Regular Article
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 39, No. 4, pp. 246-253, December, 2023
https://doi.org/10.7747/JFES.2023.39.4.246

JFES
Journal of Forest and Environmental Science

Evaluation of Drought Tolerance for Biomass Production of *Salix gracilistyla* Miq.

Hyun Jin Song[1], Seong Hyeon Yong[2], Hak Gon Kim[3], Kwan Been Park[2], Do Hyeon Kim[2], Seung A Cha[2], Ji Hyun Lee[2] and Myung Suk Choi[2,*]

[1]*Department of Plant Variety Examination, National Forest Seed and Variety Center, Chungju 27495, Republic of Korea*
[2]*Division of Environmental Forest Science & Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea*
[3]*Department of Forest Research, Gyeongsangnam-do Forest Environment Research Institute, Jinju 52615, Republic of Korea*

Abstract

Salix gracilistyla is widely distributed along riversides in Korea and very good for biomass production by SRC because of its excellent germination ability, but it is necessary to measure drying tolerance for cultivation. The drought tolerance of *S. gracilistyla* was tested using cuttings, and growth and physiological analysis were performed after irrigation was stopped. The growth inhibition of *S. gracilistyla* was observed from the day irrigation was stopped, and the soil moisture content decreased to less than 10% on the 25th day after irrigation was stopped. Over 50% of the seedlings turned brown 25 days after watering was stopped. The chlorophyll content of *S. gracilistyla* decreased dramatically after 25 days of stopping of irrigation. RWC values were unchanged until day 12 after irrigation was stopped but decreased rapidly until day 21, but there was a slightly decreasing trend after that. RWL levels increased slightly during irrigation stops. The proline content of plants subjected to drought stress was 0.91-2.63 mg/0.05 g, 2.75 times higher than that of the control treatment. The sugar content of the drought stress treatment group was 29.77 to 350.66 mg/0.05 g, which increased 12.24 times that of the control treatment. As a result of this study, *S. gracilistyla* was found to have a drought tolerance almost comparable to that of evergreen broad-leaved trees growing on the land. This study is expected to contribute to the resource utilization *S. gracilistyla*, a native willow tree of Korea, and the mass production of biomass by SRC.

Key Words: drought tolerance, irrigation, *Salix gracilistyla*, proline, reducing sugar

Introduction

Among environmental stresses, drought stress is the most important stressor that changes the moisture status of plants and severely restricts plant growth and development (Munns and Tester 2008). Drought is the most frequent occurrence that restricts the growth, development and production of trees and crops (Boyer 1982). In particular, drought stress is considered one of the most important factors limiting crop yield worldwide (Munns 1993). Dehydration of plant tissue under drought stress can lead to increased oxidative stress that causes degradation of chloroplast structure and associated loss of chlorophyll. Drought stress eventually leads to a decrease in photosynthetic activity.

The dehydration process during drought is characterized by fundamental changes in water relations, biochemical and physiological processes, membrane structure and microstructure of subcellular organelles (Aziz et al.

Received: June 14, 2023. Revised: September 4, 2023. Accepted: October 31, 2023.

Corresponding author: Myung Suk Choi

Division of Environmental Forest Science & Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
Tel: +82-55-772-1856, Fax: +82-55-772-1859, E-mail: mschoi@gnu.ac.kr

1999; Watanabe et al. 2000). The response of wild plants and crops to drought occurs in all tissues across space and time and is driven by a complex set of stress response factors. Drought stress on plants shows various growth patterns and biochemical and physiological responses (Ortiz et al. 2015). In particular, the stress caused by drought causes biochemical changes such as proline and soluble sugars.

About 400 species of plants belonging to the genus *Salix* are distributed worldwide (Mabberley 2017), and in Korea, *Salix chaenomeloides* is generally the most representative species, and *Salix babylonica*, *Salix gracilistyla*, and *Salix koriyanagi* are present (Kim 2018). Willow is the most commonly used short-rotation shrub (SRC) and produces biomass sustainably when planted at densities of 10,000-20,000 clones ha^{-1} (Weih et al. 2019; Baker et al. 2022). SRC systems using willow are already commercially produced in European countries such as Sweden, England, Ireland, and Denmark (Guidi et al. 2013; Lindegaard et al. 2016).

S. gracilistyla Miq. is known to be predominantly distributed in the riparian vegetation zone of natural wetlands in Korea (Chun et al. 1999). In addition, *S. gracilistyla*s is widely used for soil improvement, such as slope greening and reclamation, because of its rapid growth and easy asexual propagation. It is also used in industry to clean up contaminated soil and wastewater, such as removing nutrients from rivers and accumulating through heavy metal absorption (Martin and Stephens 2006). Studies on *S. gracilistyla* are limited to biomass production, such as the measurement of *S. gracilistylas* biomass production in the Japanese river basin (Sasaki and Nakatsubo 2003) and the selection of Korean native *S. gracilistylas* excellent clones (Lee et al. 2018), but there are no studies on abiotic stress resistance.

S. gracilistyla is a willow tree that grows a lot in Korea, but it needs management due to changes in the river ecosystem due to climate change. In addition, it is necessary to construct an SRC system using excellent germination ability. For biomass production by SRC, optimal management conditions such as moisture should be established. However, the evaluation of drought tolerance of *S. gracilistyla* has yet to be studied. This study aimed to conduct various physiological and biochemical assays to evaluate the drying tolerance of *S. gracilistyla*.

Materials and Methods

Plant material and drought stress treatment

Plants used in the drought stress experiment were *Salix gracilistyla* cuttings. The cuttings of *S. gracilistyla* were collected from the academic forest of Gyeongsang National University. After starting cuttings in early March, growth was induced, and plants of the same size as in Table 1 were used in the experiment.

Drought-stress experiments were conducted on potted plants in a greenhouse. Plants were respectively exposed to drought stress. The drought stress assignment did not water the plants. All potting was done in a greenhouse controlled under natural light conditions, 50% RH±10% and 25±5℃. All treatments were grown under the conditions described above for 5 weeks. All experiments were performed in five replicates for each treatment.

Measurement of soil moisture content

The pot size used in the experiment is L 14.0×W 14.0×H 12.5 cm. After drought treatment on the plant, the pot's soil is collected on each measurement date, put in a desiccator, and dried for three days based on 70 degrees. After weighing that dry soil, the moisture content of the soil was measured according to the formula below.

Moisture content was calculated as the mass (g) of the wet soil sample (wet weight−tare weight, Mw) and the mass (g) of the dry soil sample (dry weight−tare weight, Md), and the formula (Eq. 1).

$$SMC\ (\%) = (M_w - M_d)/M_d \times 100 \quad \text{(Eq. 1)}$$

Table 1. Characteristics of *S. gracilistyla* used in this study

Shoot length (cm)	Shoot number (No.)	Leaf number (No.)	Leaf width (cm)	Leaf length (cm)	Leaf thickness (mm)	Diameter (mm)
30.46±3.38	3.80±0.83	31.00±4.52	1.20±0.26	6.04±1.09	0.15±0.01	0.88±0.13

Measurement of leaf wilting and chlorophyll content

Axillary bud growth was measured at 3-day intervals for 5 weeks after drought treatment. The chlorophyll content of the dry treatment and control treatment was measured at noon (11:00-13:30 h) using a SPAD measuring instrument (SPAD 502, Spectrum Technologies Plainfield, IL, USA). Chlorophyll measurements were taken from the second leaf of the shoot.

The visual condition, or degree of wilting, of the leaves of experimental trees is used as a stress indicator. (Anderegg et al. 2013). The wilting rate visually expressed the discolored portion of the total leaves of the tree as a percentage.

Measurement of leaf water content in drought stressed plant

Leaf relative water content (RWC) was determined using the equation below (Turner 1986; Boutraa and Sanders 2001), where FW is leaf fresh weight, DW is leaf dry weight, and TW is saturated leaf fresh weight. The samples for RWC were also weighed immediately as fresh weight (FW), then sliced into 2 cm sections and floated on distilled water for 4 h. The turgid leaf discs were then rapidly blotted to remove surface water and weighed to obtain turgid weight (TW). The leaf discs were dried in the oven at 60°C for 24 h, then dry weight (DW) was obtained. The RWC value was obtained by Eq. 2.

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100 \quad (Eq.\ 2)$$

Relative water loss (RWL) was measured as follows; The leaf samples were weighed (FW), wilted for 4 hours at 35°C, reweighed (W4h), and oven dried for 24 hours at 72°C to obtain dry weight (DW). The RWL was calculated using the following formula (Eq. 3):

$$RWL\ (\%) = [(FM - W4h)/(FW - DW)] \times 100 \quad (Eq.\ 3)$$

Analysis of proline content in drought stressed plant

Proline content was measured according to the method of Bates et al. (1973). Plant leaf material (0.05 g) was ground into 5 mL of 3% sulfosalicylic acid. The homogenate was filtered (Whatman no. 1 filter paper), and 2 mL glacial acetic acid and 2 mL acid ninhydrin reagent was added to 2 mL filtrate. The mixture was then shaken by hand and incubated in a boiling water bath at 100°C for 1 h. After that, it was transferred to an ice bath and warmed to room temperature. 4 mL toluene was added to the mixture, and the top toluene layer was measured at 520 nm using a UV spectrophotometer. After preparing a standard curve, the proline content was quantified. "$Y = 0.6998x - 0.6441$ ($R^2 = 0.981$)" for leaves (Fig. 1).

Measurement of reducing sugar in drought stressed plant

The DNS method determined the total sugar content concentration of glucose (Miller 1959). DNS (3,5-dinitrosalicylic acid) reagent was prepared by dissolving 1 g of DNS and 30 g of sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45°C. After dissolution, the solution was cooled to room temperature and diluted to 100 mL with the help of distilled water. For the measurement, 2 mL of DNS reagent was pipetted into a test tube containing 1 mL of plant extract (1 mg/mL) and kept at 95°C for 5 min. After cooling, 7 mL of distilled water was added to the solution, and the absorbance of the resulting solution was measured at 540 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800). Various concentrations (50, 100, 150, and 200 μg/mL) of glucose were measured for the calibration curve. The sugar content of the hydrolyzed biomass was calculated using the formula $Y = 0.0045x - 0.044$ ($R^2 = 0.988$) for the stem, where "Y" represents the refractometer index, "x" is the glucose concentration (Fig. 2).

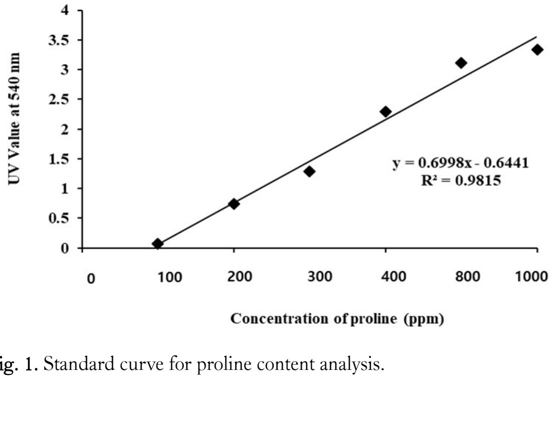

Fig. 1. Standard curve for proline content analysis.

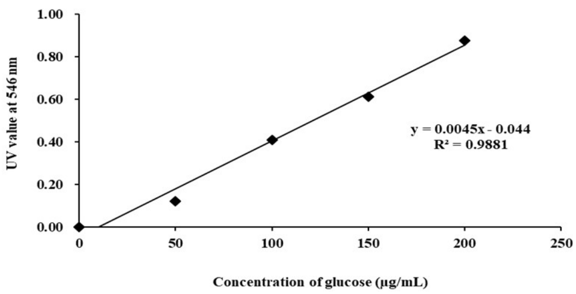

Fig. 2. Standard curve of glucose for reducing sugar assay.

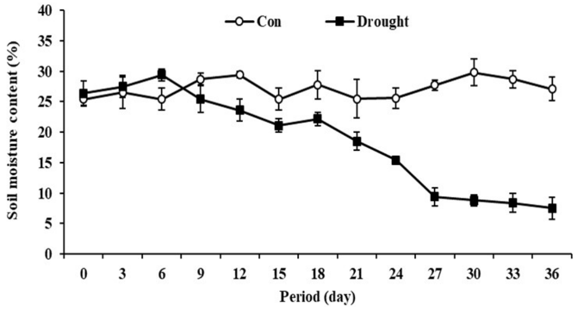

Fig. 3. Soil moisture content of pots planted with drought stressed plants. Results are expressed as the mean±standard deviation ($p < 0.05$) obtained from 5 replicates.

Statistical analysis

Mean values were taken from measurements of five replicates, and the Standard Error of the means was calculated. Differences between means were determined by one-way ANOVA and Turkey's multiple-range tests ($p < 0.05$). Analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 27.0).

Results

Soil moisture content

The soil moisture content was measured in a drought stress treatment port (Fig. 3). The water content of a control treatment after 36 days was determined from 25.4 to 29.8%. Soil moisture was lower from the day irrigation stopped irrigation and lower than 10% after 25 days from irrigation stops. Soil moisture was dramatically decreased after 18 days of irrigation stops.

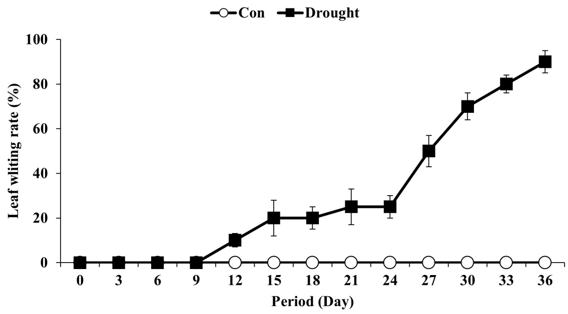

Fig. 4. Leaf wilting rate of *S. gracillstyla* based on drought stress treatment.

Fig. 5. Appearance of drought stressed *S. gracilistyla*. (A) After 1 week, (B) after 2 weeks, (C) after 3 weeks, (D) after 4 weeks, (E) 5 weeks after stopping of irrigation.

Growing characterization of drought stressed plants

Drought stress treatment affected the plant survival of *S. gracilistyla* (Figs. 4, 5). Leaf wilting was observed during the drought stress period. Wilting of drought-stressed plants was not observed until 9 days after irrigation was stopped, and wilting gradually increased until the 24th day thereafter. Wilting of *S. gracilistyla* leaves increased rapidly 24 days after irrigation was stopped. After stopping irrigation, the leaf wilting rate was 10-20% by the 18th day, 20-30% by the 24th day, 50% by the 25th day, and 90% by the 36th day. Four weeks after irrigation was stopped, the willow trees stopped growing completely and their leaves shriveled due to drought stress.

Chlorophyll content was influenced by the degree of drought stress (Fig. 6). As a result, the chlorophyll content decreased, and irrigation was hardly initiated. Chlorophyll content gradually decreased until 25 days after irrigation was stopped and then rapidly decreased. Before day 25, the chlorophyll content was observed to be more than 20% and

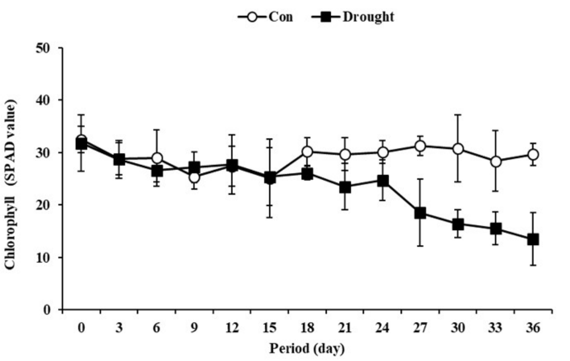

Fig. 6. Chlorophyll content (SPAD) of drought treated plants. Results are presented as the mean±standard error ($p < 0.05$) obtained from 5 replicates.

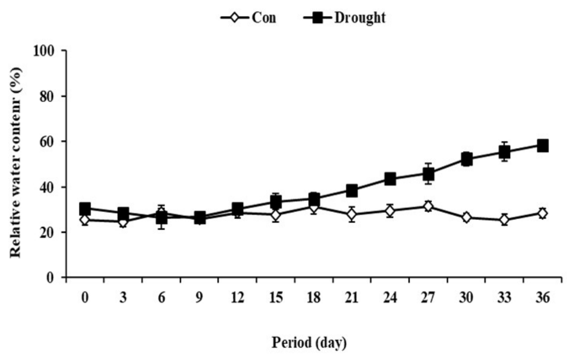

Fig. 8. Relative water loss in drought stress-treated plants. Results are expressed as the mean±standard error ($p < 0.05$) obtained from 5 replicates.

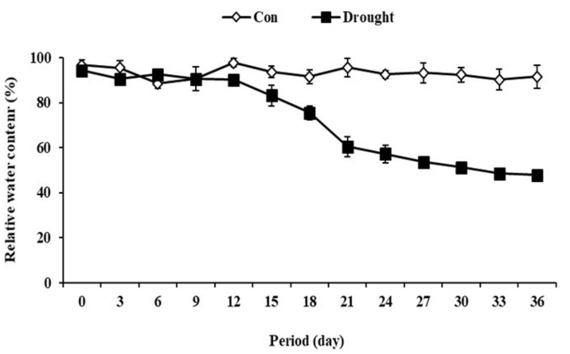

Fig. 7. Relative water content of plants treated with drought stress. Results are expressed as the mean±standard error ($p < 0.05$) obtained from 5 replicates.

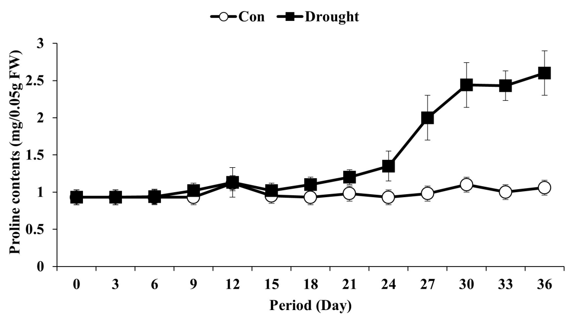

Fig. 9. Proline content of drought stress-treated plants. Results are presented as the mean±standard error ($p < 0.05$) obtained from 5 replicates.

less than 20% after day 25.

Leaf water content of drought stressed plant

Dry stress treatment affected the water content of *S. gracilistyla* (Fig. 7). RWC values showed different results between the control and drought stress treatments. RWC values were unchanged until day 12 after irrigation was stopped but decreased rapidly until day 21, but there was a slightly decreasing trend after that. The RWC values of the control group were 87.84-98.14% until day 36, but the RWC values of the drought stress treatments were 80% on day 15, 70-80% on day 15-18, and 70-80% and 47.71% on day 36 after irrigation was discontinued.

RWL showed different results between control and drought stress treatments (Fig. 8). RWL gradually increased with increasing time after cessation of irrigation. RWL levels ranged from 24.62 to 31.51% for 36 days after stopping irrigation.

Proline content of drought stressed plant

Drought-stressed plants showed increased proline content (Fig. 9). The proline content in control without drying was 0.91-0.98 mg/0.05 g. Proline content increased slightly by 24 days after irrigation was stopped. However, proline content increased dramatically after 25 days. The proline content of plants subjected to drought stress was 0.91-2.63 mg/0.05 g, 2.75 times higher than that of the control treatment.

Reducing sugar content of drought stressed plant

The total sugar content also increased in the drought stress treatments (Fig. 10). The sugar content of the dry stress untreated group was 28.45-29.94 mg/0.05 g. However, when drought stress was applied, the sugar content slightly increased until the 24th day and rapidly after the 25th day after irrigation. Sugar contents ranged from 29.77 to 350.66 mg/0.05 g during drought stress treatment. The

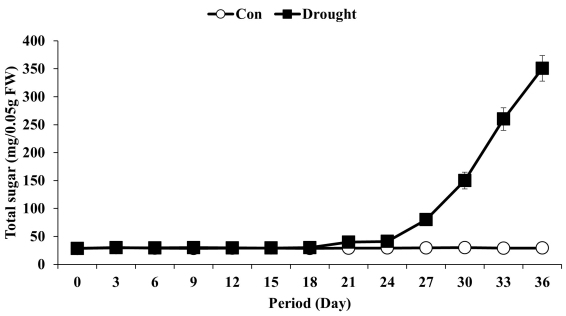

Fig. 10. Total reducing sugar content of drought stress-treated plants. Results are presented as the mean ± standard error ($p < 0.05$) obtained from 5 replicates.

sugar content of the dry stress treatment group increased 12.24 times that of the control treatment.

Discussion

Characterization of drought stressed plants

The drought tolerance of *S. gracilistyla* was tested using cuttings. In *S. gracilistyla*, growth inhibition and leaf wilting were observed from the day irrigation was stopped. In particular, the chlorophyll content decreased with the stopping of irrigation. These results indicate that soil and leaf moisture deprivation progressively inhibited photosynthesis in *S. gracilistyla*.

Wilting of leaves is known as one of the ways plants overcome a lack of moisture. Plants reduce canopy by reducing the growth and shedding of older leaves to minimize water loss (Fischer and Turner 1978) is included. Accelerated leaf senescence and leaf shedding are associated with natural drought to reduce canopy size (Rivero et al. 2007). This strategy contributes to plant survival and completion of the plant life cycle under drought stress in perennial plants.

Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, affecting chlorophyll components and damaging the photosynthetic apparatus (Iturbe-Ormaetxe et al. 1998). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff 1995). Decreased or unchanged chlorophyll levels during drought stress have been reported to vary with the duration and severity of drought (Kyparissis et al. 1995). A decrease in total chlorophyll due to drought stress means reduced light-harvesting ability. Since the production of reactive oxygen species is primarily driven by excessive energy absorption in the photosynthetic apparatus, it can be avoided by breaking down the absorbing pigment (Herbinger et al. 2002).

Leaf water content in drought stressed plant

The RWC of leaves decreased as the drought passed. On the other hand, relative water loss from leaves increased. Moisture deprivation has been found to reduce plant leaves' relative water content (RWC). These aspects appeared similar to this study. The RWC value of drought stress treatment of willow was 80% for 15 days.

RWC and RWL are related to drought tolerance, and these parameters have been proposed as more critical indicators of water status than other water potential parameters under drought stress (Kane et al. 1999; Rout et al. 2000; Sudherson et al. 2003). RWL and RWC values showed opposite results. The RWL values of *S. gracilistyla* plants subjected to drought stress increased as the drying period increased.

Moisture transpiration of plants first procures moisture from leaves and nearby sapwood. It is known that in the same environment, the lower the relative moisture content, the weaker the drought tolerance, and the higher the relative moisture content, the stronger the drought tolerance (Zhenhua et al. 2012). The RWC value (50%) after 36 days of drought stresstreatment is judged to be similar to *Cinnamomum camphora*, *Dendropanax morbifera*, and *Daphniphyllum macropodum*, which are known to have poor drought tolerance (Jin et al. 2017).

Proline and sugar content in drought stressed plant

Proline and sugar contents increased in *S. gracilistyla* plants subjected to drought stress. Jin et al. (2017) showed that *Daphniphyllum Macropodum*, *Cinnamomum camphora*, and *Dendropanax morbifera* species with low drought tolerance had high proline content, but the proline content of *Camellia japonica*, *Machilus thunbergi*, and *Quercus glauca*, which are species with high drought tolerance, were relatively low. Compared to the study by Jin et al. (2017), the proline content of *S. gracilistyla* treated with drought stress was similar to or slightly higher than that of *D. macropodum*, *C. camphora*, and *D. morbifera*, which had low drying tolerance. It shows drying tolerance comparable to that of temperate broad-leaved trees.

Proline is a very common amino acid in nature and has the function of regulating osmotic pressure, and is known to affect plasma cell membrane protection, carbon and nitrogen sources, and removal of reactive oxygen species (Ludlow and Muchow 1990). Proline indicates the degree of stress by controlling the absorption and movement of K^+ ions in plants (Liu and Zhu 1997).

The soluble sugar content of *S. gracilistyla* increased with drought stress treatment. Various authors have pointed out the role of soluble sugars as a protective mechanism against stress (El-Tayeb 2006). The soluble sugar content was also reported to increase as drought stress increased in corn and durum wheat (*Triticum durum* Desf) (Dhanda and Sethi 1998; Mohammadkhani and Heidari 2008).

In plants, the amount of proline and sugar is observed differently depending on the degree of drought tolerance of the plant. Thus, proline and sucrose can assess plant drought tolerance or susceptibility (Golestani Araghi and Assad 1998; Keles and Oncel 2004). As can be seen from these results, the measurement of proline and sugar was a valuable tool to measure the drought tolerance of *S. gracilistyla*.

As a result of this study, *S. gracilistyla* was found to exhibit drought tolerance similar to terrestrial evergreen broad-leaved trees such as *D. macropodum, C. camphora*, and *D. morbifera*, and it was judged to be suitable as an SRC for biomass production. However, this study is not a comparative experiment through direct planting with evergreen broad-leaved trees, so further detailed research is required. However, this study is expected to contribute to establishing an SRC system for resource utilization and high biomass production of *S. gracilistyla*, a willow native to Korea.

Acknowledgements

This work was supported by the "Multiple stress tolerance assessment and customized mapping of domestic major afforestation tree species to prepare for climate change (No. 2017R1D1A1B04036320)" of National Research Foundation of Korea.

References

Anderegg WRL, Kane JM, Anderegg LDL. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Clim Change 3: 30-36.

Aziz A, Martin-Tanguy J, Larher F. 1999. Salt stress-induced proline accumulation and changes in tyramine and polyamine levels are linked to ionic adjustment in tomato leaf discs. Plant Sci 145: 83-91.

Baker P, Charlton A, Johnston C, Leahy JJ, Lindegaard K, Pisano I, Prendergast J, Preskett D, Skinner C. 2022. A review of Willow (Salix spp.) as an integrated biorefinery feedstock. Ind Crops Prod 189: 115823.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207.

Boutraa T, Sanders FE. 2001. Influence of water stress on grain yield and vegetative growth of two cultivars of bean (*Phaseolus vulgaris* L.). J Agron Crop Sci 187: 251-257.

Boyer JS. 1982. Plant productivity and environment. Science 218: 443-448.

Chun SH, Hyun JY, Choi JK. 1999. A study on the distribution patterns of *Salix gracilistyla* and Phragmites japonica communities according to micro-landforms and substrates of the stream corridor. J Korean Inst Landsc Archit 27: 58-68.

Dhanda SS, Sethi GS. 1998. Inheritance of excised-leaf water loss and relative water content in bread wheat (Triticum aestivum). Euphytica 104: 39-47.

El-Tayeb MA. 2006. Differential response of two Vicia faba cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agron Hung 54: 25-37.

Fischer RA, Turner NC. 1978. Plant productivity in the arid and semiarid zones. Annu Rev Plant Physiol 29: 277-317.

Golestani Araghi S, Assad MT. 1998. Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. Euphytica 103: 293-299.

Guidi W, Pitre FE, Labrecque M. 2013. Short-rotation coppice of willows for the production of biomass in Eastern Canada. In: Biomass Now-Suitable Growth and Use (Matovic MD, ed). InTech, London, pp 421-448.

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A, Grill D. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiol Biochem 40: 691-696.

Iturbe-Ormaetxe I, Escuredo PR, Arrese-Igor C, Becana M. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol 116: 173-181.

Jin EJ, Cho MG, Bae EJ, Park J, Lee KS, Choi MS. 2017. Physiological responses to drought stress of seven evergreen hardwood species. J Korean Soc For Sci 106: 397-407.

Kane ME, Davis GL, McConnell DB, Gargiulo JA. 1999. In vitro propagation of Cryptocoryne wendtii. Aquat Bot 63:

197-202.

Keles Y, Oncel I. 2004. Growth and solute composition in two wheat species experiencing combined influence of stress conditions. Russ J Plant Physiol 51: 203-209.

Kim MH. 2018. Antioxidant activity and anti-inflammatory effects of Salix Koreensis andersson branches extracts. J Korean Soc Food Cult 33: 104-111.

Kyparissis A, Petropoulou Y, Manetas Y. 1995. Summer survival of leaves in a soft-leaved shrub (Phlomis fruticosa L., Labiatae) under mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. J Exp Bot 46: 1825-1831.

Lee H, An C, Kang J, Lee W, Yi JS. 2018. Investigation of growth characteristics of *Salix gracilistyla* clones for promoting woody biomass resources. J Korean Soc For Sci 107: 16-24.

Lindegaard KN, Adams PW, Holley M, Lamley A, Henriksson A, Larsson S, von Engelbrechten HG, Esteban Lopez G, Pisarek M. 2016. Short rotation plantations policy history in Europe: lessons from the past and recommendations for the future. Food Energy Secur 5: 125-152.

Liu J, Zhu JK. 1997. Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of Arabidopsis. Plant Physiol 114: 591-596.

Ludlow MM, Muchow RC. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43: 107-153.

Mabberley DJ. 2017. Mabberley's plant-book: a portable dictionary of plants, their classification and uses. Cambridge University Press, Cambridge.

Martin PJ, Stephens W. 2006. Willow growth in response to nutrients and moisture on a clay landfill cap soil. I. Growth and biomass production. Bioresour Technol 97: 437-448.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426-428.

Mohammadkhani N, Heidari R. 2008. Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl Sci J 3: 448-453.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651-681.

Munns R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ 16: 15-24.

Ortiz N, Armada E, Duque E, Roldán A, Azcón R. 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. J Plant Physiol 174: 87-96.

Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E. 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci U S A 104: 19631-19636.

Rout GR, Samantaray S, Das P. 2000. In vitro manipulation and propagation of medicinal plants. Biotechnol Adv 18: 91-120.

Sasaki A, Nakatsubo T. 2003. Biomass and production of the riparian shrub *Salix gracilistyla*. Ecol Civil Eng 6: 35-44.

Smirnoff N. 1995. Environment and plant metabolism: flexibility and acclimation. BIOS Scientific Publishers, Oxford.

Sudherson C, AboEl-Nil M, Hussain J. 2003. Tissue culture technology for the conservation and propagation of certain native plants. J Arid Environ 54: 133-147.

Turner NC. 1986. Adaptation to water deficits: a changing perspective. Aust J Plant Physiol 13: 175-190.

Watanabe S, Kojima K, Ide Y, Sasaki S. 2000. Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. Plant Cell Tissue Organ Cult 63: 199-206.

Weih M, Glynn C, Baum C. 2019. Willow short-rotation coppice as model system for exploring ecological theory on biodiversity-ecosystem function. Diversity 11: 125.

Zhenhua X, Jun L, Jingping X, Ting Z, XinYan Y. 2012. The study on drought resistance of roof greening woody plants. Chin Agric Sci Bull (1): 311-316.