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Genetic Diversity and Physiological Response to Drought Stress of *Chamaecyparis obtusa* from Six Geographical Locations

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ABSTRACT

Water deficit is a critical factor obstructing the growth and survival of plant. Therefore, researchers have been trying to develop drought-resistant varieties. To find indicators of drought stress-tolerance of cypress (*Chamaecyparis obtusa*), we analyzed the response of cypress seedlings from six provenances of Korea (Jeju, Suwon, Seoul, Seongnam, Yong-in, and Osan) to drought stress. Additionally, the genetic diversity of *C. obtusa* from the six provenances were determined using microsatellite markers. We confirmed that populations from Suwon and Seongnam were relatively separated from other populations through genetic distance and cluster analysis. We examined their physiologic and metabolic responses after drought treatment for five weeks. Almost all of the cypress seedlings showed a reduced shoot growth rate under drought treatment compared to controls. In addition, temperature of drought treated cypress seedling leaves was 1.2-2℃ higher than that of the controls. Almost all of the drought stress-treated cypress showed increased carbon metabolite contents and pigments. In particular, the cypress seedlings from Osan showed the highest increase in all of the measured metabolites. Therefore, it is suggested that the seedlings from Osan are susceptible to drought stress. Conversely, the seedlings from Jeju, Suwon, and Yong-in showed a lower sensitivity to drought treatment. These results indicate that the cypress trees from the six provenances have a different response to drought stress. In addition, it is confirmed that previously identified indicators of drought stress, especially those that measure total soluble sugar, carotenoid, and H2O2, can be used in the selection of drought resistance cypress. These findings may useful in studies concerned with the metabolic and physiological responses of young cypress to drought.

MAIN OBJECTIVES

Compared the 6 *C. obtusa* populations in terms of their genetic differences and physiological differences in their response to drought for the selection and development of drought-resistant varieties.

RESULTS

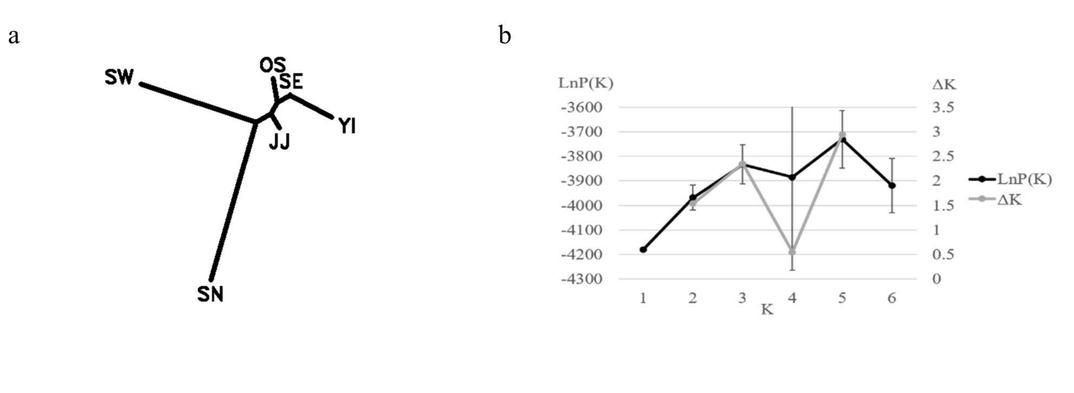
Genetic diversity and population differentiation of cypress seedlings from six different provenances

Locus	N_A	N_E	H_O	H_E	F_{IS}	F_{ST}	G_{ST}
Co31	5	2.5	0.562	0.599	0.065	0.144**	0.121
Cos0319	20	6.4	0.924	0.840	-0.101*	0.052**	0.043
Cos1536	27	5.7	0.688	0.822	0.162**	0.070**	0.058
Cos1951	27	8.4	0.868	0.873	0.006*	0.089**	0.075
Cos2224	11	4.0	0.797	0.755	-0.055	0.105**	0.090
Cos2610	12	4.1	0.778	0.735	-0.059**	0.110**	0.094
Cos2680	7	2.9	0.637	0.641	0.007	0.140**	0.120
Overall	8.3	4.87	0.750	0.752	0.002	0.099	0.084

Table 1 Genetic characteristics of *C. obtusa* in Korea based on a microsatellite analysis using seven microsatellite loci. N_A: Number of alleles, H_O: Observed heterozygosity, H_E: Expected heterozygosity (Unbiased), F_{IS}: Fixation index, G_{ST}: coefficient of gene differentiation (Nei, 1973); * $p < 0.05$; ** $p < 0.01$)

Population	N_A	N_E	H_O	H_E	F_{IS}
Jeju	9.3	5.8	0.744 (0.055)	0.802(0.042)	0.073**
Osan	9.9	5.5	0.725(0.066)	0.790(0.039)	0.081**
Seoul	10.6	7.2	0.818(0.043)	0.833(0.043)	0.019*
Seongnam	7.6	3.4	0.726(0.073)	0.683(0.047)	-0.063
Suwon	4.7	3.1	0.780(0.051)	0.672(0.034)	-0.164**
Yong-in	7.8	4.2	0.710(0.098)	0.733(0.057)	0.028**

Table 2 Genetic characteristics of the population of *C. obtusa* in Korea based on microsatellite analysis N_A: Number of alleles, H_O: Observed heterozygosity, H_E: Expected heterozygosity (Unbiased), F_{IS}: Fixation index, G_{ST}: coefficient of gene differentiation (Nei, 1973); * $p < 0.05$; ** $p < 0.01$)



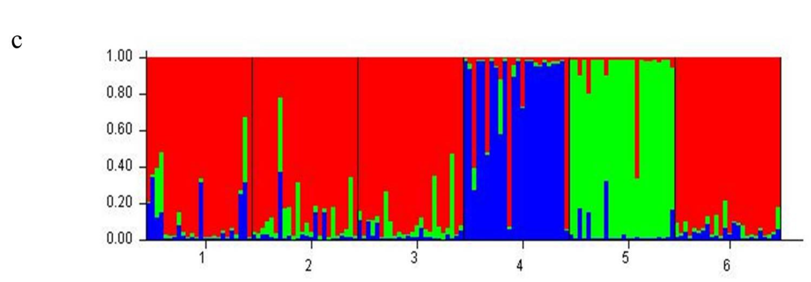


Fig. 1. Genetic relationship and population structure of the *C. obtusa* from the 6 regions in Korea.
(a) Unrooted Neighbor-joining tree based on the Nei's genetic distance (Nei 1978). OS: Osan; SE: Seoul; JJ: Jeju; YI: Yong-in; SW: Suwon; SN: Seongnam. (b) The median of LnP(K) and ΔK (Evanno *et al.* 2005) for each K, (c) Bar plot representation for K = 3, the number on the x-axis indicates the population in Jeju, Osan, Seoul, Seongnam, Suwon, and Yong-in in the ascending order.

⇒ The populations from Suwon and Seongnam were relatively apart from the other populations in terms of their Nei's genetic distance.

Growth phenotype analysis of C. obtusa seedlings under drought treatment

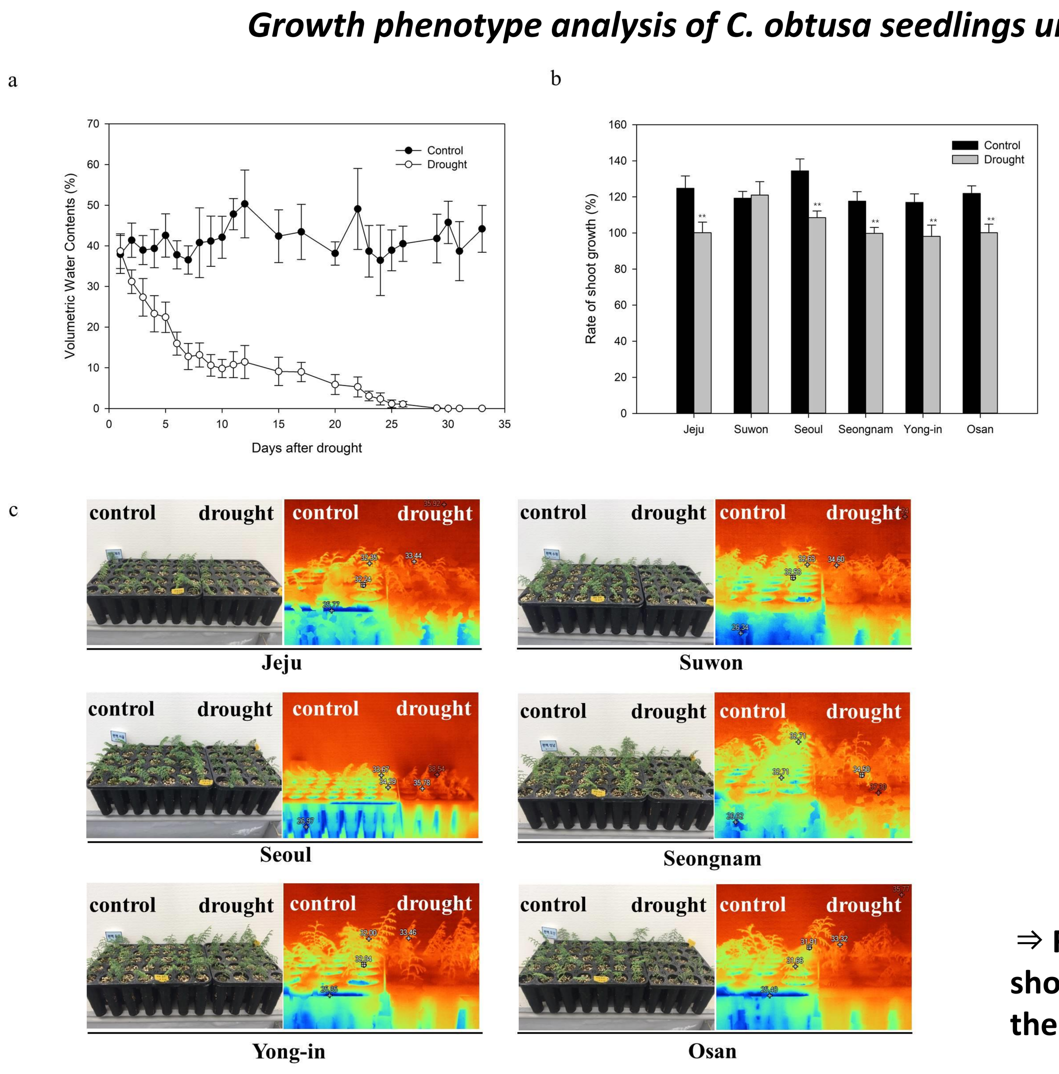


Fig. 2. Height growth phenotype analysis and thermal images of *C. obtusa* seedling under drought treatment.
(a) Volumetric water content in the soil of the control- and drought-treated plant pots. The soil moisture was measured every 2 days for 33 days. The control plants were watered throughout the experiment. (b) The effect of drought on the shoot growth of the *C. obtusa* seedlings from Jeju, Suwon, Seoul, Seongnam, Yong-in, and Osan. The values are the means ± SD (n = 10). (c) Growth phenotypes of *C. obtusa* Jeju, Suwon, Seoul, Seongnam, Yong-in, and Osan seedlings were subjected to drought treatment. Control plants (left) and drought-treated plants (right) after 33 days. Infrared thermal images.

⇒ For almost of seedlings, the growth rate showed a slight reduction in the heights of the drought-treated seedlings

Analysis of leaf chlorophyll contents

	treatment	Chl a (mg/g FW$^{z)}$)	Chl b (mg/g FW$^{z)}$)	total Chl (mg/g FW$^{z)}$)	carotenoids (mg/g FW$^{z)}$)	Chl a/b$^{z)}$	Chl/Car$^{z)}$
Jeju	control	0.83±0.05	0.21±0.02	1.05±0.04	0.23±0.03	3.92±0.65	4.56±0.73
	drought	1.55±0.04**	0.53±0.10**	2.08±0.11**	0.32±0.02*	3.00±0.71	6.37±0.77
Suwon	control	0.94±0.13	0.21±0.10	1.15±0.23	0.23±0.008	5.06±2.23	4.90±0.90
	drought	1.69±0.06**	0.60±0.10*	2.29±0.15**	0.35±0.02**	2.85±0.49	6.46±0.69
Seoul	control	0.93±0.08	0.33±0.09	1.27±0.17	0.2±0.002	2.86±0.49	6.31±0.93
	drought	2.03±0.05**	0.68±0.13*	2.72±0.18**	0.44±0.02**	3.03±0.52	6.18±0.80
Seongnam	control	0.83±0.04	0.30±0.09	1.13±0.14	0.17±0.007	3.00±1.00	6.47±1.08
	drought	2.03±0.10**	0.75±0.20*	2.78±0.29**	0.41±0.03**	2.83±0.77	6.71±1.13
Yong-in	control	0.85±0.07	0.30±0.07	1.15±0.14	0.18±0.006	2.88±0.48	6.24±0.95
	drought	1.41±0.12**	0.44±0.04*	1.86±0.16**	0.32±0.02**	3.16±0.13	5.70±0.08
Osan	control	1.01±0.03	0.41±0.03	1.42±0.06	0.2±0.01	2.43±0.15	7.00±0.47
	drought	2.57±0.09**	0.90±0.07**	3.47±0.17**	0.51±0.007**	2.85±0.14	6.71±0.23

Table 3 Effects of drought-treatment on the photosynthetic pigments in *C. obtusa* seedlings.
$^{z)}$ The values are the means ± SD (n = 4); The asterisks indicate significant differences (*t* test; $^{*}p$ < 0.05, $^{**}p$ <0.01) between the control and drought-treated seedlings.

⇒ The photosynthetic pigment level in *C. obtusa* sensitively reacts to drought stress. In particular, the Osan seedling showed the greatest increase in all chlorophyll contents.

Determination of soluble sugars

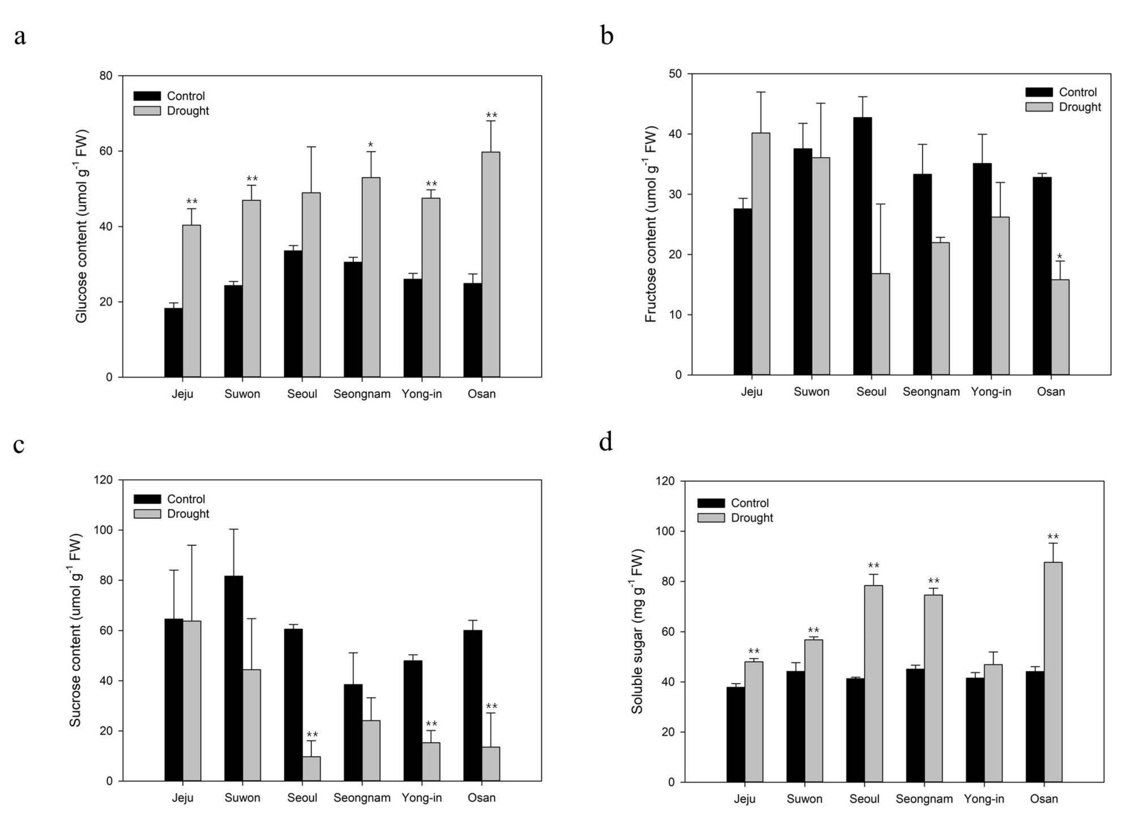


Fig. 3. Effects of the carbohydrates in the *C. obtusa* seedlings subjected to drought treatment.
(a) Glucose. (b) Fructose. (c) Sucrose. (d) Soluble sugars. The values are the means ± SD of three independent measurements. The asterisks indicate significant differences (*t* test; **P* < 0.05, ***P* < 0.01) between the control and the drought-treated seedlings.

⇒ Under drought treatment, the soluble sugar content of the Osan, Seoul, and Seongnam seedlings increased significantly, compared with the controls.

MDA, proline, and H_2O_2 levels in C. obtusa seedlings

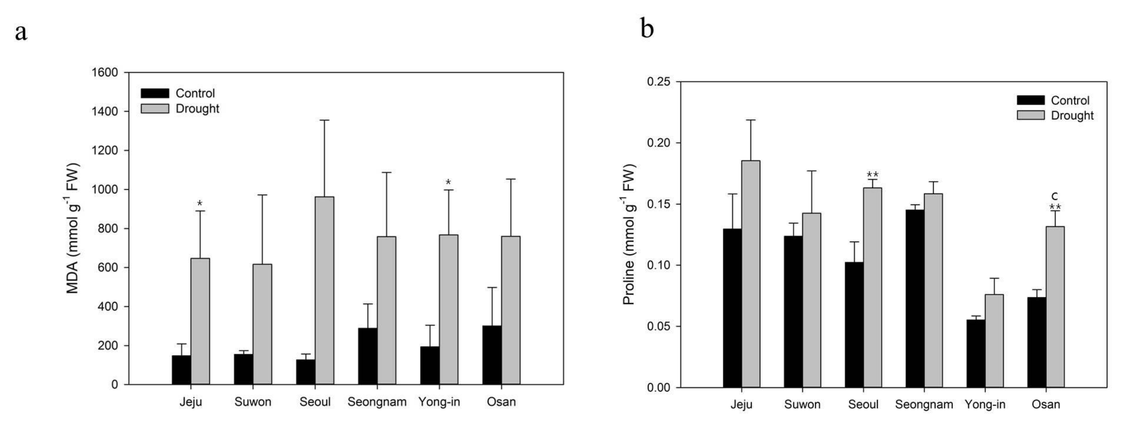
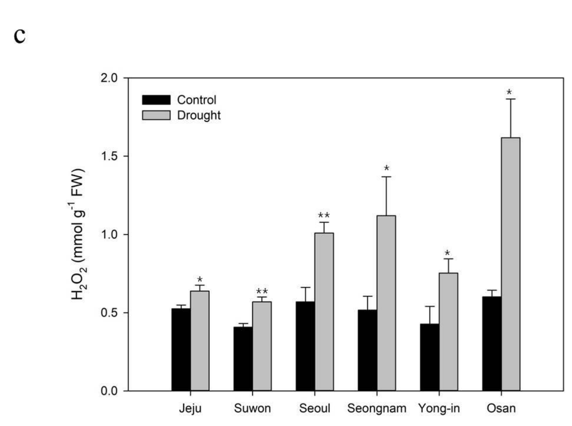


Fig. 4. Measurement of the MDA, proline, and H2O2 levels.
(a) MDA. (b) proline. (c) H2O2. The asterisks indicate significant differences (*t* test; **P* < 0.05, ***P* < 0.01) between the control and drought-treated seedlings.



⇒The stress indicators showed difference changes according to seedlings.

CONCLUSIONS

⇒ The genetic distance and cluster analysis showed the genetic differentiation of the populations in Seongnam and Suwon from the others.
⇒ The measurements of carotenoid, total soluble sugar, and H_2O_2 showed increases in all populations under drought treatment.
⇒ The seedlings of Seoul, Seongnam, and Osan sensitively react to drought stress, showed a significant increase in the level of contents, compared with the seedlings of Jeju, Suwon, and Yong-in.