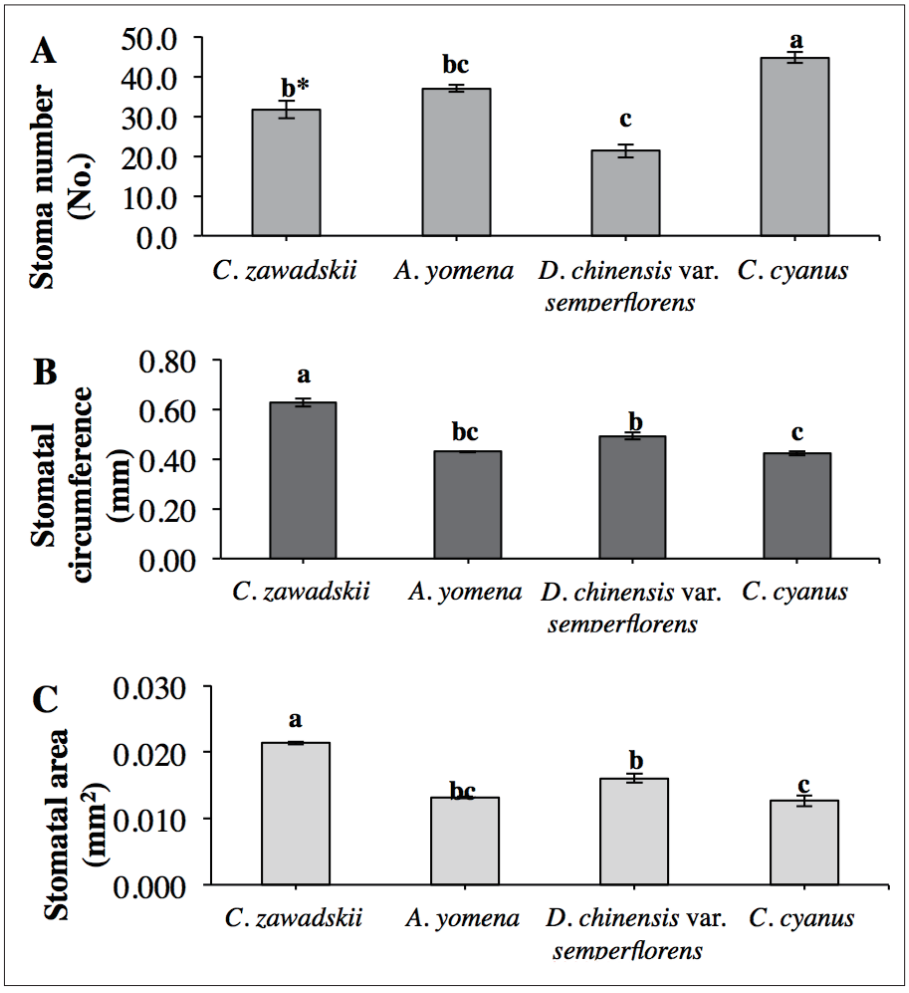

Fig. 5. Stoma numbers, circumference and area of the drought tolerant and sensitive plant species. (A) Stoma numbers, (B) Stomatal circumferences, (C) Stomatal areas. *Different letters indicate Duncan's multiple range tests (Significant at $p \leq 0.05$).

leaf area between *D. chinensis var. semperflorens* (1.74±0.04 cm^2) and *C. zawadskii* var. *latilobum* (0.89±0.12 cm^2) was also observed. Root length of *D. chinensis var. semperflorens* (16.74mm) was the least length of the selected plants. The root lengths of *C. zawadskii* var. *latilobum* seedlings were longer than that of *D. chinensis var. semperflorens*. It was considered that there is no common characteristic in growth.

Drought tolerances in plants are influenced by stomata in leaves and their transpiration rates (Farooq et al., 2009). The stomatal characteristics of the drought tolerant and sensitive plants were observed (Fig. 5 and 6). Differences in stomatal number, circumference and area were observed between tolerant plant group and sensitive plant group (Fig. 5). Of the tolerant group, the numbers of stomata (per 25mm2) were 23.5±2.6 in *D. chinensis var. semperflorens* and 31.8±2.8 in C. *zawadskii*, respectively. In the other hands, the numbers of stomata of plants in the sensitive group were comparatively higher than that of the tolerant group: 37.8±1.2 in *A. yomena*, and 46.2±1.9 in *C. cyanus*. The stomata circumferences of the plants in the tolerant group were 0.64±0.11mm (C. *zawadskii*), and 0.49±0.12mm (*D. chinensis var. semperflorens*) whereas those of *A. yomena* and *C. cyanus* were 0.44±0.0mm, and 0.44±0.09mm, respectively. Lager stomatal areas were shown in the plants of the tolerant group than the sensitive group. The stomatal areas of *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* were 0.022±0.000mm^2, and 0.017±0.001mm^2, respectively. However, the stomatal area of *A. yomena* and *C. cyanus* in the sensitive group were 0.013±mm^2, and 0.013±mm^2, respectively.

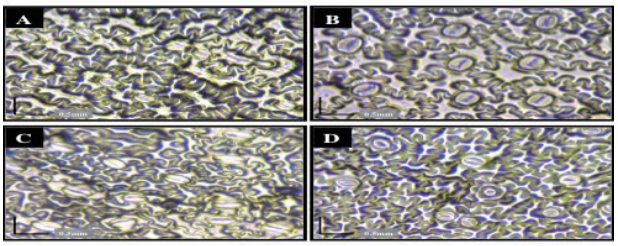

Fig. 6. Guard cells of 4-week old plantlets of (A) *D. chinensis var. senperflorens*, (B) *C. zawadskii*, (C) *A. yomena,* and (D) *C. cyanus*. Samples of stomata were prepared from top 2-4 leaves of each 4-week old plantlet by peeling the back side of leaves using corrosion solution, and observed using an optical microscope under a hundred magnification.

A transpiration ability of tolerant and sensitive plant groups was given in Fig. 7. No tendencies were observed

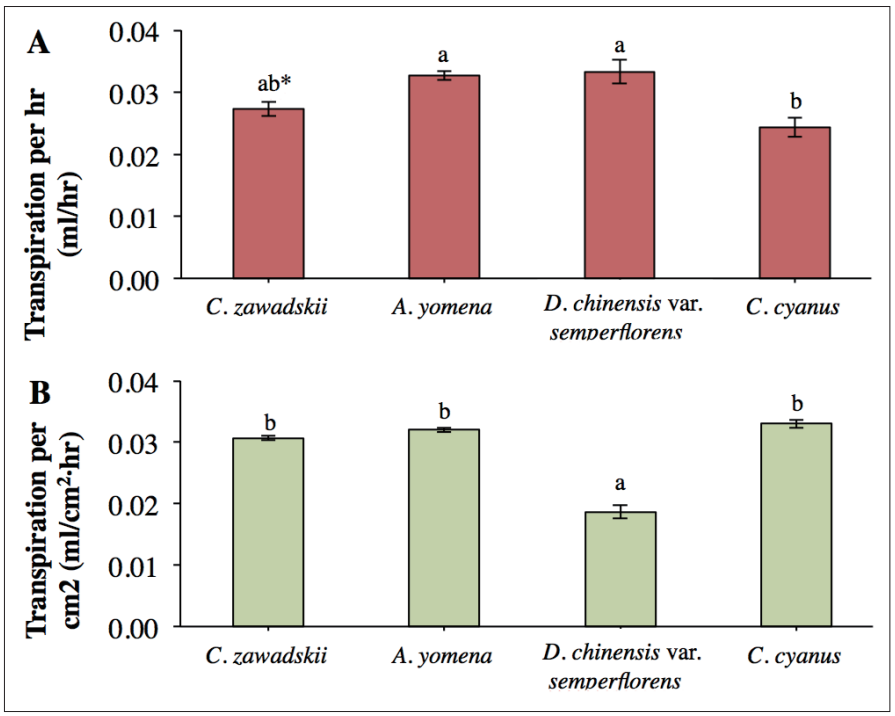

Fig. 7. Characterization of transpiration abilities in the drought tolerant and sensitive plant species. (A) Transpiration per hr, (B) Transpiration per cm^2. *Different letters indicate Duncan's multiple range tests (Significant at $p \leq 0.05$).

in transpiration levels per leaf of the plant. The transpiration level per leaf was found highest in *D. chinensis var. semperflorens* and lowest in *C. cyanus*. In contrast, transpiration level per cm2 was lowest in *D. chinensis var. semperflorens* (0.018±0.0010). Even though there was no statistical significance between *C. zawadskii* var. *latilobum* and the plants of the sensitive group, transpiration level per cm^2 of *C. zawadskii* var. *latilobum* (0.031±0.0001) was lower than those of the sensitive group: *A. yomena* (0.033±0.0001), *C. cyanus* (0.034±0.0002).

A method using plants has been suggested to recover devastated lands (Jeon 2002; Kim et al. 2008). Because of extreme environmental conditions in the devastated lands, the planting of environmental stress tolerant plants have been suggested (Atif 1988). Drought, a major environmental stress factor, can cause a problem for recovering devastated lands because it inhibited plant growths and productivities. For this reason, drought tolerant plants can be the first option for the recovery of devastated lands. This study was carried out to establish *in vitro* method for selecting drought tolerant plants using osmotic stress agents.

Growth parameters including shoot height, root length, leaf area, fresh weight and dry weight of rice plant, which was grown under water deficit and salt stress were decreased relating to osmotic pressure in the culture media (Hien et al. 2003). The growth characteristics in osmotic stress tolerant plant species are maintained better than in sensitive genotypes (Ahmad et al. 2007). The growths of *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* plants were not inhibited severely in both *in vitro* conditions, which were treated with Mannitol and PEG, in comparison to the growths of *A. yomena* and *C. cyanus* (Table 1 and 2). The plants of *A. yomena* and *C. cyanus* were also damaged significantly in their morphology when exposed to osmotic agents (Fig. 1 and 3). Same results found ex vitro test too. The plantlets of *C. zawadskii* var. *latilobum* and *D. chinensis var. semperflorens* endured longer days with the non-irrigation condition than the plantlets of *A. yomena* and *C. cyanus* (Fig. 4). These results indicated that the osmotic tolerant plants can also effectively tolerate arid condition. It has been reported that drought tolerant clones of *Tagetes minute* were selected by *in vitro* method using mannitol (Mohamed et al. 2000). Bibi et al. (2012) reported that PEG was effective for screening drought tolerant Sorghum bicolor var. moench. In addition, an *in vitro* culture method with both osmotic agents was applied for selection of drought tolerant tomato (Abdel-Raheem et al. 2007). Therefore, it has been suggested that an *in vitro* selection method using osmotic agents can be used for screening drought tolerant plant species.

There were no tendencies in growth characteristics of drought tolerant and drought sensitive plant groups (Table 3). However, there were some differences in stomata numbers, circumference, and area (Fig. 5). The number of stomata in *D. chinensis var. semperflorens* and *C. zawadskii* var. *latilobum* were lower than in *A. yomena* and *C. cyanus*. In addition, the stomatal circumferences and area of former two were larger than later two. Transpiration efficiency is an important trait for drought tolerance in *Arachishy pogaea* (Ratnakumaret et al. 2009). An effective plant drought acclimation or adaptation strategy is used to reduce water loss on transpiration, which allows plants to maintain an adequate water status to sustain critical physiological and biochemical processes (Chaves et al. 2003). In this study, the transpiration level of *D. chinensis var. semperflorens* was lower than *A. yomena* and *C. cyanus* (Fig. 7). This result indicated that the ability of drought resistance in *D. chinensis var. semperflorens* is acquired by their lower transpiration levels.

Mannitol and polyethylene glycol have been used as stimulating osmotic stress in some previous studies (Abdel-Raheem et al. 2007; Bibi et al. 2012; Mohamed et al. 2000; Hissouand and Bouharmont 1994). In this study, the growth parameters were more severely inhibited in PEG-treated plants in comparison to mannitol-treated. The result suggested that mannitol is comparatively more suitable than PEG for assessing candidate plants with drought tolerance at the early growth stage. Hassanein (2010) found a similar result for *Pelargonium* species that mannitol treatments were more appropriate than PEG for selecting drought tolerant.

In this study, it was observed that C. zawadskii var. latilobum and *D. chinensis var. senperflorens* were more tolerant to drought stress than A. yomena and C. cyanus in both in-vitro- and in-vivo-systems. *C. zawadskii var. latilobum* (*Compositae*) and *D. chinensis var. senperflorens* (*Caryophyllaceae*) are Korean native plants. The results of this study suggest that both native plants can be used for

efficient re-vegetation in devastated lands.

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