Evaluation of Sargent's cherry (*Prunus sargentii* Rehder) drought stress level through photosynthesis and chlorophyll fluorescence parameters and proline content analysis

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Sargent's cherry tree (*Prunus sargentii* Rehder) is widely planted as an ornamental, climate change-sensing species. This study investigated the changes in soil moisture content, fresh weight, photosynthesis and chlorophyll fluorescence properties, chlorophyll and proline content according to irrigation and no watering for 30 days in 4-year-old seedlings of *P. sargentii* tree. In the dry treatment area, the soil moisture content decreased and the fresh weight of the above-ground part also decreased. However, there was no significant difference in the root growth of the dried plants. Among the photosynthesis parameters, $P_{n\,max}$, E, and g_s showed a significant ($p<0.000$) decrease after 15 days in dry-stressed seedlings, and in the case of WUE, there was no difference between treatments until 20 days, but there was a significant ($p<0.000$) effect after 24 days. Chlorophyll fluorescence parameters F_v/F_m, ΦPSII, R_{fd}, NPQ, $P_{n\,max}$ also increased after 10 days in dry-stressed seedlings, but there was no significant difference in the control treatment. These results may suggest that drought stress has a high correlation between photosynthesis and chlorophyll fluorescence parameters. Chlorophyll content also showed a significant decrease in the seedlings under drought stress compared with the control group. The proline content decreased until the 10th day of the drying treatment, and increased after the 15th day, showing an increase of 10.9% on the 15th day and 57.1% on the 30th day compared to the control treatment. These results suggest that photosynthesis, chlorophyll fluorescence parameters, and proline content can be usefully used to evaluate drought stress in trees. In addition, it seems that it will greatly contribute to the management of forests, such as irrigation of trees at a time when pore control ability and photosynthesis ability decrease.

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Abstract

Sargent's cherry tree (*Prunus sargentii* Rehder) is widely planted as an ornamental, climate change-sensing species. This study investigated the changes in soil moisture content, fresh weight, photosynthesis and chlorophyll fluorescence properties, and chlorophyll and proline content according to irrigation and no watering for 30 days in 4-year-old seedlings of *P. sargentii* tree. In the dry treatment area, the soil moisture content decreased, and the fresh weight of the above-ground part decreased. However, there was no significant difference in the root growth of the dried plants. Among the photosynthesis parameters, $\underline{Pn}_{MAX}$, E, and gs showed a significant ($p<0.000$) decrease after 15 days in dry-stressed seedlings, and in the case of WUE, there was no difference between treatments until 20 days. However, there was a significant ($p<0.000$) effect after 24 days. Chlorophyll fluorescence parameters Fv/Fm, ΦPSⅡ, R_{fd}, NPQ, and $\underline{Pn}_{MAX}$ also increased after 10 days in dry-stressed seedlings, but there was no significant difference in the control treatment. These results may suggest that drought stress highly correlates with photosynthesis and chlorophyll fluorescence parameters. Chlorophyll content also significantly decreased the seedlings under drought stress compared with the control treatment. The proline content decreased until the 10th day of the drying treatment and increased after the 15th day, showing an increase of 10.9% on the 15th and 57.1% on the 30th day compared to the control treatment. These results suggest that photosynthesis, chlorophyll fluorescence parameters, and proline content can be used to evaluate drought stress in trees. In addition, it seems that it will significantly contribute to the management of forests, such as the irrigation of trees when pore control ability and photosynthesis ability decrease.

Subjects Ecology, Soil Science, Biogeochemistry, Forestry

Keywords Sargent's cherry, drought stress, photosynthesis, chlorophyll fluorescence, proline

Introduction

According to the Climate Change Commission (IPCC, 2014), the frequency of high-temperature events has increased in many countries worldwide, and the index surface temperature change is projected to exceed 2°C by the end of the 21st century compared to 1850–1900. This scenario is a range of environmental stress that can cause severe consequences for the growth of crops and trees (Mittler and Blumwald, 2010; Lee et al., 2018). In particular, from January 1 to June 30, 2017, the cumulative precipitation throughout South Korea was 224.4 mm, accounting for 48.5% of the average precipitation, the worst drought since 1973 (Korea Forest Service, 2017). As such, it is difficult to accurately predict and respond to water shortages according to weather conditions every year, and a lack of water in plant growth can impair healthy growth and lead to death. Plants have a variety of survival strategies to cope with water stress. This ability is a quantitative trait of a plant and exhibits moisture tolerance through the interaction of various mechanisms (Oh et al., 2005). Therefore, a complex study is required to understand tree species' physiological damage and adaptation mechanisms caused by predicted climate change.

In the initial response of plants to drought, factors affected by turgor pressure, such as leaf expansion and shoot elongation, are reduced, and mechanisms such as leaf detachment and stomata closure increase water conservation and water use efficiency in the body and then reduce extreme stress. When received, it causes a significant decrease in the photosynthetic rate, loss of osmoregulation, and severe disturbances in significant intracellular metabolism, resulting in permanent damage (Taiz and Zeiger, 2006). As the drought stress increased, the maximum photosynthetic rate (Pn_{MAX}) of *the Dendropanax morbiferus* tree decreased (Lee, 2018). decreased and has been shown to affect photosynthetic capacity (Lee and Lee, 2017). According to Kim and Park(2013), dark respiration and net proton yield tended to decrease rapidly as the unwatered period increased, while water utilization efficiency increased, showing resistance to maintaining photosynthetic ability even under poor moisture conditions. A recent study comparatively analyzed stomatal and non-stomatal limitations to predict the photosynthetic response to water deprivation (Drake et al., 2017; Salmon et al., 2020) as a valuable indicator for predicting the effect of water stress. (Campos et al., 2014; Chen et al., 2015; Gimeno et al., 2019).

The photosynthetic ability of plants can be quantified through chlorophyll fluorescence and is used as a representative non-destructive assay to evaluate plant health. The energy absorbed by

chlorophyll is 1) used for photosynthesis, 2) emitted as a long wave by heat dissipation, and 3) the remaining dissipated energy is emitted as fluorescence (Mishra et al., 2012). Due to the competition between these three processes, chlorophyll can be used to obtain photosynthesis information (Maxwell and Johnson, 2000; Murchie and Lawson, 2013). Recently, researchers have been able to quickly and easily measure changes in the structure and function of Photosystem II through the measurement of chlorophyll fluorescence in various environments to diagnose early abiotic stresses (moisture, drought, high temperature, low temperature, salt and nutrient deficiency) of plants. Although it has been widely used as a physiological indicator (Iqbal et al., 2019; Xu et al., 2020), the selection of chlorophyll fluorescence index as an indicator according to drought stress and evaluation of the possibility of supplementation of moisture management for the possibility of using the selection index is also necessary.

Several pigments are involved in photosynthesis; the most important pigment is chlorophyll. Leaves have two fluorescence emission peaks located the 685nm of the red region(LD685) and 740nm of the far-red region(LD740)(Buschmann, 2007), which are closely related to the chlorophyll content(Kalmatskaya et al., 2016; Nyachiro et al., 2001). LD685 and LD740 are good indicators of chlorophyll and have been demonstrated to reflect photosynthetic activity (Baker, 2008; D'ambrosio et al., 1992). However, a comprehensive study on fluorescence kinetic parameters and fluorescence spectrum that can be used to evaluate the response of leaves to drought stress is still insufficient (Magney et al., 2017).

In addition, plants that are resistant to poor environments have various organic substances in the cytoplasm, such as turgor pressure caused by drought stress, intracellular concentration (Lichtenthaler 1996; Bray, 1997), and alleviation of osmotic stress (Kishor et al., 1995) to maintain moisture in cells, and regulates gene expression according to unfavorable environments. Proline plays an essential role in osmotic pressure regulation as an osmoprotectant in many plants affected by various environmental stresses such as salinity and drought stress (Giri, 2011; Semida et al., 2015; Arteaga et al., 2020). In particular, storage of energy and amino nitrogen has been reported to play an important role in the rapid restoration of cellular homeostasis and recovery after drought stress (Verbruggen et al., 1996), and proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio et al. 2002).

The Sargent's cherry tree (*Prunus sargentii* Rehder) is a Korean native tree belonging to the Rosaceae deciduous broad-leaved tree (Figure 1a). It has strong cold resistance, so it can be

adapted anywhere in the country and grows well on the seaside. In addition, as an intolerant shade tree, it thrives in flat, fertile soil with high humidity, grows very quickly and has strong resistance to air pollution (Cho and Choi, 1992). In the meantime, Sargent's cherry have been selected and planted as a tree species recommended for reforestation by the Korea Forest Service, considering their growth characteristics. According to statistical data from the Korea Forest Service (2020), Sargent's cherry trees are currently the second most planted after ginkgo trees, accounting for 11.1% of 2003(859,000 trees, second place) among street trees in Korea, and pine trees (37.9%) in the National Preferred Tree Survey (Korea Forest Service, 2022). they are followed by cherry trees (16.2%, 3rd place) for their high scenic value.

Recently, due to climate change, a withering of Sargent's cherry trees frequently occurs in street planting sites. However, it is still very difficult to plant and manage roadside trees due to the significant lack of abiotic and physiological data such as drying and light of wild cherry trees. Various physiological responses to drought stress will be very useful for selecting and managing street tree planting species. In other words, the following hypotheses were established to investigate the physiological mechanism of drying Sargent's cherry trees. 1) There will be significant correlations between soil moisture content and growth, photosynthesis, and chlorophyll fluorescence parameters of grafted Sargent's cherry trees, and 2) as a result of prevalence studies, Sargent's cherry trees will increase water utilization efficiency while maintaining photosynthetic efficiency in dry conditions. 3) The degree of drought resistance of Sargent's cherry trees could be identified by analyzing soil moisture content, chlorophyll fluorescence response, and proline content. Therefore, this study was conducted to find out the optimal moisture environmental conditions and drought resistance mechanism by examining various physiological responses according to continuous drought stress targeting wild cherry trees, where the planting of street trees is increasing.

Materials & Methods

Planting materials, experimental design, and environmental variables

The four-year-old *Sargent's cherry* (*Prunus sargentii* Rehder) used in the experiment was 1 in January 2017 at the Sargent's cherry Tree Genetic Resource Conservation Center (E 126˚56'03", N 33˚31'06") of the Warm Temperate and Subtropical Forest Research Center of the National Institute of Forest Science. Annual branches were harvested and grafted. Grafted seedlings were grown in a greenhouse (E 128˚10'08", N 35˚16'33") in the Forest Biomaterials Research Institute of the National Institute of Forest Science, and 100 seeds were transplanted into a 40 L air pot in March 2021. The transplanted soil was mixed with Masato and bed soil in a ratio of 1:1, and it was used in the experiment after being acclimatized in a greenhouse for 5 months before drying.

Dry stress was induced through artificial water treatment for about 1 month from August 1, 2021, to August 31. Among the 100 transplanted individuals, 66 individuals (root diameter 13.0±2.6 cm, height 2.0±0.4m) were selected for control (10 individuals) and treatment (56 individuals: 8 individuals x 7 times measurement). Separated by In the case of the control treatment, direct irrigation was conducted to maintain the soil moisture content at 15.0±0.5% until the end of the survey (Fig. 1).

After stopping the irrigation, a temperature and humidity measuring device (HOBO H08-004-02, ONSET, USE) was installed at 1m above the ground to measure environmental factors in the greenhouse during the period of the experiment. Photon Systems Instruments Co., Drasov, Czech) was used every day from 13:00 to 14:00. During the experiment, the average temperature was 24.2±5.7°C, the highest temperature was 37.6°C, the lowest temperature was 12.5°C, and the average daily temperature difference the experiment was 15.3℃ (18.1~28.4℃), which was relatively large (Fig. 2a). The average relative humidity was set to 68.6±18.9% (Fig. 2b). The average solar radiation was set to 468.1 $W \cdot mm^{-2}$.

Measurement of growth parameters and soil water content

In order to compare the effect on growth according to the control and dry stress treatment period during the cultivation period, three specimens were collected at intervals of 5 days, divided into aboveground parts (stems, leaves) and underground parts (roots), and the fresh weight of each part was measured. Dry weight measurement was divided into aboveground parts (stems, leaves) and

underground parts (roots), washed thoroughly with tap water, and then dried in a dry oven at 70 ° C for 48 hours, and then the weight of each part was measured. -SMD-M005, ONSET, USA) was measured 5 times every 20 minutes at a depth of 10 cm on the soil surface.

Analysis of photosynthetic characters

Photosynthesis was measured for healthy leaves per unit leaf area using a portable photosynthesis system (Portable Photosynthesis system, Li-6400, Li-Cor Inc., USA) from 09:00 to 15:00 when photosynthesis is active on a sunny day. Maximum photosynthesis rate ($P_{n\ MAX}$), stomatal transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), etc. Photosynthesis was measured at intervals of 5 days, and 15 repetitions per object (3 objects x 5 leaves) were measured 7 times.

PPFD controls the light intensity (Photosynthetic Photon Flux Density) using an LED light source attached to a portable photosynthetic measuring device in 8 steps (0, 100, 200, 400, 800, 1,000, 1,400, and 1,800 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

At this time, the common measurement conditions were measured while maintaining the inflow air flow into the chamber at 500 $\mu mol \cdot s^{-1}$ and the temperature at 20±2°C. All measured data are automatically saved in the Date Logger, and the maximum photosynthetic rate, stomatal transpiration rate, and stomatal conductivity per unit leaf surface area were automatically calculated using the formulas of Von Caemmerer and Farquhar (1981). It is expressed as the value obtained by dividing the transpiration rate, $\mu molCO_2 \cdot mmol\ H_2O^{-1}$.

Analysis of chlorophyll fluorescence

A total of 105 measurements were taken, 15 repetitions each (5 leaves of 3 individuals) every 5 days for 30 days from the point of no watering. The measurement time was from 13:00 to 14:00, and the position of the measured leaves was measured at the 13th to 15th leaves from the growing point until the 10th day of treatment, and after the 15th day, the 7th to 10th leaves from the growing point was investigated. The same leaf as the photosynthesis measurement was used as the target, and the leaf clip was bitten on the plant leaf before measurement and irradiated after 20 minutes of dark treatment. Fv/Fm, YPSII, RFd, and NPQ were measured by quenching kinetics analysis after 20 minutes of dark treatment in a chlorophyll fluorescence analyzer chamber using a Handy Cam (FlorCam, CZ) (Barbagallo et al., 2003; Genty et al., 1989). Actinic and continuous light was

used to induce chlorophyll fluorescence in the measurement. At this time, the analysis conditions of the Handy Cam are actinic light (red LED); 200 μmol·m-2·s-1 , saturating light, moderate light; It was 1,250 μmol·m-2·s-1. The measured data were analyzed by the method presented by Gorbe et al. (2012). In addition, PIABS was calculated using a Chlorophyll fluorescence meter (FP-100, Photon System Instruments, Czech Republic) according to the method of JIP-TEST (Stirbet and Govindjee, 2011) (Table 1).

Analysis of chlorophyll contents

Chlorophyll content measurement was compared and analyzed after collecting leaves before drying treatment and every 5 days for 30 days (7 times in total) after drought stress. Chlorophyll was extracted from leaves using dimethyl sulfoxide (DMSO) as an extraction solvent according to Hiscox and Israelstam (1978). The extract was obtained by measuring the absorbance at wavelengths of 663 nm and 645 nm using a UV-Vis spectrophotometer (Nicolet Evolution 100, Thermo Electrom Co., USA), and the contents of chlorophyll a and b were obtained by the following formula (Arnon, 1949; Mackinney, 1941).

Analysis of proline contents

Proline analysis was performed according to the method of Bates et al.(1973). The leaves were collected before the drying treatment and every 5 days for 30 days(7 times in total) after the drying treatment. After collecting 0.1 g(15 total repetitions) of each leaf, 10 mL of a sulfosalicylic acid solution (3%, w/v) was added, followed by a mortar grinding. The grinding solution was filtered with two layers of filter paper (Whatman No. 42). After adding 1 mL of glacial acetic acid and 1 mL of ninhydrin reagent to 1 mL of the filtrate, the test tube was capped, reacted in boiling water (100°C) for 1 hour, and then stored at room temperature (21.0°C) for 5 minutes. 2 mL of toluene was added to that, stirred for 20 seconds, the supernatant was taken, and the wavelength was measured at 520 nm using a UV spectrophotometer (X-ma 2000, Human Crop.). Quantitation was calculated according to a calibration curve prepared using proline (Sigma-Aldrich Co., USA) as standard material and expressed as μmol proline/g FW.

Statistical analysis

The homogeneity of data variance was tested using Levene's test. Data on physiological

indicators were analyzed using SPSS software (ver. 27.0; SPSS Inc., Chicago, IL, USA) by one-way ANOVA, which takes the elapsed time after a single treatment as a factor. At this time, Duncan's multiple range test performed the difference between averages at the 5% significance level (DMRT, $p<0.05$). Before performing the analysis of variance, the data sets were checked for homogeneity of error variances using the Shapiro-Wilk test in SPSS software to ensure that the homogeneity assumption was not violated. In addition, Pearson's correlation analysis by the R statistical package (R-x64-4.0.4) was performed on the correlation between each physiological indicator according to the drying treatment.

Results

Effect of drought stress on plant growth and changes in soil moisture content

As a result of examining the soil moisture content and fresh weight during the experiment period, the decrease in soil moisture content was significantly higher in the drought stress treatments than in the control (Fig. 3). Immediately after irrigation, it was 20.1%, and from the 2nd day to 10 days after irrigation was stopped. It was in the range of 22.7~18.4% until the 15th~19th day, and it was in the range of 10% (9.6~5.4%) or less, and it decreased to the level of 1% or less after the 20th day. In comparison, in control, the above-ground part increased by 4.3% and the underground part by 3.5%, whereas in the drought stress treatments, the above-ground part decreased by 17.9% and the underground part by 7.2%. Soil moisture content was found to be less than 10%.

Effect of drought Stress on response of leaf photosynthetic traits

The maximum photosynthetic rate, stomatal transpiration rate, stomatal conductivity, and water utilization efficiency measured in *P. sargentii* leaves showed significant differences between treatments as the experimental period increased ($p<0.05$)(Fig. 4). The maximum photosynthetic rate in the drought stress treatments showed a significant decrease from the 15th day of no watering, showing a 29.8% decrease compared to before drought treatment to 7.22±0.66 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. On the 30th day, 2.15±0.79 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. showed an 80.1% reduction rate (Fig. 4a, b). In particular, the maximum photosynthetic rate decreased sharply after 15 days of drying treatment when the soil moisture content decreased by 9.6 to 5.4%.

In addition, there was no significant difference in stomatal conductance on days 25 and 30 after drought stress treatment (Fig. 4c, d, e, f). The stomatal transpiration rate showed a significant difference after 15 days between treatments, and compared to the control (1.35±0.04 mol $CO_2 \cdot m^{-2} \cdot s^{-1}$.), the dry treatments were 0.97±0.02 mol $CO_2 \cdot m^{-2} \cdot s^{-1}$. before treatment. It showed a decrease rate of 33.0% compared to that. This decreased at the same time as the maximum photosynthetic rate when the soil moisture content was reduced to less than 10%. The pore conductivity also showed a significant difference after 15 days of drying, and the dry treatment zone was 0.04±0.00 mol CO_2·m-2·s-1 which decreased by 77.7% compared to the control (0.16±0.01 mol $CO_2 \cdot m^{-2} \cdot s^{-1}$.).

Compared to the control treatment, water utilization efficiency temporarily increased after 10 days of drying treatment. Still, it decreased from the 15th day when the maximum photosynthetic rate decreased (Fig. 4g, h).

Effect of drought Stress on response of leaf chlorophyll fluorescence

The four indices of the measured chlorophyll fluorescence response decreased after the 15th day of the drought stress treatment, showing a significant difference between the treatments. After the 20th day, the drought stress treatments showed a sharp decrease (Fig. 5a, b). F_v/F_m, which shows the maximum quantum yield of photosystem II in the dark adaptation state, was 0.84±0.02 in the control treatment before treatment and 0.80±0.01 in the dry stress treatments. After 15 days, it was 0.82±0.02 in the control treatment and 0.57±0.04 in the dry stress treatments, respectively. 0.2% and 2.88% decreased. After 30 days of drying treatment, the control treatment showed a decrease of 4.8% and 43.4%, respectively, to 0.78±0.03 and 0.45±0.02 in the dry treatments.

The decrease in chlorophyll fluorescence (R_{fd}) was 5.16±0.32 in control and 5.21±0.09 in the dry stress treatments before dry treatment. After 15 days, the control was 5.20±0.09, and the drought treatment was 2.35±0.15, which was 54.9 in the dry stress treatments compared to the control treatment. % decreased. After 30 days, the control was 5.30±0.11 and that of the dry stress treatments, 0.89±0.02, which increased by 2.6% in control but decreased by 83.0% in the dry treatments, showing sensitivity to drought stress(Fig. 5e, f).

Non-optical fluorescence extinction (NPQ) also showed a difference between treatments after 15 days of drying. Compared to before drying treatment, the control decreased by 1.0% to 3.02±0.65, whereas the dry treatments decreased by 43.3% to 1.02±0.05. After 30 days, the control

treatment increased by 6.7% to 3.31±0.11, but the dry treatments showed a decrease of 83.2% (Fig. 5g, h).

In addition, PI_{ABS} showed a significant decrease in the dry treatments compared to the control treatment, decreased from 5.48±1.07 after 15 days of drying to 32.4% compared to before treatment, and showed a high reduction rate of 82.1% after 30 days (Fig. 5i, j).

Overall, the chlorophyll fluorescence reaction showed a significant decrease in the dry treatments compared to the control treatment. In particular, after the 15th day of the drying treatment, the energy captured for use in the photochemical process decreased. The energy not used for electron transfer increased, so the activity was shown to reduce.

Effect of drought stress on response of leaf chlorophyll

The chlorophyll content showed a significant difference between treatments ($p<0.05$), and the chlorophyll a and b content showed similar values until the 10th day of the drying treatment(Fig. 6). Still, after the 15th day, the dry treatment showed a significantly lower value than the control treatment. The total chlorophyll content decreased by 13.8% to 4.98±0.21 mg g^{-1} in the control treatment after 15 days compared to before drying treatment, and a 42.1% decrease in the dry treated to 3.80±0.11 mg g-1 and dried after 30 days. The treatments showed a decline of 77.8% to 1.46±0.25 mg g^{-1}.

306 **Effect of drought stress on response of leaf proline**

307 The proline content of the control treatment was 2.24 mg at the start of the test and 1.67 mg
308 after 30 days of drying treatment (Fig. 7a, b). There was no statistically significant difference in
309 the proline content of the control during the drought stress application period. On the other hand,
310 in the dry treatments, there was no significant difference at 1.52±0.02 to 1.51±0.06 mg from before
311 the treatment to 10 days, and 1.35±0.08 mg on the 15th day, which decreased by 11.6% compared
312 to before treatment, and was 3.90±0.18 mg after 30 days, which was 61.1%. Increased these results
313 were the same as when the fresh weight decreased, and the photosynthesis and chlorophyll
314 fluorescence values decreased when the soil moisture content was less than 10%.
315
316 **Correlation among each factors**

317 Correlation analysis between photosynthesis, chlorophyll fluorescence, chlorophyll and proline
318 content activity of *P. sargentii* trees according to drought stress was performed (Fig. 8 & Table 2).
319 $P_{n\,MAX}$ is E($r = 0.98^{***}$), PI_{ABS} ($r = 0.96^{***}$), R_{fd} ($r = 0.93^{***}$), g_s ($r = 0.90^{***}$) is a positive There was
320 a correlation, and the proline content($r = -0.74^{*}$) showed a negative correlation. Proline content
321 negatively correlated with other parameters except for WUE ($r = 0.69$). Among the chlorophyll
322 fluorescence parameters, F_v/F_m is g_s ($r = 0.78^{*}$), ΦPSII is g_s ($r = 0.97^{***}$), E ($r = 0.91^{***}$), $P_{n\,MAX}$ (r
323 $= 0.89^{**}$), R_{fd} is g_s($r = 0.98^{***}$), E($r = 0.94^{***}$), $P_{n\,MAX}$ ($r = 0.93^{***}$), NPQ is g_s($r = 0.95^{***}$), E($r =$
324 0.87^{*}), $P_{n\,MAX}$ ($r = 0.88^{**}$), PI_{ABS} is g_s ($r = 0.86^{**}$), E ($r = 0.97^{***}$), $P_{n\,MAX}$ ($r = 0.96^{***}$) for
325 photosynthetic properties and chlorophyll fluorescence. There was a significant correlation
326 between chlorophyll content showed a significant positive correlation with E, F_v/F_m, ΦPSII, R_{fd},
327 and negative correlation with proline content.
328
329

330 ## Discussion

331 **Changes in plant growth and soil moisture content by drought stress**

332 Moisture and temperature are important factors affecting tree growth and physiological
333 characteristics (Wu et al., 2011; Rustad et al., 2001). Dry stress is a significant limiting factor in
334 plants' initial growth and establishment stages, affecting both cell length growth and hypertrophy
335 (Kusaka et al., 2005; Shao et al., 2004). In general, when plants are under drought stress, they
336 reduce the ratio of above-ground to underground parts and develop deeper roots to reduce water

consumption and enhance uptake (Pallar and Rhoads, 1993). In this study, as the soil moisture content decreased, the above-ground fresh weight decreased in the drying treatments compared to the control treatment. However, the underground fresh weight was higher than the control treatment until the 20th day of the drying treatment. However, there was a tendency to decrease after 25 days of drying treatment, but there was no significant ($p>0.99$) difference between treatments. According to Zang et al. (2014), beech trees were divided into a normal drying zone and a strong drying zone, and as a result, root production increased in the normal drying zone, but root production decreased in the strong drying zone. Ratio increased. In the case of Sargent's cherry trees, the above-ground fresh weight did not show much change until the soil moisture content was 5.0% or more (25 days of no irrigation). However, it decreased compared to the control from 1.0% or less soil moisture content (30 days of no irrigation). As seen, damage due to drying stress appeared in the above-ground and underground parts. In previous studies, poor root respiration in plant growth affects the synthesis of new plant tissues and the preservation of living tissues (Ryan and Law, 2005; Lee et al., 2012), and the decrease in root respiration results in the loss of anabolic capacity. It was reported that root growth was restricted as it led to a decrease (Bengough et al., 2006). This study judged that more assimilation materials were invested in the underground part rather than the above-ground part as the production of substances necessary for plant growth decreased due to a short-term response due to drying stress. In addition, it is judged that continuous research on the pattern and mechanism of sensitivity to dryness throughout the ecosystem over a long period is necessary.

Response of leaf photosynthetic traits to drought stress

In general, drought stress induces plants to close their stomata, reducing the CO_2 concentration in the mesophyll, thereby inhibiting photosynthesis or directly inhibiting carbon metabolism, resulting in reduced photosynthesis (Gimenez et al., 1992; Conric, 2000). In this study, the photosynthetic rate, transpiration rate, and stomatal conductance of the treatments subjected to drought stress decreased compared to the control treatment (Fig. 4). This causes physiological disorders in various parts or organs of the plant due to drought stress. In particular, it may cause much damage to the photosynthetic mechanism to cause a decrease in plant growth. Many research results have been reported so far on the effect of drought stress on photosynthesis, and the decrease in photosynthetic efficiency is known to be due to various causes (Chaves and Oliveira, 2004). In

general, it is consistent with studies that drought stress inhibits photosynthesis by inducing plants to close their stomata and reducing the concentration of CO_2 in the mesophyll or directly inhibits carbon metabolism and causes a decrease in photosynthesis (Gimenez et al., 1992; Cornic, 2000). Abscisic acid (ABA) is synthesized when plant roots sense water stress. ABA moves through the xylem, induces various actions such as stomatal control (Zhang and Davies, 1990), and activates defense mechanisms against stress. Promote In this study, and it was also confirmed that the resistance to drought stress was increased by quickly controlling the opening and closing reaction of the stomata by drought stress. Water utilization efficiency is closely related to plant growth, and plants close their stomata to increase their efficiency in a water-poor environment, reducing the transpiration rate more than photosynthesis. However, this efficiency increase in power negatively correlates with plant growth (Richards and Condon, 1993). In the results of this study, it was found that the water utilization efficiency was increased by reducing the transpiration rate by closing the stomata due to drought stress. However, the growth deteriorated due to the decrease in the photosynthetic rate.

Leaf chlorophyll fluorescence response to drought stress

In general, drought stress is an abiotic stress that affects photosynthesis in the short and long term due to the stomatal closure of plants and the inactivation of RuBisCo (Gorbe and Calatayud, 2012). Fv/Fm is a representative chlorophyll fluorescence index that can evaluate the photosynthetic level of plants during dark adaptation and is used to detect various abiotic and biotic stresses (Rungrat et al., 2016). In this study, the Fv/Fm value was also found to decrease after 15 days of drying treatment (Fig. 5a). It is presumed that drought stress inhibited the photochemical activity of photosystem II and reduced the Fv/Fm of leaves. PSII can be damaged under drought stress, inhibiting the primary reactions of photosynthesis (Lichtenthaler and Rinderle, 1988). Fluorescence parameters in leaves are known to be altered in two ways under stress conditions. Minimal fluorescence (Fo) increases due to obstruction of electron flow through PSII, and plastoquinone receptor (QA-) cannot be fully oxidized during stress. Concomitantly, the decrease in Fm during stress may be influenced by the reduced activity of water lyase complexes and accompanying cyclic electron transport in or around PSII (Porcar-Castell et al., 2014).

In PSⅡ, the maximum fluorescence value (FM_LSS) is measured by irradiating saturated light. At the same time, the plant is photosynthesizing, and in this state, when actinic light (light that

causes photosynthesis) is continuously illuminated, the fluorescence decreases and reaches a steady state. It consists of Ft_LSS, the fluorescence value at this time, and represents the photochemical energy conversion efficiency of photosystem II (Schreiber and Bilger, 1993; Stepien and Klobus, 2006; Krause and Weis, 1991; Baker, 2008; Boughalleb et al., 2008). After 15 days of drying treatment, the Apsii value decreased by 56.0%, and it was more sensitive to drought stress than Fv/Fm (Fig. 5c, d). A significant decrease in Bpsii after 15 days of drying treatment means that CO_2 supply as a chloroplast was reduced due to stomatal closure (Zhou et al., 2017). Chlorophyll fluorescence reduction (R_{fd}) reflects the photosynthetic performance or CO_2 fixation rate, and the Rfd value measured under saturated light significantly correlates with the CO_2 fixation rate (Lichtenthaler et al., 2005) and decreases with increasing drought stress. It is known (Méthy et al., 1994). In this study, a significant decrease was confirmed after 15 days of drying treatment, when the photosynthetic rate began to decrease significantly. This is considered to reduce photosynthetic efficiency by reducing the water potential of the leaves and the photosynthetic rate (Lawlor and Cornic, 2002; Chaves and Oliveira, 2004).

NPQ, which refers to the thermal loss of energy in the photosynthetic mechanism during photochemical energy conversion, is known to increase under stress conditions (Genty et al., 1990), but in this study, photosynthesis and transpiration rates began to decrease after 15 days of drying treatment. After that, it showed a high decrease (Fig. 5g, h). This was consistent with previous studies that showed that damage to photosynthetic pigments reduced chlorophyll fluorescence and decreased NPQ (Shin et al., 2021; Kim et al., 2020). In the case of the former, a decrease in Fm_LSS means that in the case of the latter, a decrease in Fm increases. Moreover, since NPQ is related to the thermal dissipation of leaves, it is judged that a comprehensive study considering leaf temperature is necessary to understand plants' growth status.

In the case of PI_{ABS}, which represents the photochemical performance index of photosystem II or plants' vitality level, the drying treatments significantly decreased as the drying treatment period increased compared to the control group. The drying treatments showed a reduced rate of 82.1% (Fig. 5i, j) after 30 days of drying treatment compared to before drying treatment. This result can suggest that, in the case of Sargent's cherry trees, when the soil moisture content is less than 5.0%, the energy captured for use in the photochemical process decreases and the energy not used for electron transfer increases, resulting in a decrease in the activity of photosystem II. PI_{ABS} represents the energy conservation efficiency in electron carrier reduction using absorbed light

energy (Holland et al., 2014). In addition, PIABS is used to evaluate the degree of stress and photosynthetic capacity of plants (Van Heerden et al., 2007), and it has been shown that the more stressed plants are, the lower the PI_{ABS} level (Wang et al., 2012). According to the results of PIABS, it was confirmed that when Sargent's cherry trees are cultivated, the soil moisture content should be managed to maintain a level of 5.0% or more for stable growth. In addition, it is judged that it is possible to evaluate the drought resistance of Sargent's cherry trees by grasping the meaning of various chlorophyll fluorescence indices and analyzing the photosynthetic efficiency using objective values.

Response of leaf chlorophyll traits to drought stress

Proline is a crucial osmotic regulator and free radical scavenger that can alleviate stress damage by reducing water potential (Hayat et al., 2012; Bala, 2000). We found that the proline content of each treatment gradually increased during drought stress. During the drying treatment period, there was no significant difference in the proline content of the control treatment, while the drying treatments showed a significant increase in proline content when the soil moisture content was less than 5.0% (Fig. 7). This is thought to be related to the osmotic adjustment mechanism (Xiao et al., 2008) that protects plants from dehydration due to drought stress and lowers osmotic potential. In addition, the proline content increased after 15 days of drying treatment, and the photosynthetic efficiency significantly decreased. This suggests that it is due to the decrease in stomatal conductance that increases the accumulation of ABA content. Several studies have shown that proline accumulates in dehydrated conditions and is rapidly lost when dehydration conditions are relieved (Blum and Ebercon, 1976; Singh et al., 1973; Stewart, 1972). When osmotic stress is removed, proline is oxidized to Δ1-pyrroline-5-carboxylate (P5C) by proline dehydrogenase, also known as proline oxidase, the first enzyme in the proline degradation pathway. P5C is then converted back to glutamate by the enzyme P5C dehydrogenase (Hare et al., 2002). Therefore, it is considered necessary to study changes in proline content and evaluate tree regeneration ability according to re-irrigation after drying treatment.

Correlation among each factor

Due to drought stress, photosynthesis and chlorophyll fluorescence showed a positive correlation with a significant decrease. A previous study showed reduced chlorophyll fluorescence

parameters following drought stress impaired photosynthetic electron transport (Zhuang et al., 2020). In this study, Pn_{MAX} showed the highest positive correlation with PIABS (r = 0.96***) and Rfd (r = 0.93***). Drought stress damages the reaction center of PSII and inhibits the electron transfer process of photosynthesis, reducing the photosystem II efficiency of light energy conversion (Brestic et al., 1995; Cornic and Fresneau, 2002; Longenberger et al., 2009). . Drought stress alters leaf chloroplast layer structure and reduces chlorophyll content (Batra et al., 2014). Chlorophyll content showed the highest positive correlation with ΦPSII (r = 0.93***), Rfd (r = 092***), and E (r = 091***). In addition, the chlorophyll content decreased as the photosynthetic efficiency and chlorophyll fluorescence parameters decreased. Hypotheses 1 and 2 were verified through the following research results. In previous studies, a decrease in chlorophyll content deteriorated the photochemical process, and the dependence of light absorption and fluorescence emission on the concentration of chlorophyll molecules in chloroplasts was demonstrated (Nyachiro et al., 2001). Proline content negatively correlated with all variables except for WUE (0.69*). Proline content increased as PIABS (-0.78**), E (-0.75**), and Pn_{MAX} (-0.740**) decreased. Proline accumulation is believed to play an adaptive role in plant stress tolerance (Verbruggen and Hermans 2008). Proline Accumulation has been advocated as a selection parameter for stress tolerance (Yancy et al., 1982; Jaleel et al., 2007). Therefore, Pn_{MAX}, E, and PIABS confirmed the drought stress level at an early stage through a significant correlation(Fig. 9.).

Conclusion

As the drought stress continued, the fresh weight of the Sargent's cherry tree tended to decrease by 20.5% compared to the control after 25 days of drying treatment. The photosynthetic efficiency was affected after 15 days of drying treatment. When the soil moisture content was below 10.0%, the decrease in Pn_{MAX}, E, and gs was striking, and WUE temporarily increased. As a result of chlorophyll fluorescence analysis, in the early stage of drought stress, energy absorbed per leaf area and energy captured by the photochemical process first appeared to decrease. It was found that there was a decrease. In particular, ΦPSII, R_{fd}, NPQ, and PI_{ABS} show a positive correlation with photosynthetic efficiency, chlorophyll content, and proline content and are considered suitable indicators for confirming the level of drought stress. In addition, Sargent's cherry trees showed an adaptive response to avoid hydraulic failure by maintaining water potential by reducing stomatal conductance when the soil moisture content was less than 10.0% to cope with drought

stress. It was found that this plant simultaneously exhibits an adaptation to temporarily increase water utilization efficiency to reduce water loss inside the leaves while maintaining photosynthetic efficiency. Through the above results, Sargent's cherry trees showed a response to increase water utilization efficiency and reduce water loss by reducing transpiration through stomatal closure at the beginning in response to drought stress. However, as the soil moisture content decreased to 5.0 to 10.0% or less, the response to drought stress reached its limit, and the loss of electrons in the process of transferring electrons from photosystem II to photosystem I increased, resulting in a significant drop in overall photosynthetic activity. Chlorophyll content was also decreased. Afterward, as the soil moisture content reached below 5.0%, the Pn_{MAX}, E, gs, and chlorophyll fluorescence parameters decreased significantly, and the proline content increased, leading to permanent damage and death. Therefore, for the healthy growth of 4-year-old Sargent's cherry trees, keeping the soil moisture content within 5.0 to 10.0% is good. It was found that improvement was needed. The results of this study identified physiological indicators that can be used to diagnose and manage the damage caused by drought stress in Sergent cherry trees early. This is expected to be widely used to select appropriate species of other woody plants to cope with climate change in the future.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Eon ju Jin and Jun Hyuck Yoon conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Myung Suk Choi authored analyzed the data, or reviewed drafts of the article, and approved the final draft.
- Hyeok Lee analyzed the data, prepared figures and/or tables, and approved the final draft.
- Eun Ji Bae conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the Supplemental Files.

Supplemental Information

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Table 1(on next page)

Chlorophyll fluorescence parameters used in this study.

Table 1: Chlorophyll fluorescence parameters used in this study.

Parameter	Formula	Description
F_v/F_m	$(F_m-F_o)/F_m$	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
ΦPSII	$(F'_m- F_s)/ F'_m$	Effective quantum yield of photochemical energy conversion in PSII
R_{fd}	$(F_{m-} F_s)/ F_s$	Ratio of fluorescence decline
NPQ	$(F_m -F'_m)/ F'_m$	Non-photochemical quenching of maximum fluorescence
PI_{ABS}	$\frac{RC}{ABS} \cdot \frac{\Phi_{Po}}{1 - \Phi_{Po}} \cdot \frac{\Psi_o}{1 - \Psi_o}$	Performance index on absorption basis

Table 2(on next page)

Table 2.Summary of analysis of variance for photosynthesis characteristics, chlorophyll fluorescence, chlorophyll, proline assay of *Prunus sargentii* at the two water levels(control, drought stress) and drought treatment times.

RMANOVA was used estimate the effect of treatment: *, **, and *** indicate significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively. NS: non-significant.

Table 2:

Summary of analysis of variance for photosynthesis characteristics, chlorophyll fluorescence, chlorophyll, proline assay of *Prunus sargentii* at the two water levels(control, drought stress) and drought treatment times.

Parameters	Water Level(W)		Treatment time(T)		W x T	
	F-Value	Significance	F-Value	Significance	F-Value	Significance
$P_{n\ max}$	344.0	***	65.7	***	57.3	***
E	305.5	***	51.7	***	29.1	***
g_s	963.6	***	213.1	***	83.7	***
WUE	29.8	***	8.1	***	6.6	***
F_v/F_m	14.8	**	14.2	**	4.8	**
ΦPSⅡ	635.7	***	87.1	***	92.8	***
R_{fd}	780.9	***	51.6	***	88.4	***
NPQ	258.8	***	14.3	***	23.2	***
PI_{ABS}	202.9	***	27.0	***	13.4	***
Chl. a	12.2	**	6.2	*	2.4	*
Chl. b	10.8	**	12.1	*	2.6	*
Total Chl.	13.4	**	7.4	*	1.8	*
Chl. a/b	6.7	*	13.9	*	2.9	*
Proline	136.7	***	132.1	***	97.9	***

RMANOVA was used estimate the effect of treatment: *, **, and *** indicate significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively. NS: non-significant.

Figure 1

Sargent's cherrytree (*Prunus sargentii*Rehder)(a) and overview of the drying treatment in this study(b).

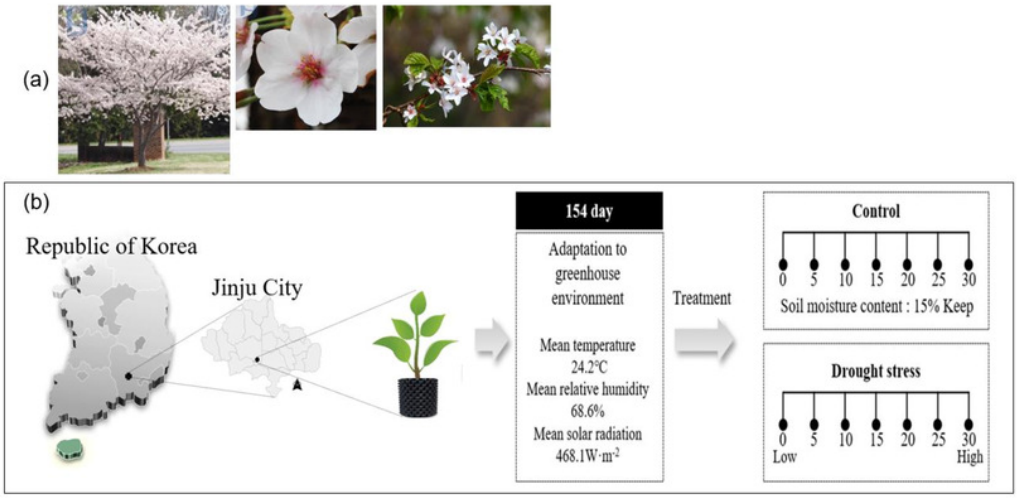
(a)
(b)
Republic of Korea
Jinju City
154 day
Adaptation to greenhouse environment
Mean temperature 24.2℃
Mean relative humidity 68.6%
Mean solar radiation 468.1W·m-2
Treatment
Control
0 5 10 15 20 25 30
Soil moisture content : 15% Keep
Drought stress
0 5 10 15 20 25 30
Low
High

Figure 2

Changes of mean air temperature(a), solar radiation(b) and relative humidity(c) green house on during the experimental period.

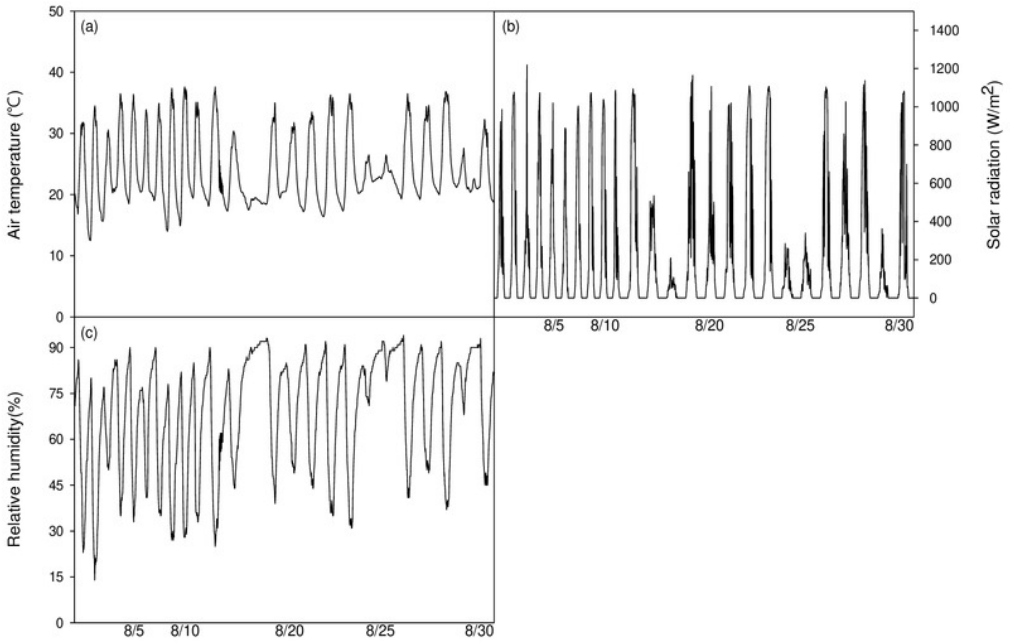
(a)
(b)
(c)
Air temperature (°C)
Solar radiation (W/m2)
Relative humidity(%)
8/5
8/10
8/20
8/25
8/30

Figure 3

Changes in visual appearance of *Prunus sargentii* seedlings (a), Soil water content(b), Shoot(c) and Root(d) fresh weight drought stress conditions during treatment time.

(a), Soil water content(b), Shoot(c) and Root(d) fresh weight drought stress conditions during treatment time. Different letters indicate a significant difference difference at $p<0.05$ by Duncan's multiple range test.

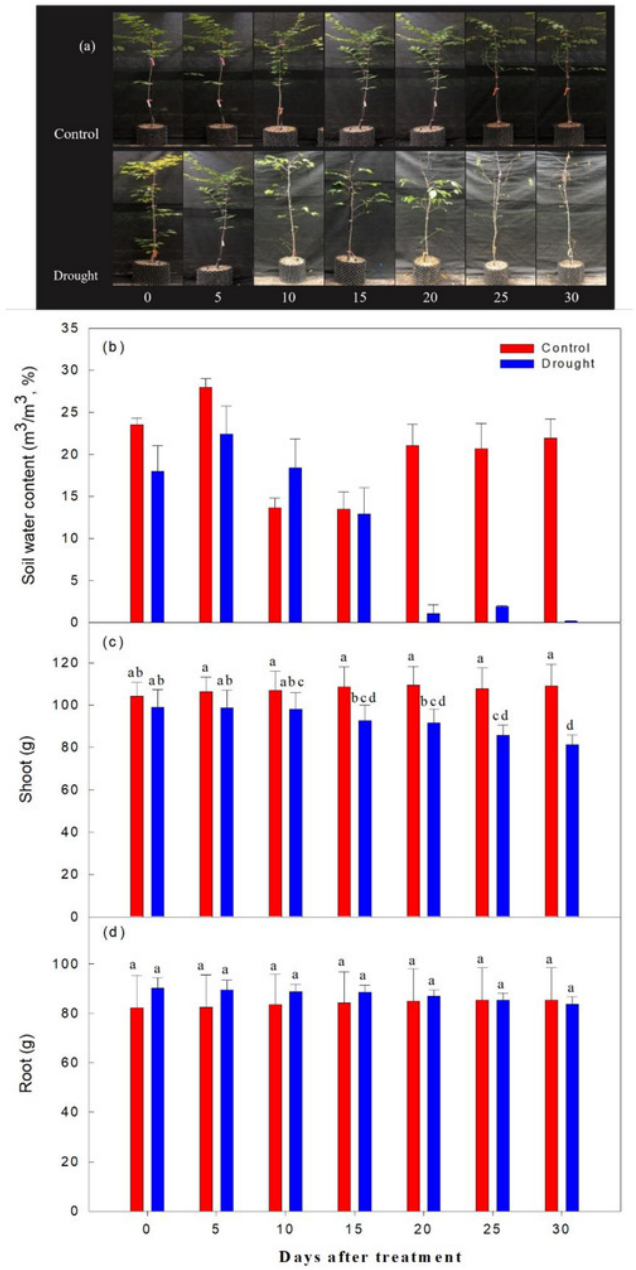
(a)
Control
Drought
0
5
10
15
20
25
30
(b)
Control
Drought
Soil water content (m3/m3, %)
(c)
Shoot (g)
(d)
Root (g)
Days after treatment

Figure 4

Variations of photosynthetic characteristics under control and drought stress of *Prunus sargentii*

(a, b) Maximum photosynthesis rate. (c, d) Stomatal transpiration rate. (e, f) Stomatal conductance. (g, h) WUE(water use efficiency).

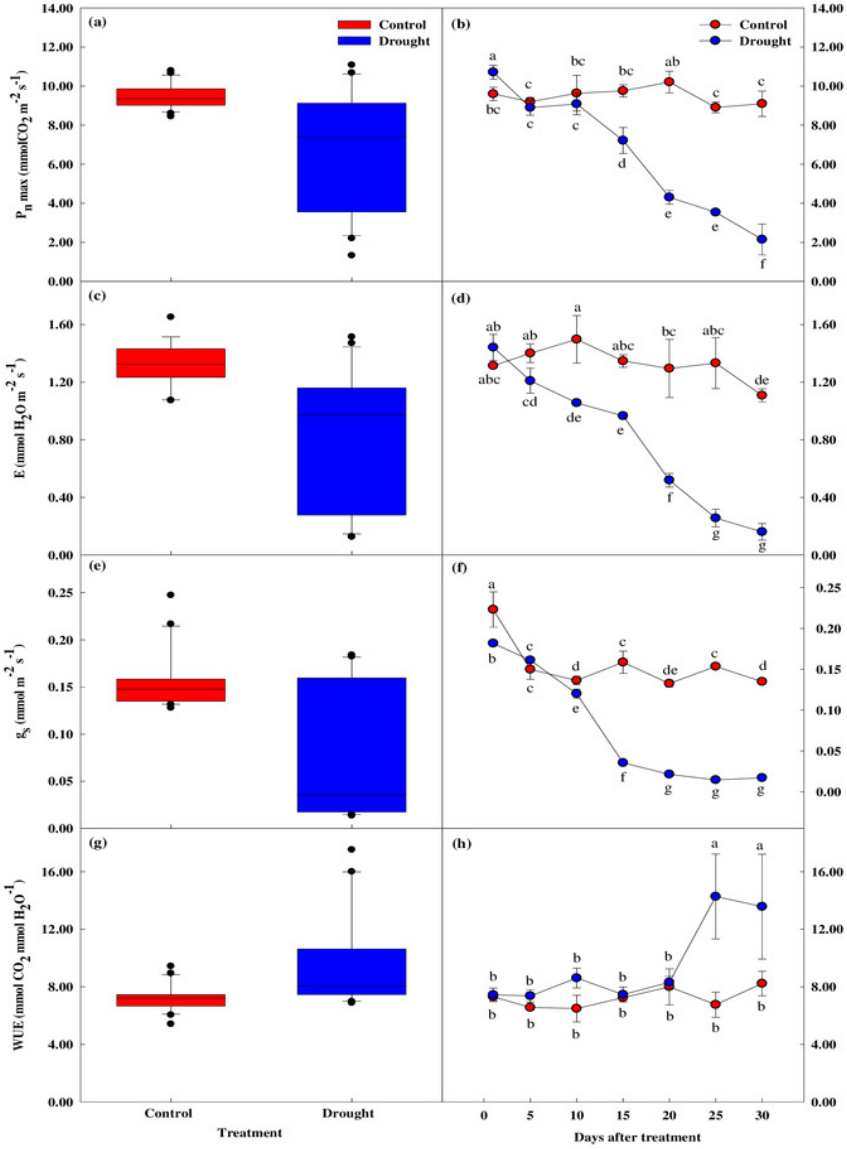
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Control
Drought
P_n max (mmol(CO_2 m^{-2} s^{-1})
E (mmol H_2O m^{-2} s^{-1})
g_s (mmol m^{-2} s^{-1})
WUE (mmol CO_2 mmol H_2O^{-1})
Treatment
Days after treatment

Figure 5(on next page)

Figure 5: Variations of photosynthetic characteristic in control and drought stress. (a, b) F_v/F_m, (c, d) ΦPSⅡ, (e, f) R_{fd}, (g, h) NPQ, (I, j) PI_{ABS} .

In the box plot, the points and short error bars represent the mean (±SE) of n = 21 per treatment, and the line and long error bars represent the median line and 95% CI, respectively. In the line chart, the points and error bars reflect the mean (±SE) of three replicates per treatment per date. The blue and red indicates the control and drought treatment, respectively.

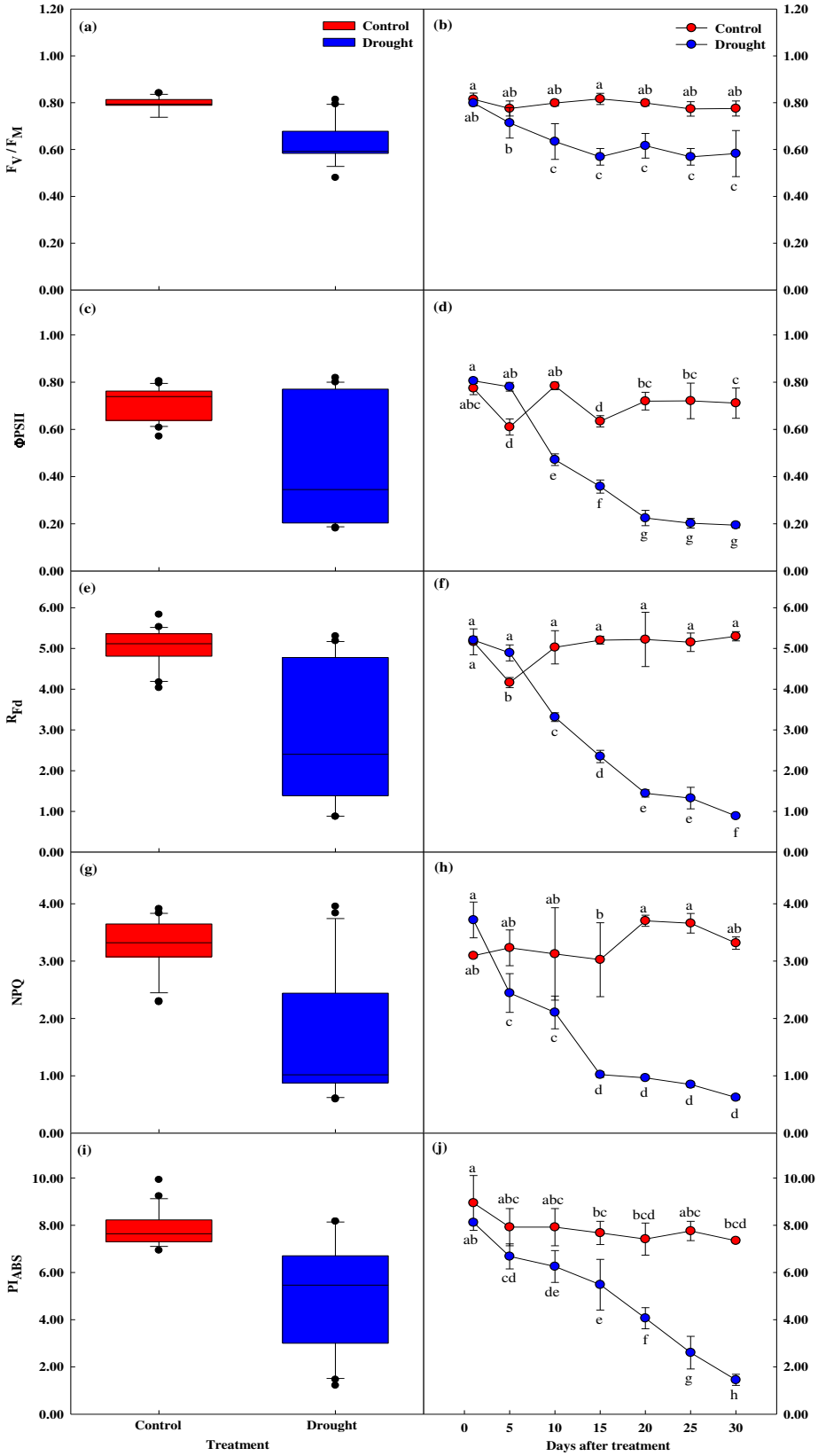
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
Control
Drought
F_V/F_M
ΦPSII
R_{Fd}
NPQ
PI_{ABS}
Treatment
Days after treatment
Control
Drought
0
5
10
15
20
25
30

Figure 6(on next page)

Variations of photosynthetic characteristic in control and drought stress. (a, b) Chl. a, (c, d) Chl. b, (e, f) Total Chl., (g, h) Chl. a/b.

In the box plot, the points and short error bars represent the mean (±SE) of n = 21 per treatment, and the line and long error bars represent the median line and 95% CI, respectively. In the line chart, the points and error bars reflect the mean (±SE) of three replicates per treatment per date. The blue and red indicates the control and drought treatment, respectively.

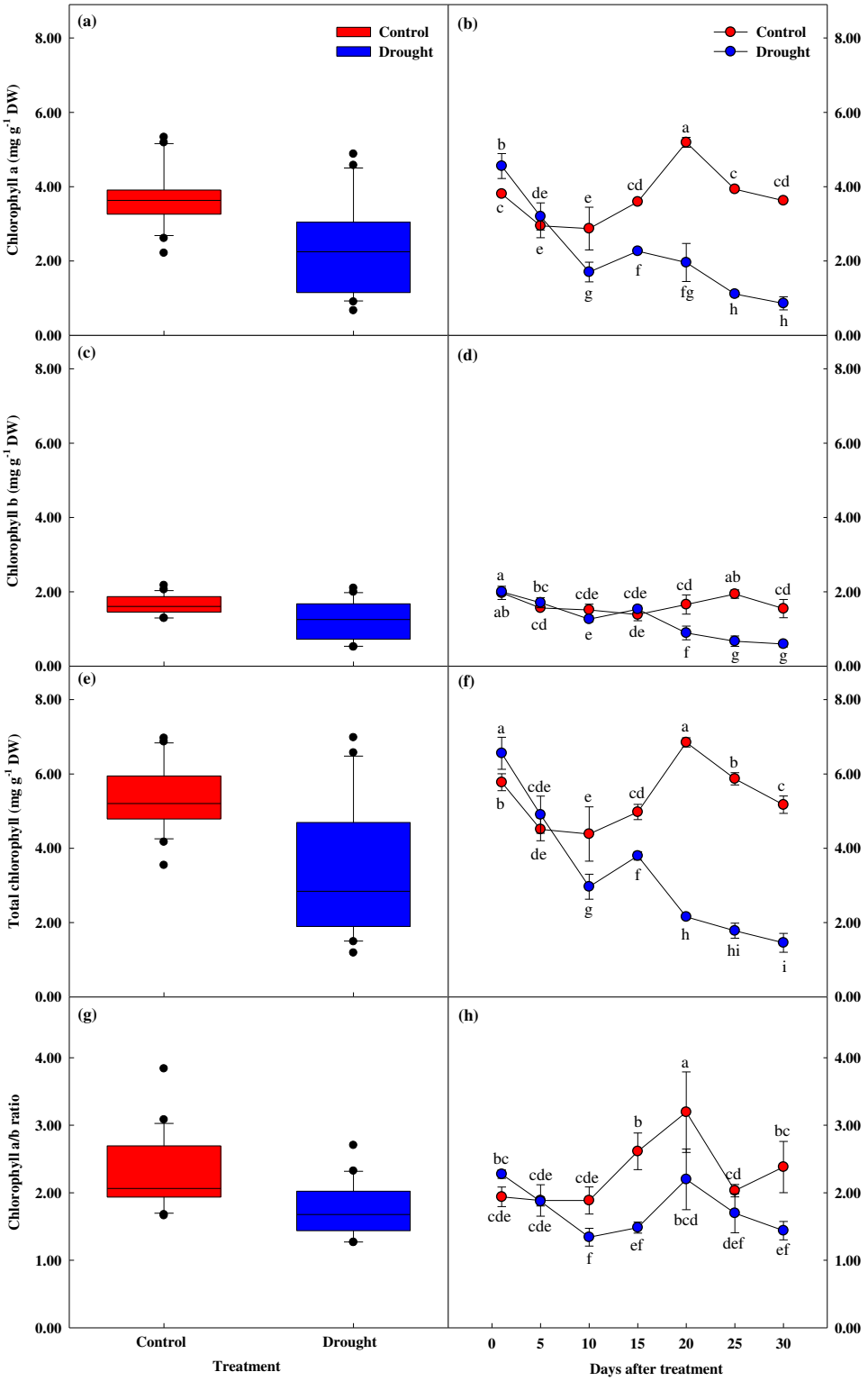
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Control
Drought
Chlorophyll a (mg g-1 DW)
Chlorophyll b (mg g-1 DW)
Total chlorophyll (mg g-1 DW)
Chlorophyll a/b ratio
Treatment
Days after treatment
0
5
10
15
20
25
30

Figure 7

Variations of proline in control and drought stress. (a, b) Proline.

In the box plot, the points and short error bars represent the mean (±SE) of n = 21 per treatment, and the line and long error bars represent the median line and 95% CI, respectively. In the line chart, the points and error bars reflect the mean (±SE) of three replicates per treatment per date. The blue and red indicates the control and drought treatment, respectively.

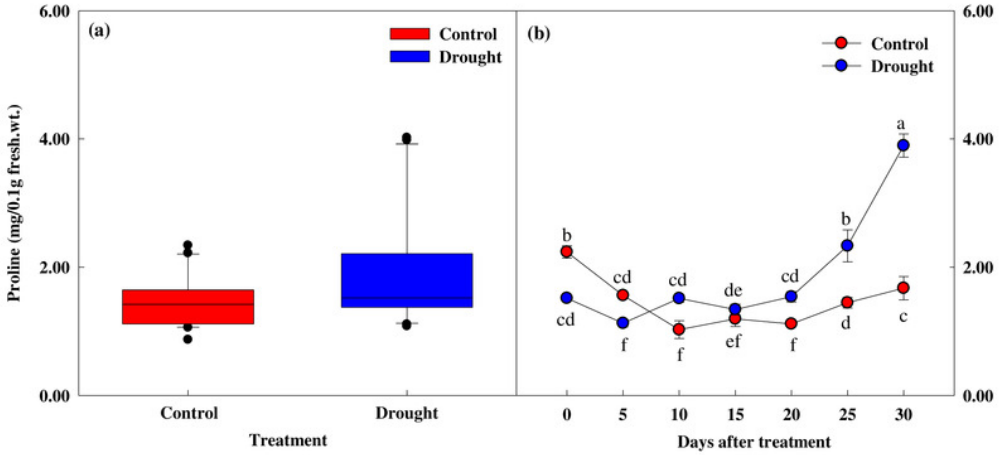
(a)
Control
Drought
Proline (mg/0.1g fresh.wt.)
6.00
4.00
2.00
0.00
Control
Drought
Treatment
(b)
Control
Drought
b
cd
cd
de
cd
b
a
cd
f
f
ef
f
d
c
0
5
10
15
20
25
30
Days after treatment

Figure 8

Correlation analysis for photosynthesis, chlorophyll fluorescence parameters, chlorophyll, and proline contents in *P. sargentii* seedlings, regardless of treatment length or drought stress.

Blue and red boxes represent positive and negative correlation, respectively. Color intensities are proportional to the correlation coefficients, as shown in the legend to the right.

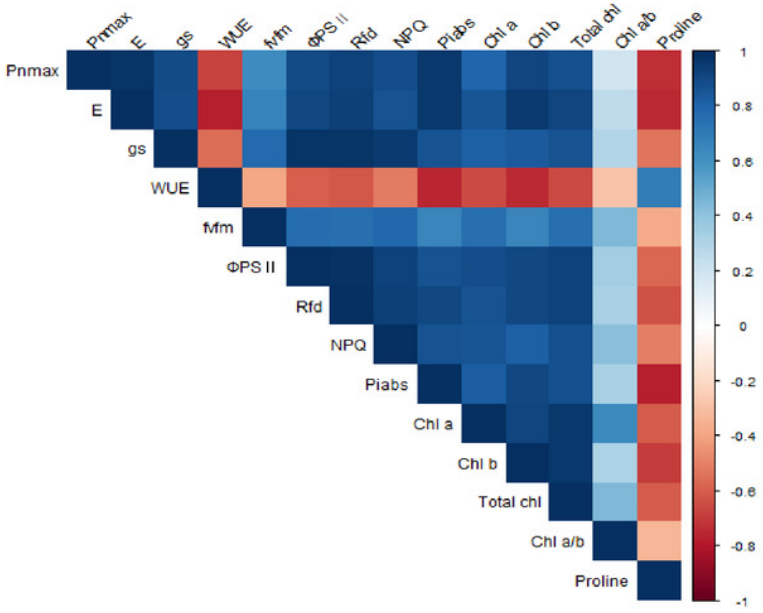
Pnmax
E
gs
WUE
fvfm
ΦPS II
Rfd
NPQ
Piabs
Chl a
Chl b
Total chl
Chl a/b
Proline
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 9

Schematic diagram of the changes of major parameters during progressive treatment time under drought stress condition.