JEE
ISSN: 2288-1220
https://doi.org/10.5141/jee.23.039
Journal of Ecology and Environment (2023) 47:10

RESEARCH

Photosynthetic characteristics and chlorophyll of *Vitex rotundifolia* in coastal sand dune

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ARTICLE INFO
Received July 3, 2023
Revised August 5, 2023
Accepted August 6, 2023
Published on August 25, 2023

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Background: This study analyzed the physiological adaptations of a woody plant, *Vitex rotundifolia*, in Goraebul coastal sand dunes from May to September 2022. Environmental factors and physiological of plants growing under field and controlled (pot) conditions were compared.
Results: Photosynthesis in plants growing in the coastal sand dunes and pots was the highest in June 2022 and July 2022, respectively. Chlorophyll fluorescence indicated the presence of stress in the coastal sand dune environment. The net photosynthesis rate (P_N) and Y(II) were highest in June in the coastal sand dune environment and July in the pot environment. In August and September, Y(NPQ) increased in the plants in the coastal sand dune environment, showing their photoprotective mechanism. Chlorophyll *a* and *b* contents in the pot plant leaves were higher than those in the coastal sand dune plant leaves; however, chlorophyll-*a*/*b* ratio was higher in the coastal sand dune plant leaves than in the pot plant leaves, suggesting a relatively high photosynthetic efficiency. Carotenoid content in the coastal sand dune plant leaves was higher in August and September 2022 than that in the pot plant leaves. Leaf water and soluble carbohydrate contents of the coastal sand dune plant leaves decreased in September 2022, leading to rapid leaf abscission. Diurnal variations in photosynthesis and chlorophyll fluorescence in both environments showed peak activity at 12:00 hour; however, the coastal sand dune plants had lower growth rates and Y(II) than the pot plants. Plants in the coastal sand dunes had higher leaf water and ion contents, indicating that they adapted to water stress through osmotic adjustments. However, plants growing in the coastal sand dunes exhibited reduced photosynthetic activity and accelerated decline due to seasonal temperature decreases. These findings demonstrate the adaptation mechanisms of *V. rotundifolia* to water stress, poor soils, and high temperature conditions in coastal sand dunes.
Conclusions: The observed variations indicate the responses of the *V. rotundifolia* to environmental stress, and may reveal its survival strategies and adaptation mechanisms to stress. The results provide insights into the ecophysiological characteristics of *V. rotundifolia* and a basis for the conservation and restoration of damaged coastal sand dunes.

Keywords: chlorophyll, chlorophyll fluorescence, coastal dune plant environmental stress, photosynthesis

Introduction

Coastal dunes are ecological transformation areas where the characteristics of coastal areas and inland areas are common (Kim and Hong 2009). The ecological and functional characteristics of coastal sand dunes include a buffering role to protect coastal ecosystems from wave action (Arun et al. 1999; Mascarenhas and Jayakumar 2008), underground freshwater storage, storage of sand carried by the wind, and prevention of sand from drifting further inland by sand dune vegetation (Dahm et al. 2005). The metabolism and survival of plant species growing in coastal sand dunes are affected by various environmental stresses, such as drought, salt, flooding, high temperatures, low water acceptability of sand, low nutrient and water availability in coastal dune environments (Hesp 1991; Lawlor and Cornic 2002; Maun 1998). In particular, water scarcity is one of the major stresses in coastal sand dunes, and frequent water scarcity is caused by high evaporation rates that limit plant growth, development, and survival (Bae et al. 2013).

Plants respond physiologically and biochemically to varying moisture stress levels by exhibiting reduced moisture content and osmotic potential, reduced relative water content (WC), osmotic regulation, leaf withering, high leaf temperatures, stomatal closure, cell enlargement, and reduced growth (Kumar and Singh 1998; Pasban Eslam et al. 2000; Shao et al. 2007). In addition mosture stress alters various physiological processes such as photosynthesis, respiration, transpiration, ion absorption, carbohydrate metabolism, stomatal conductivity, and electron transport (Acevedo et al. 1971; Angelopoulos et al. 1996; Flexas et al. 1998; Lu and Zhang 1998; Clifton-Brown et al. 2002; Munns 2002; Silva et al. 2007). Coastal sand dune plants are an important factor in the formation of coastal dunes, understanding coastal dunes' responses to environmental stresses such as dryness, salinity, temperature, and light changes is important for explaining and predicting the distribution of plant colonies in coastal sand dunes, and can also play an important role in protecting natural vegetation through effective management (Hwang and Choo 2017). Plants continuously exposed to environmental stimuli have developed various adaptation strategies to settle and perpetuate within their habitats (Flowers and Colmer 2008), and in general, both stress avoidance and stress tolerance strategies in plants include various plant mechanisms that provide plant viability under environmental stress conditions (Levitt 1980). In this study, research was conducted through various analysis items to identify the physiological characteristics of coastal dune plants. Photosynthesis is the most basic physiological process in which plants obtain energy using light, and is affected by various environments such as atmospheric temperature and rainfall (Kratsch and Wise 2000). Photosynthetic ability is used as an indicator to investigate physiological properties of plants, and chlorophyll fluorescence, which can quantify changes in photosynthetic ability, such as pore opening and water utilization efficiency and photochemical reactions, can provide useful information for investigating plant reactions (Baker 2008; Gunderson 2000; Krause and Weis 1991; Murchie and Lawson 2013).

Coastal sand dune plants are known to be resistant to high-temperature dry environments that can occur for a variety of reasons, including salt, drying, high evaporation, and osmotic bonding of water (Ishikawa et al. 1990; Larcher 2003; Mooney et al. 1983). However, defining factors is a challenge because plant species avoid or adapt to environmental stress through various mechanisms.

Therefore, this study aimed to investigate seasonal variations in physiological characteristics, including photosynthetic raates, chlorophyll fluorescene, and chlorophyll contents, of the perennial sand dune plant species *Vitex rotundifolia* growing under field (coastal sand dunes) and controlled (pots) conditions to determine its adaptive mechanisms to environmental stress. This species was selected because it is a dominant species in the Goraebul coastal sand dune area of the East Sea in Korea.

Furthermore, sand plants are essential for the formation of coastal sand dunes, and *V. rotundifolia* is one of the key species (Park et al. 2009). The results of this study are expected to provide valuable information to guide coastal sand dune conservation and management in the future.

Materials and Methods

Study area and plant materials

Vitex rotundifolia, which is distributed acoss the Goraebul coatal sand Dunes, a representative coastal sand dune area of the Korean East Coast (36°35′03.5″N 129°24′41.4″E), was planted in a three large pots (R = 1.0 m, H = 0.9 m) in a greenhouse at the Biology Center of Kyungpook National University (35°53′12.0″N 128°36′20.8″E) and allowed to settle for approximately 1 year. In addition, water was supplied periodically to maintain the moisture content of the soil at approximately 5%, and 2 L of modified Hoagland's solution was modified and treated at 2 L per pot every week (0.5 mM NH_4NO_3, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM KH_2PO_4, 0.5 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM K_2SO_4, 19 mM Fe-EDTA and trace elements) was added to each pot every week. The physiological traits of plants growing in coastal sand dunes and pots were analyzed. The experiments were repeated at least three times for individual plants under good growth conditions to account for seasonal changes.

Physiological characteristics according to seasonal changes

(1) Photosynthesis indicators

To investigate the monthly photosynthesis pattern of *V. rotundifolia*, from May 2022 to September 2022, a portable photosynthesis measurement device, LCi Portable, was used by using three good leaves from each individual in the coastal sand dune environment were selected every month and subjected to this analysis. After stabilization for approximately 5 minutes with the Photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, UK), net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and inter-cellular CO_2 concentration (C_i) were measured, and water use efficiency (WUE) was analyzed as the ratio of P_N and E (P_N/E), and carboxylation efficiency (CE) was analyzed as the ratio of P_N and C_i (P_N/C_i).

(2) Chlorophyll fluorescence

To investigate the monthly chlorophyll fluorescence pattern, a portable chlorophyll fluorescence measuring device, Portable Chlorophyll Flourometer (PAM-2500; Heinz Walz GmbH, Effeltrich, Germany), was used to measure the follwing chlorophyll fluorescence-related parameters: minimum fluorescene (F_o), maximum fluorescence (F_m), maximum photochemical quantum yield of PSII (F_v/F_m), quantum yield of photochemical energy conversion in PSII (Y[II]), quantum yield of regulated nonphotochemical energy loss in PSII (Y[NPQ]), and quantum yield of nonregulated nonphotochemical energy loss in PSII (Y[NO]).

(3) Leaf moisture content and sample solution extraction

The measured leaves were collected, the fresh weight (FW) was measured, dried in a dryer at 70°C for more than 3 days, and the dry weight (DW) was measured. The WC of the leaves was calculating with the formula.

$$WC (\%) = [(FW - DW)/ FW] \times 100$$

The plant sample liquid was ground into a homogeneous powder by grinding dried plant leaves with a pulverizer UDY cyclone sample mill (UDY Corperation, Fort Collins, CO, USA), and then 1 g of the sample was put into a 25 mL measuring flask and then incubated in a water bath at 95°C for 1 hour. After sufficient cooling at room temperature, the final volume was adjusted to 10 mL and extracted by filtering with a GF/C filter (pore size 1.2 μm).

(4) Chlorophyll content

A certain area (cm^2) of the leaf was collected and chlorophyll was extracted for 48 hours using 5 mL of dimethyl sulfoxide. The extracted chlorophyll was measured using a UV/VIS Spectrophotometer OPTIZEN 2120 (Mecasys, Daejeon, Korea), and the content of chlorophyll *a* and *b* was calculated using the formula of Wellburn (1994) based on the absorbance at 663 nm and 645 nm, and the absorbance at 480 nm for the content of carotenoids.

$$C_a = 12.47\ A_{665.1} - 3.62\ A_{649.1}$$
$$C_b = 25.06\ A_{649.1} - 6.5\ A_{665.1}$$
$$C_{x+c} = (1{,}000\ A_{480} - 1.29\ C_a - 53.78\ C_b) / 220$$

(5) Osmolarity, total ion content, soluble carbohydrate content

The osmotic concentration was measured with an Osmometer (Micro-Osmometer 3MO; Advanced Instruments, Norwood, MA, USA) using the principle of freezing point enhancement by taking 50 μL of the extracted sample solution. The total ion content was measured using the conductivity method (MX300 X-mate pro: Mettler Toledo, Columbus, OH, USA) by diluting 4 mL of distilled water in 1 mL of the extracted sample solution, and the value was obtained using the NaCl equivalent of Na^+ and Cl^- ions. Soluble carbohydrate content was measured by adding 400 μL of 5% phenol solution and 2 mL of H_2SO_4 stock solution to a solution of 20 μL of plant extract sample solution and 780 μL of distilled water, mixing well after 10 minutes, cooling at room temperature for 30 minutes. Absorbance at 490 nm was measured using a UV/VIS Spectrophotometer OPTIZEN 2120. Glucose (20–800 μL in 1,000 μL) was used as a standard solution, and based on this, the sugar content was quantified (Chaplin and Kennedy 1994).

(6) Environmental factors

Meteorological data for the study period was obtained from local meteorological stations, and soil moisture content in areas inhabited by *V. rotundifolia* was measured using the ML3 ThetaProbe Soil Moisture Sensor (Delta-T Devices, Cambridge, UK).

Physiological characteristics of V. rotundifolia in response to diurnal variations

(1) Photosynthesis

To analyze the diurnal change pattern, three good leaves of plant growing in the coastal sand dunes environment on August 26, 2022 and in the large pot were collected on August 26, 2022 and August 28, 2022, respectively, at 3-hour intervals from 06:00 to 18:00 (i.e., five times a day). After stabilization for approximately 5 minutes, P_N, g_s, E, and C_i in mesophyll cells were measured using the LCi Portable Photosynthesis system (ADC Bioscientific Ltd.), a portable photosynthesis measuring device. WUE was analyzed as P_N/E and CE was analyzed as P_N/C_i.

(2) Chlorophyll fluorescence

The diurnal change pattern in the coastal sand dunes on August 26, 2022 and in the large pot on August 28, 2022 was measured at 3 hours intervals (5 times in total) from 06:00 hour to 18:00 hour using a portable chlorophyll fluorescence measuring device, Portable Chlorophyll Flourometer PAM-2500. The foloowing chlorophyll fluorescence-related parameters were measured: (1) F_o, (2) F_m, (3) F_v/F_m, (4) the quantum yield of photochemical energy conversion in PSII (Y[II]), (5) the quantum yield of regulated non-photochemical energy loss in PSII (Y[NPQ]), and (6) the quantum yield of non-regulated, non-photochemical energy loss in PSII (Y[NO]). F_v/F_m was calculated as $(F_m - F_o) / F_m$.

(3) Leaf moisture content, chlorophyll content and solutes

Leaves were collected from plants growing in coastal sand dunes on August 26, 2022 and from plants growing in pots on August 28, 2022. Leaf moisture content, chlorophyll content, osmolarity, total ion content, and soluble carbohydrate content were determined.

Results

Seasonal changes in photochemical indicators

Environmental factors during the seasonal change measurement period

The meteorological conditions of the coastal dune area during the study period are shown in Figure 1A, B. The average temperature was the highest in July and August 2022 (24.7°C) and the lowest at 14.4°C in October 2022 (14.4°C). The average maximum temperature was the highest in July 2022 (29.6°C), whereas the average minimum temperature was the lowest in May 2022 (11.8°C). Monthly precipitation was the lowest in May 2022 (5.4 mm) and the highest in September 2022 (141.5 mm). In addition, it was investigated that the soil moisture content in the coastal sand dunes was considerably low (0.1%–0.5%) (Fig. 1A, B).

The weather conditions of Daegu, where the pot experiments were performed, are shown in Figure 1C, D. The average temperature was the highest in July 2022 (27.4°C) and the lowest in May 2022 (20.7°C). The average maximum temperature was the highest in July 2022 (32.4°C), whereas the average minimum temperature was the lowest in May 2022 (14.1°C). Monthly precipitation was the lowest in May 2022 (4.2 mm) and the highest in August 2022 (143.3 mm), (Fig. 1C, D). In the pot experiments, the soil moisture content was maintained at 4.5%–5.5% through regular water supply, and it was higher than that in the coastal sand dune environment (Fig. 2A). Photosynthetically active radiation (PAR) values for the pot plants were higher than those for the coastal sand dune plants, except for July 2022, and PAR values had a similar range at the time of measurement in both environments (Fig. 2B).

Photosynthesis indicators

Photosynthesis patterns in coastal dunes and large pot environments measured from May to September 2022 are shown in Figure 3. Light is essential for photosynthesis, and plants can convert solar energy into chemical energy through a metabolic process. The net photosynthetic rate is highly dependent on the amount of light absorbed by plants (Ueda et al. 2000). The P_N of plants growing in coastal sand dunes exhibited an increasing trend from May to June 2022 and then decreased. The P_N of plants growing in pots exhibited an increasing trend from May to July 2022; however, a decreasing trend was observed after August 2022 (Fig. 3A). A high photosynthetic rate was observed in *V. rotundifolia* grown in pots with continuous water supply due to a higher soil moisture content than that in the coastal sand dunes (0.1%–0.5%) (Figs. 2A and

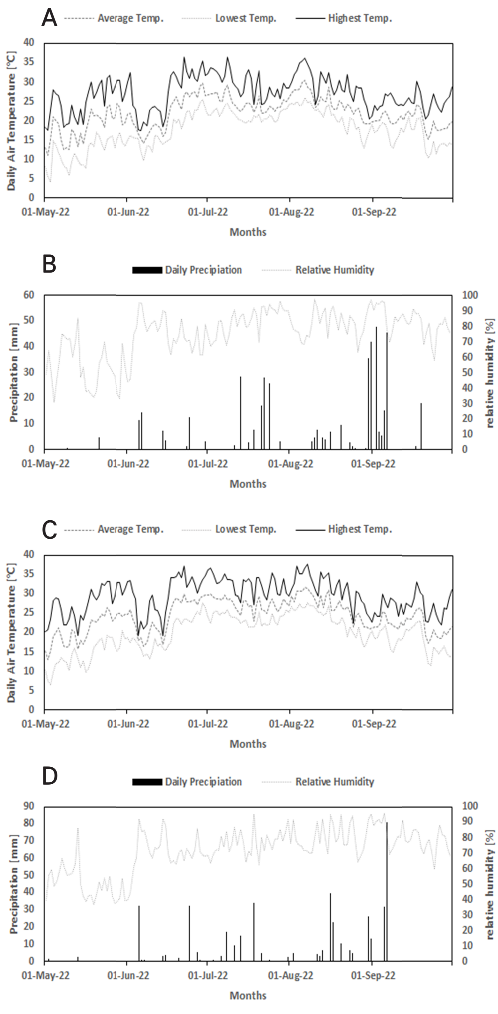


Fig. 1 Seasonal variations in (A) average air temperature (°C), lowest air temperature (°C), highest air temperature (°C), (B) daily precipitation (mm), relative humidity (%) during the survey period (May–September 2022), (C) average air temperature (°C), lowest air temperature (°C), highest air temperature (°C), (D) daily precipitation (mm), relative humidity (%) during the study period (May–September 2022). Meteorological data were obtained from the Yeongdeok and Daegu meteorological observatory.

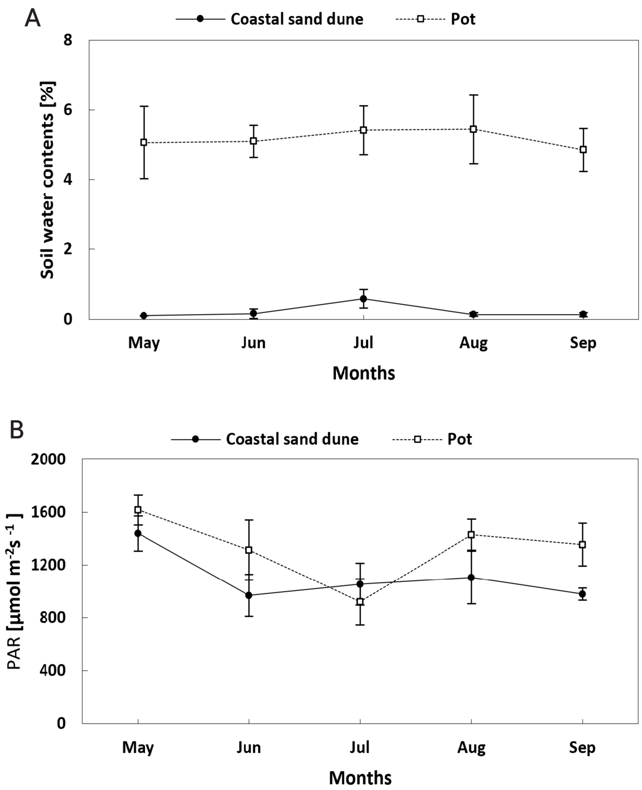


Fig. 2 Seasonal variations in environmental factors in the coastal sand dune (solid line) and the pot (dashed line). (A) Soil moisture content (%), (B) photosynthetically active radiation (PAR) (μmol m^{-2}s^{-1}). Data are presents as mean values with standard deviation.

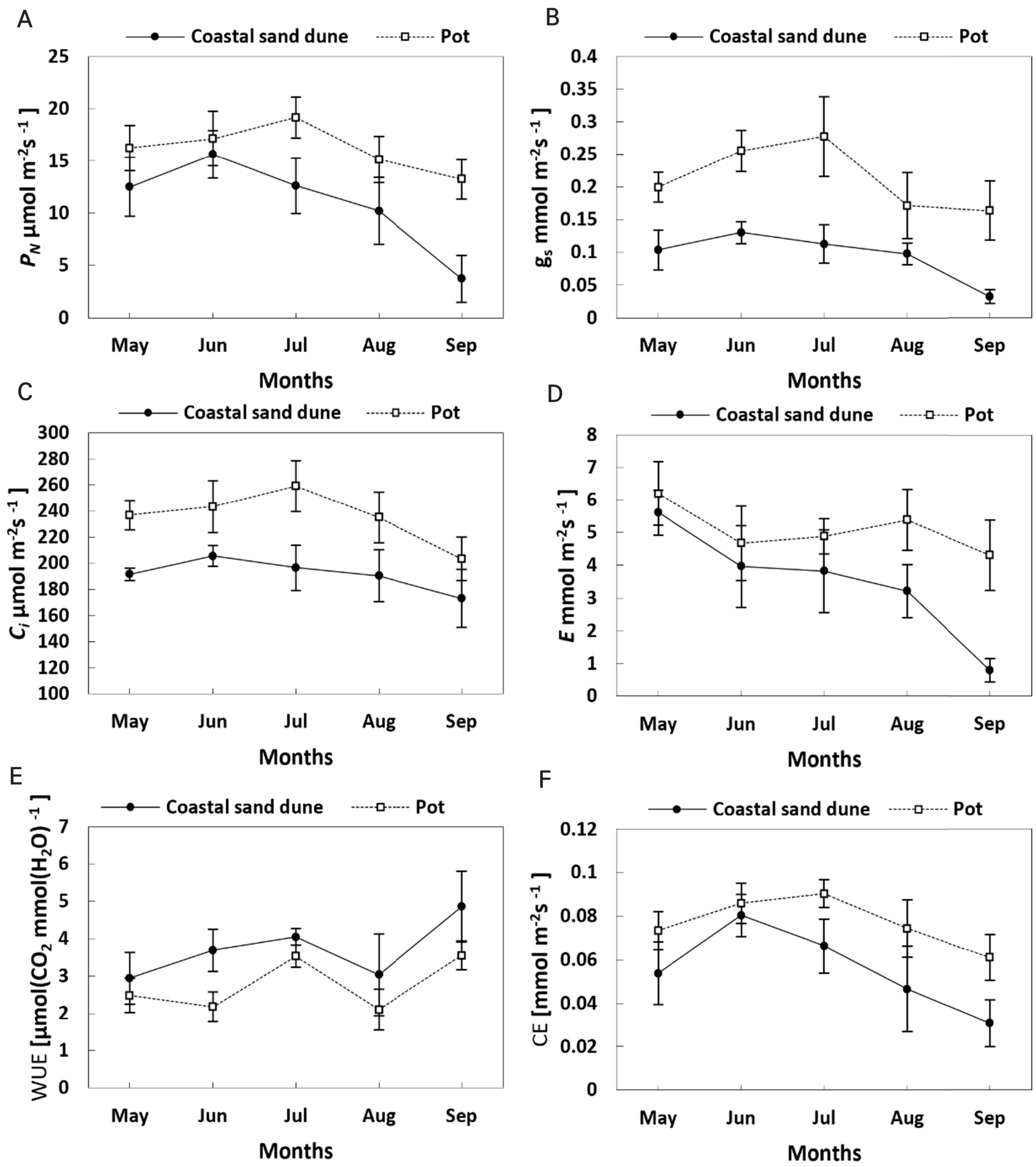


Fig. 3 Seasonal variations in (A) net photosynthetic rate (P_N), (B) stomatal conductance for CO_2 (g_s), (C) intercellular CO_2 concentration (C_i), (D) stomatal transpiration rate (*E*), (E) water use efficiency (WUE), and (F) carboxylation efficiency (CE) of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented as mean values with standard deviation.

3A). The P_N, g_s, and C_i of plants growing in pots increased from May to July 2022 with an increase in temperature but decreased after August 2022 with a decrease in temperature (Fig. 3A-C). In contrast, the P_N, g_s, and C_i of plants growing in coastal sand dunes increased from May to June 2022 but decreased after July and August 2022 when the temperature was high (Fig. 3A-C). Water is essential for photosynthesis, but transpiration, which cause water loss in plants, occurs during CO_2 absorption through the stomata (Pallardy 2010). The Moisture content of leaves is regulated by the opening and closing of stomata, and stomata are known to regulate the rate of transpiration and photosynthesis of plants through gas and water exchange (Sim et al. 2021). In addition, stomatal conductance is a key factor that influences the resistance to diffusion of water, which affects transpiration in leaves. The degree of stomatal opening and closing varies with the physiological status of plants in various environments and it can be determined (Collatz et al. 1991). The stomatal conductance of *V. rotundifolia* leaves under pot conditions increased from 0.255 mmol $m^{-2}s^{-1}$ to 0.278 mmol $m^{-2}s^{-1}$ in July 2022, and transpiration was also recovered accordingly, resulting in an increase in the net photosynthetic rate from 17.1 μmol $m^{-2}s^{-1}$ to 19.2 μmol $m^{-2}s^{-1}$ (Fig. 3A, B). Conversely, stomatal conductance of plant leaves in the coastal sand dunes decreased from 0.130 mmol $m^{-2}s^{-1}$ to 0.113 mmol $m^{-2}s^{-1}$, and transpiration decreased accordingly, resulting in a decrease in the net photosynthetic rate from 15.6 μmol $m^{-2}s^{-1}$ to 12.6 μmol $m^{-2}s^{-1}$ (Fig. 3A, B, and D). Plants close their stomata under water stress conditions to reduce water loss from leaves, which prevents CO_2 entry into the leaves, thereby reducing intracellular CO_2 concentration and photosynthetic rate (Gimenez et al. 1992). The plant hormone abcisic acid (ABA) promotes stomatal closure via a signaling pathway (Arena et al. 2008). The intracellular CO_2 concentration of *V. rotundifolia* leaves in the coastal dunes and pots exhibited the highest values in June 2022 and July 2022, respectively (Fig. 3C). WUE of *V. rotundifolia* growing in coastal sand dunes was higher than that of plants growing in pots (Fig. 3E). The high WUE observed in plants growing in coastal sand dunes suggests that these plants closed their stomata to overcome water strss due to the low soil moisture content of the habitat, CE exhibited a trend similar to that of P_N (Fig. 3A, E, and F). The indicators of photosynthetic activity in the pot plants exhibited higher values than those in the coastal sand dune plants during the study period. The highest values of photosynthetic activity indicators were observer in plants growing in coastal sand dunes and pots in June 2022 and July 2022, respectively, indicating that CO_2 consumption was efficiently achieved by photosynthesis (Fig. 3).

Chlorophyll fluorescence

The light energy absorbed by chlorophyll is released in the form of heat or fluorescence or used for photosynthesis, and the physiological state of the plant can be determined by calculating the ratio. This ratio is calculated as the values of Y(II), Y(NPQ), and Y(NO), which represent the ratio of energy use in photosystem II, and the value of Y(II) + Y(NPQ) + Y(NO) is 1 (Klughammer and Schreiber 2008). This rate of energy use provides crucial information about plant photosynthesis (Klughammer and Schreiber 2008). Y(II) means the quantum yield of photochemical energy conversion in PSII and has a high correlation with the rate of photosynthesis (Baker 2008). It means the mechanism of photoprotection that protects leaves by releasing excited energy generated under stress environment in the form of heat by active dissipation of regulated non-optical energy (Müller et al. 2001). In this study, *V. rotundifolia* exhibited a similar photosynthetic efficiency, with Y(II) ratios of 0.34 and 0.39 in May 2022 in the coastal sand dunes and large pot environments (Fig. 4). However, the Y(II) ratio (0.41) of the coastal sand dune plants was the highest in June 2022 when the photosynthetic rate was the highest, whereas that of pot plants was the highest 0.56 in July 2022 (Fig. 4A, B). The Y(II) rations in both environments initially increased to a peak and subsequently decreased, indicating a decrease in photosynthetic efficiency. The Y(NPQ) ratio of coastal sand dunes plants tended to increase. Studies suggest that more energy is used for the photoprotection mechanism that protects leaves by releasing the energy received in the form of heat to overcome the stress caused by the high temperature. Fluorescence emitted from chlorophyll is extremly sensitive to environmental stress and is widely known as an indicator of the physiological state of plants in response to environmental changes. In particular, it is known that the ratio of F_v/F_m ration indicaters the photochemical efficiency and the state of photosystem II (Falqueto et al. 2010). The F_v/F_m values of plants that are not under environmental stress ranges from 0.75 to 0.85 (Peterson et al. 1988). No stress was observed in *V. rotundifolia* grown in pots when F_v/F_m values were relatively low 0.70–0.75 in the coastal sand dune plants (Fig. 5). The results could be associated with the stress caused by insufficient soil moisture content and high temperature conditions in the coastal sand dunes. Furthermore, it could be due to the fact that leaf abscission was more rapid in the coastal sand dune plants than in the pot plants.

Chlorophyll content

Chlorophyll is an essential pigment for photosynthesis in plants and is a key factor regulating plant physiological processes. Plant leaves consist of chlorophylls *a* and *b*, which are the main elements constituting the light harvesting complex (LHC), and the chlorophyll *a*/*b* ratio indicates their composition. Chlorophyll *a* constitutes the reaction center and the LHC of photosystems I and II, whereas

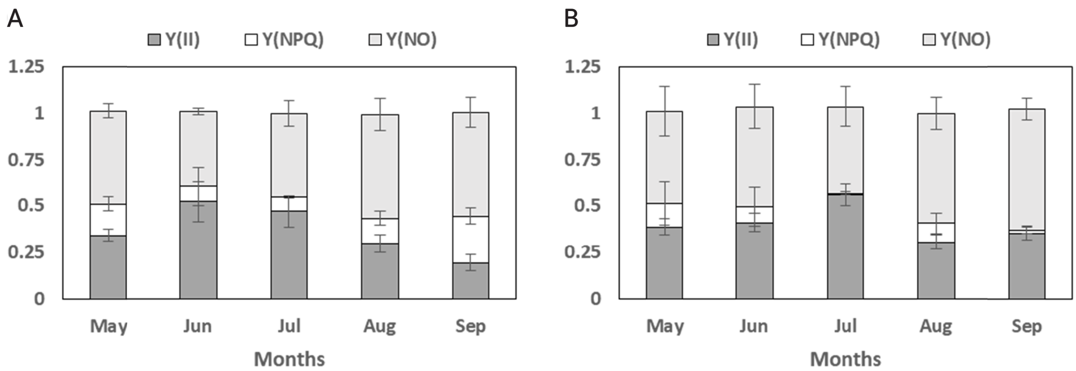


Fig. 4 Seasonal variations in chlorophyll fluorescence parameters; (A) Y(II), Y(NPQ), and Y(NO) of *Vitex rotundifolia* in coastal sand dune, (B) Y(II), Y(NPQ), and Y(NO) of *V. rotundifolia* in the pot. Data are presented as mean values with standard deviation. Y(II): the quantum yield of photochemical energy conversion in PSII; Y(NPQ): the quantum yield of regulated non-photochemical energy loss in PSII; Y(NO): the quantum yield of non-regulated, non-photochemical energy loss in PSII.

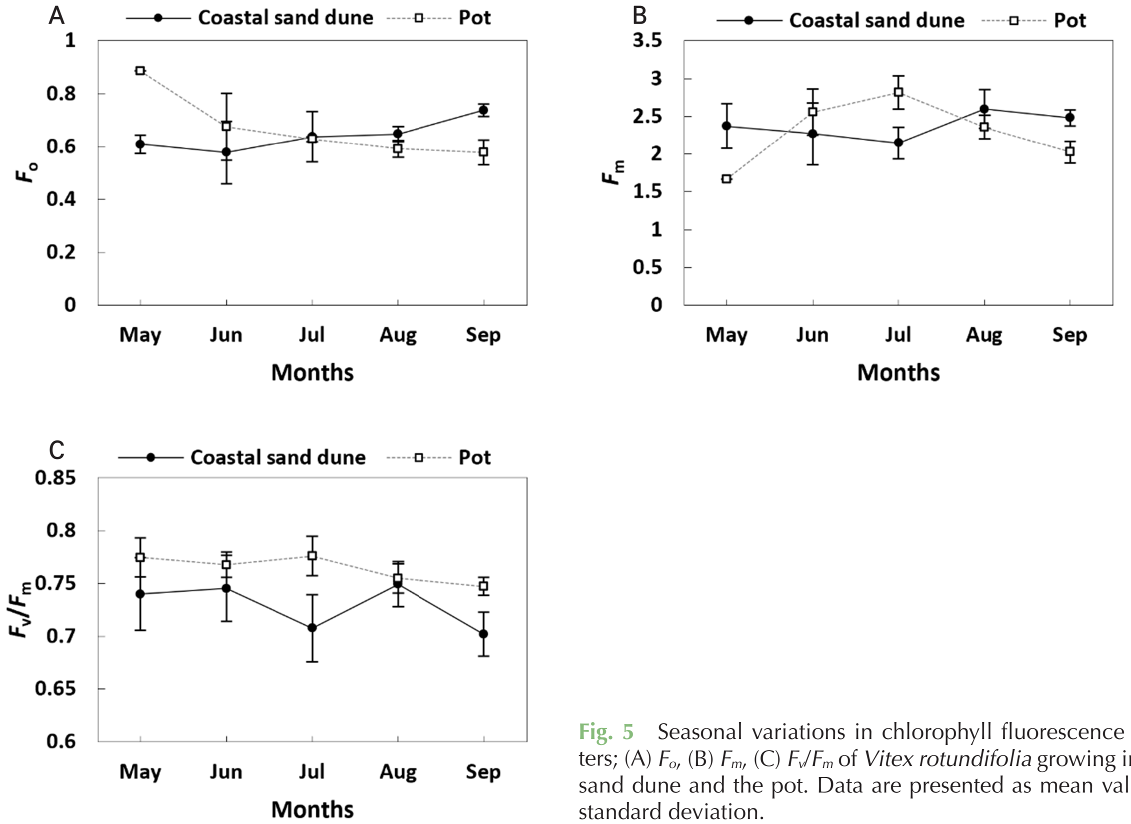


Fig. 5 Seasonal variations in chlorophyll fluorescence parameters; (A) F_o, (B) F_m, (C) F_v/F_m of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented as mean values with standard deviation.

chlorophyll *b* serves as an antenna pigment that absorbs light and transmits it to the reaction center (Taiz and Zeiger 2006). According to the results, chlorophylls *a*, *b*, *a* + *b* contents in leaves of plants grown in pots were higher than those of plants growing on the coastal sand dunes during the study period, except for September 2022 (Fig. 6A-C). Chlorophyll *a*/*b* ratio, which is used as an indicator of photosynthetic capacity, was higher value in the coastal sand dune plants than in pot plants during the study period, with a marked increase being observed in September 2022 (Fig. 6D). The results suggest that *V. rotundifolia* growing on the coastal sand dunes maintains a high photosynthetic rate by increasing the photosynthetic efficiency under water stress conditions. Carotenoids assist chlorophylls in absorbing sunlight and transferring the energy to the reaction center. Carotenoid also enhence photoprotection of plants by preventing the formation of singlet oxygen through photooxidation in a high light intensity environments (Sarijeva et al. 2007). In this study, carotenoid contents of pot plants were higher between May and July 2022 than those of coastal sand dune plants (Fig. 6E). However, carotenoid content of coastal sand dune plants was relatively high in August and September 2022 (Fig. 6E). The high considered content is considered to be a photoprotective mechanism against the high temperature and intense light on coastal sand dunes (Kyparissis et al. 2000) and may be associated with the high Y(NPQ) value observed during the same period (Figs. 4 and 6).

Leaf water content, osmolality, total ion content, and soluble carbohydrate content

Various in leaf water content (LWC) are not only used to indicate plant health, but also tolerance to dry environmental conditions (Chyliński et al. 2007). The LWC of pot plants was constant during the study period, whereas that of coastal sand dune plants increased in August 2022 and then decreased substantially in September 2022 (Fig. 7A). This observation could be attributed to water stress and high temperature in the coastal sand dunes, and is also believed to influence early leaf detachment.

Variations in osmolality, total ion content, and soluble carbohydrate content in the leaves of *V. rotundifolia* growing in the coastal sand dune and pot environments are shown in Figure 7. Osmolality in coastal sand dune plants was higher than that in pot plants between May and September 2022. Both environments exhibited increased osmolality and total ion content in September 2022 when the temperature was low (Fig. 7B, C). Generally, plants cope in stressful environments by increasing the content of readily available inorganic solutes and decreasing water potential by accumulating various ions in their vacuoles (Edwards and Dixon 1995). Specifically, leaf water potential increases with a decrease in WC under water stress, which makes it difficult to regulate the osmotic pressure of cells. Consequently, the stomata are closed and photosynthetic activity reduces, thereby inhibiting carbohydrate and nitrogen metabolism (Hsiao and Bradford 1983). To overcome water stress, plants have developed various adaptive mechanisms

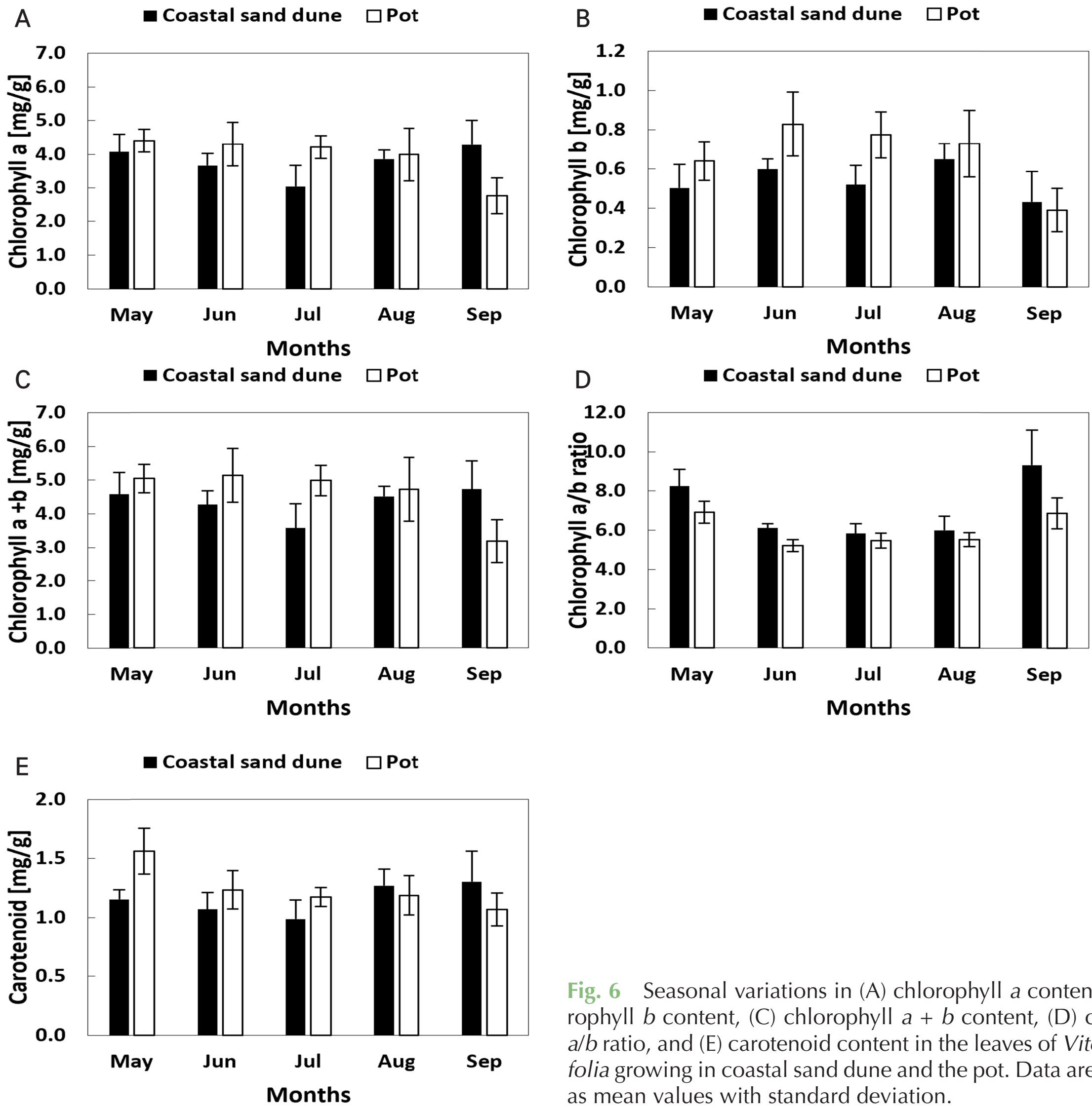


Fig. 6 Seasonal variations in (A) chlorophyll *a* content, (B) chlorophyll *b* content, (C) chlorophyll *a* + *b* content, (D) chlorophyll *a*/*b* ratio, and (E) carotenoid content in the leaves of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented as mean values with standard deviation.

(Chaves et al. 2003). We speculate that *V. rotundifolia* maintains WC and responds to water stress by accumulating ions or osmotic compounds, such as soluble carbohydrates. Osmolarity and total ion content of the coastal sand dune plants increased in September 2022, although the soluble carbohydrate content decreased (Fig. 7B-D). Osmolarity, total ion content, and soluble carbohydrate content of the pot plants increased during the same period (Fig. 7B-D). *Vitex rotundifolia* may have adapted to low moisture content and low temperatures in low stress environments through osmotic adjustment and accumulation of soluble carbohydrates.

Physiological characteristics in response to diurnal variations

Environmental factors during diurnal variation

The PAR values for plants growing in coastal sand dunes and pots based on measurement time was the highest at 12:00 hour in August 2022, except for the values observed at 09:00 hour, which were similar (Fig. 8A). The PAR value for the pot plants was lower than that for the coastal sand dune plants at 09:00 hour, and higher in pot plants at 15:00 hour (Fig. 8A). The leaf temperature of *V. rotundifolia* growing on the coastal sand dunes ranged from 21.9°C to 34.7°C, with the highest value being observed at 12:00 hour (Fig. 8B). The leaf temperature of *V. rotundifolia* grown in pots ranged from 23.2°C to 39.5°C, with the lowest value being observed at 09:00 hour and the highest value at 15:00 hour under the influence of PAR (Fig. 8B).

Diurnal variations in photosynthetic parameters

The diurnal variations in photosynthetic parameters of *V. rotundifolia* in August 2022 are shown in Figure 9. The net photosynthetic rate exhibited a bell-shaped normal distribution, with the highest value being observed at 12:00 hour in both environments (Fig. 9A). Furthermore, stomatal conductance and transpiration rate exhibited a similar trend (Fig. 9B, D). The photosynthetic rate of the coastal sand dune plants was lower than that of the pot plants (Fig.

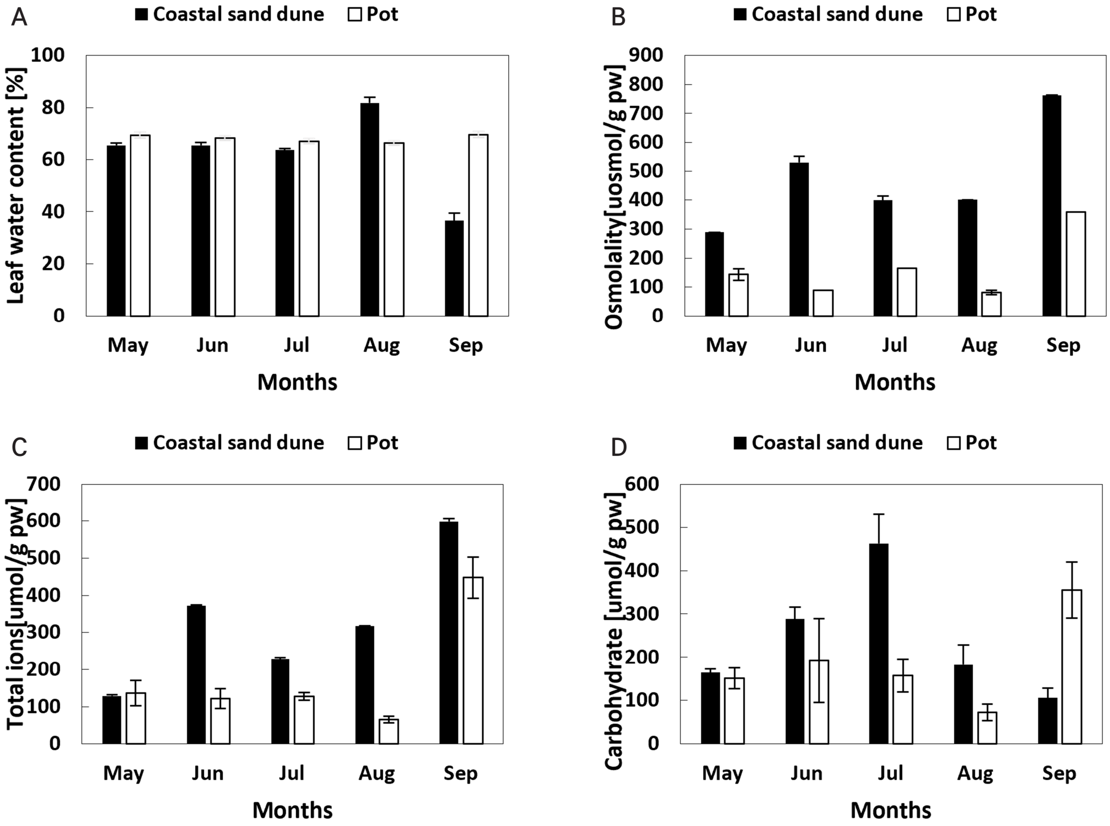


Fig. 7 Seasonal variations in (A) leaf water content (B) osmolality, (C) total ion content, and (D) carbohydrate content in the leaves of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented as mean values with standard deviation.

9A). A similar trend was observed for g_s and E, which affected P_N (Fig. 9B, D). CE also exhibited a trend similar to that of P_N, with the highest value being observed at 12:00 hour in the coastal sand dune plants and at 15:00 hour in the pot plants and decreasing thereafter (Fig. 9F).

Diurnal variations in chlorophyll fluorescence

Chlorophyll fluorescence and related parameters of *V. rotundifolia* leaves were measured in August 2022, and the results revealed that the diurnal F_v/F_m value in both environments ranged from 0.75 to 0.81, indicating that the plant was not affected by stress (Fig. 10). The diurnal Y(II) ratio of pot plants ranged from 0.31 to 0.37, and the photosynthetic rate of coastal sand dune plants was lower than that of pot plants at 12:00 hour (Figs. 9A and 11). Y(II) decreased, whereas the Y(NO) ratio increased (Fig. 11). *Vitex rotundifolia* was affected by the high temperature at 12:00 hour (Fig. 8). The results imply that most of the absorbed light energy is not used as effective photochemical energy and the ratio of energy lost passively in the form of fluorescence in the reaction center of PSII increases (Sim et al. 2021).

Chlorophyll content

Comparison of the chlorophyll contents of *V. rotundifolia* in the coastal dune environment and those in the large

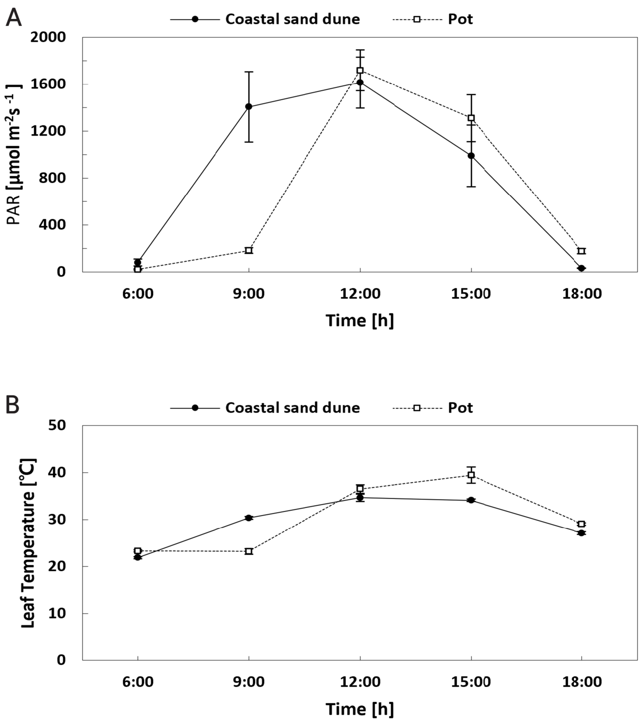


Fig. 8 Diurnal variations in environmental factors of *Vitex rotundifolia* growing in coastal sand dune and the pot. (A) Photosynthetically active radiation (PAR) (μmol m^{-2}s^{-1}), (B) leaf temperature (°C). Data are presented as mean values with standard deviation.

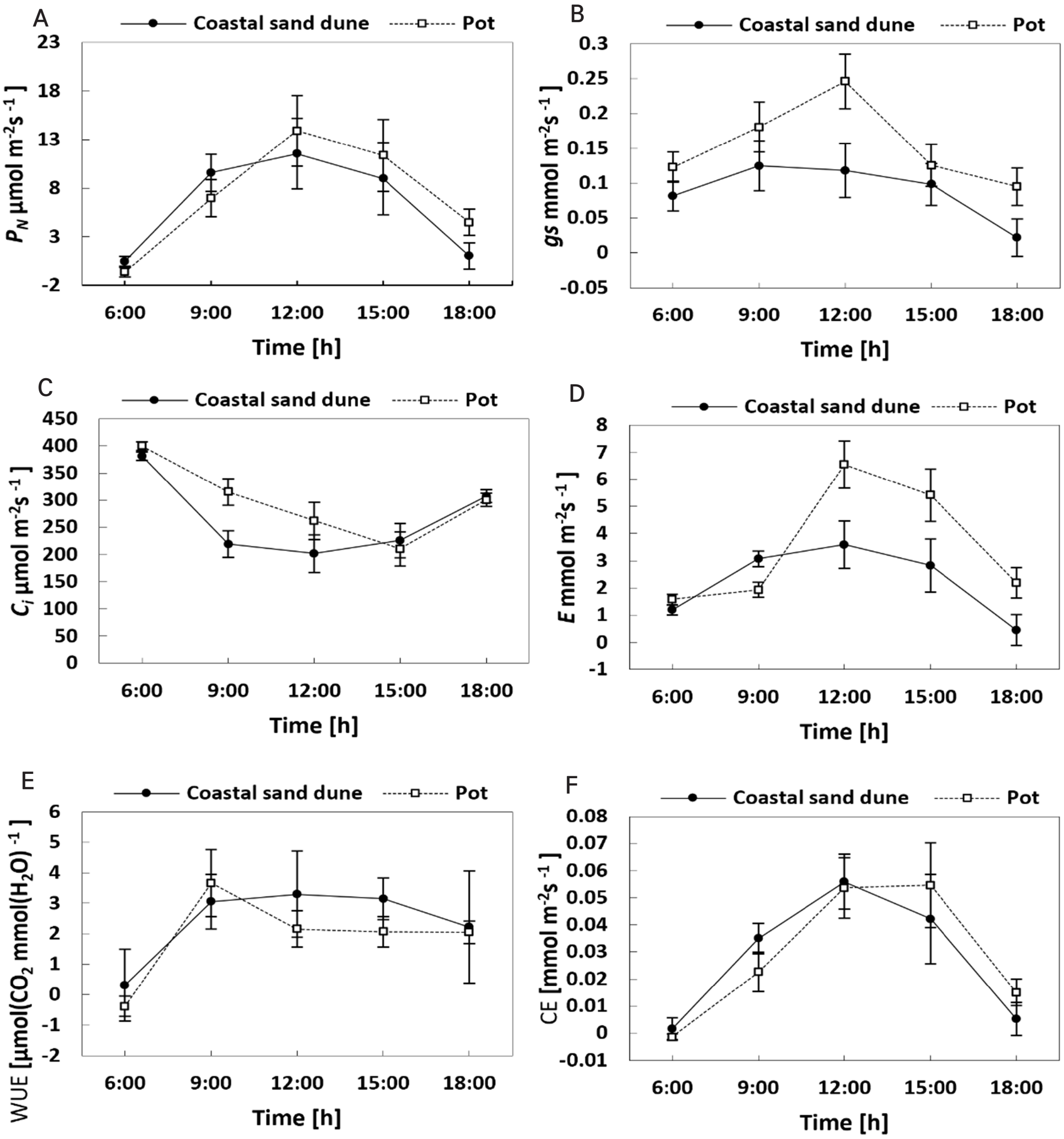


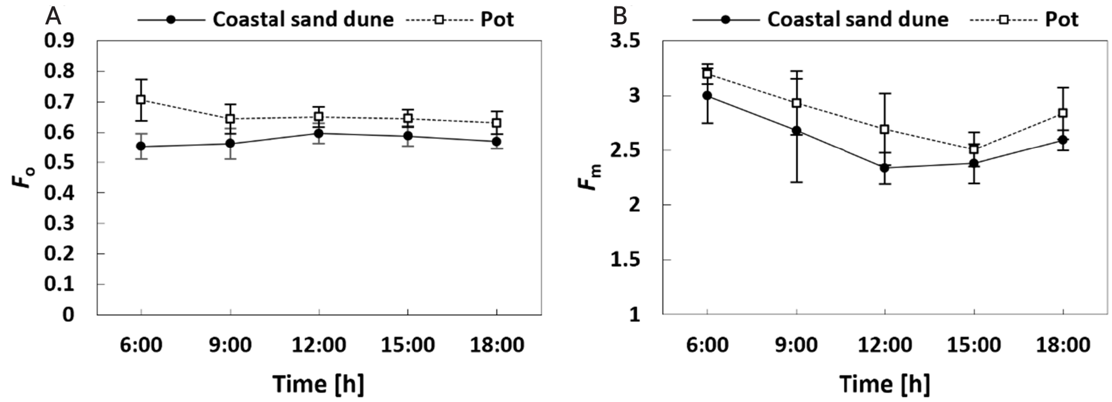
Fig. 9 Diurnal variation of (A) net photosynthetic rate (P_N) , (B) stomatal conductance for CO_2 (g_s), (C) intercellular CO_2 concentration (C_i), (D) stomatal transpiration rate (*E*), (E) water use efficiency (WUE), and (F) oxylation efficiency (CE) of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented as mean values with standard deviation.

pot environment, revealed that the contents of chlorophyll *a*, chlorophyll *b*, and chlorophyll *a* + *b* were higher in *V. rotundifolia* in the plants in the pot environment than in those in the coastal sand dune environment (Table 1). However, the chlorophyll *a*/*b* ratio, which is used as an indicator of photosynthetic ability, was higher in the coastal sand dune environment than in the pot environment (Table 1). It is believed that the ratio of chlorophyll *a* increased to increase the photosynthetic efficiency in the coastal sand dune environment where environmental stress existed. In addition, the carotenoid content was higher in the coastal sand dune environment than in the pot environment, indicating a photoprotective mechanism against high light intensity in the coastal sand dune environment (Table 1).

Leaf water content, osmolarity, total ion content, and soluble carbohydrate content

The leaf LWC of *V. rotundifolia* in the coastal dune environment was 82.8% and that in the pot environment was 66.5% (Table 2). Therefore, it was found that more water was contained in the coastal dune environment.

In addition, revealed that osmolality, total ion content, and soluble carbohydrate content of *V. rotundifolia* growing on the coastal dunes were higher than those of plants grown in pots (Table 2). Therefore, In the coastal sand dune environment where water stress is present due to low soil WC, it is believed that *V. rotundifolia* maintains the WC and responds to water stress through the accumulation of ions or osmotic substances such as soluble carbohydrates.



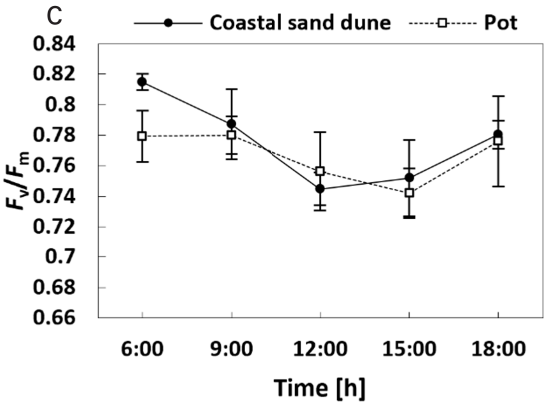


Fig. 10 Diurnal variation in chlorophyll fluorescence parameters: (A) F_o, (B) F_m, (C) F_v/F_m of *Vitex rotundifolia* growing in coastal sand dune and the pot. Data are presented mean values with standard deviation.

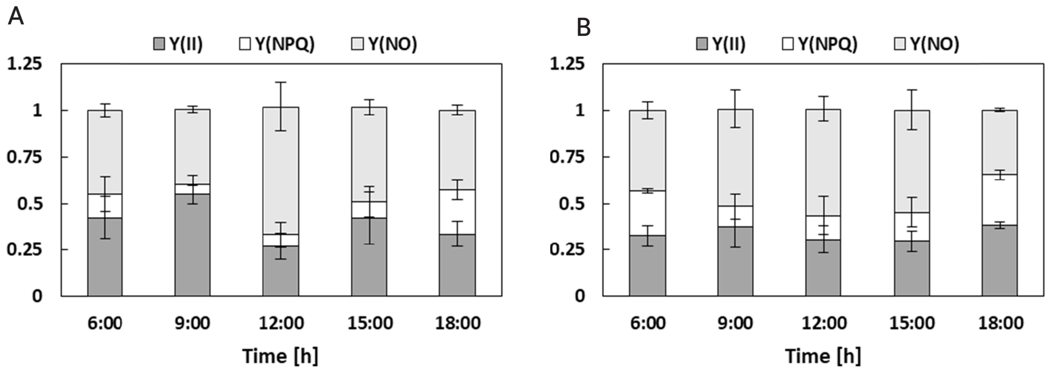


Fig. 11 Diurnal variations in chlorophyll fluorescence parameters; (A) Y(II), Y(NPQ), and Y(NO) of *Vitex rotundifolia* in coastal sand dune. (B) Y(II), Y(NPQ), and Y(NO) of *V. rotundifolia* in the pot. Data are presented as mean values with standard deviation. Y(II): the quantum yield of photochemical energy conversion in PSII; Y(NPQ): the quantum yield of regulated non-photochemical energy loss in PSII; Y(NO): the quantum yield of non-regulated, non-photochemical energy loss in PSII.

Table 1 Chlorophyll contents in the leaves of *Vitex rotundifolia* growing in coastal sand dune and the pot

Chlorophyll contents	Coastal sand dune	Pot
Chl *a* (μg ml⁻¹)	3.852 ± 0.268	3.988 ± 0.787
Chl *b* (μg ml⁻¹)	0.649 ± 0.081	0.729 ± 0.170
Chl *a* + *b* (μg ml⁻¹)	4.501 ± 0.295	4.717 ± 0.952
Chl *a*/*b* ratio	5.997 ± 0.727	5.518 ± 0.361
Carotenoid (μg ml⁻¹)	1.268 ± 0.141	1.186 ± 0.166

Data are presented as mean ± standard deviation.

Table 2. Leaf water content and solute contents in *Vitex rotundifolia* leaves in coastal sand dune and the pot

Parameter	Coastal sand dune	Pot
Leaf water content (%)	82.8 ± 2.2	66.5 ± 0.8
Osmolality (uosmol/g pw)	400.77 ± 0.00	81.41 ± 7.05
Total ions (umol/g pw)	316.84 ± 0.95	65.60 ± 8.87
Carbohydrate (umol/g pw)	182.65 ± 45.97	72.73 ± 19.10

Data are presented as mean ± standard deviation.

Discussion

After transplanting the primary colony of *V. rotundifolia* in the Goraebul sand dunes located on the east coast of Gyeongsangbuk-do, to a large pot in a greenhouse at the Kyungpook National University and allowing it to settle for 1 year, data for the natural habitat and controlled environment were collected. Moreover, physiological characteristics such as photosynthetic rates and variations in chlorophyll fluorescence, leaf chlorophyll content, carotenoid content, LWC, osmolality, total ion content, and soluble carbohydrate content were investigated.

Seasonal variations in photosynthetic activity indicators, including g_s and P_N, were observed between plants growing in coastal sand dunes and pots. g_s and P_N of *V. rotundifolia* growing in coastal sand dunes were the highest in June 2022 but decreased in July 2022 when the temperature was the highest. However, g_s and P_N of *V. rotundifolia* grown in pots initially increased, reached a peak in July 2022 when the temperature was the highest, and subsequently decreased. In both environments, there was a temporal difference, but after the peak of photosynthetic activity in *V. rotundifolia*, it showed a tendency to decrease according to the change of seasonal time. Photosynthetic activity of the coastal sand dune plants was rapidly reduced when compared to that of pot plants. In addition, a high WUE was observed in plants growing on the coastal sand dunes with low soil moisture content than in plants grown in pots, suggesting the physiological characteristics of *V. rotundifolia* that facilitate its adaptation to water stress environments.

Based on the chlorophyll fluorescence results, plants in the coastal sand dune environment were stressed, whereas those grown in pots were not substantially stressed, and the Y(II) ratio exhibited a trend similar to that of P_N. In addition, it exhibited a decreasing trend over time after peaking in June 2022 in the sand dune environment; however, it of plants grown in pots exhibited a decreasing trend after reaching a peak in July 2022, which was consistent with the trend of photosynthetic rate. The results revealed a decrease in photosynthetic efficiency of the coastal sand dune plants when compared to that of pot plants. The Y(NPQ) of the coastal the sand dune plants increased in August and September 2022, and the energy absorbed was not used for photosynthesis but was instead released as heat as a photoprotective mechanism. The observation is associated with the effect of environmental stress caused by high temperature and high light intensity on the coastal sand dunes.

Chlorophyll *a*, *b*, and *a* + *b* contents in the leaves of pot plants were higher than those of the coastal sand dune plants during the study period, except for September 2022; however, chlorophyll *a*/*b* ratio, which is used as an indicator of photosynthetic efficiency, was higher in the coastal sand dune plants than in the pot plants during the study period. The observation is a response mechanism of *V. rotundifolia* to environmental stress, where an increase in the chlorophyll *a*/*b* ratio increases photosynthetic efficiency. According to the results of LWC, osmolarity, total ion content, and soluble carbohydrate content, *V. rotundifolia* growing on the coastal sand dunes did not exhibit osmotic adjustment. The results revealed that leaf abscission in the coastal sand dune plants was more rapid than that in pot plants.

Furthermore, the diurnal variations in photosynthetic parameters of *V. rotundifolia* revealed physiological differences based on the plant environment, with photosynthetic parameters of plants grown in pots having high values at 12:00 hour (when photosynthetic rate was the highest) when compared to those of plants growing on the coastal sand dunes. Stomatal conductance (g_s) and transpiration rate (E) also exhibited a similar trend. Chlorophyll fluorescence analysis revealed that the Y(II) ratio of the coastal sand dune plants decreased at 12:00 hour. Therefore, we conclude that *V. rotundifolia* has adapted to the coastal sand dune environment, which experiences water stress due to insufficient soil moisture, by accumulating osmotic compounds in the cells.

Conclusions

This study revealed that the photosynthetic rate of *V. rotundifolia* grown in pots initially increased owing to relatively low environmental stress but decreased in July 2022 when the temperature was the highest. Based on the diurnal variations in photosynthetic parameters, the photosynthetic rate increased substantially at 12:00 hour. Conversely, the photosynthetic rate of plants growing in coastal sand dunes, where environmental stress exists, was the highest in June 2022 and then decreased in July and August 2022 when the temperature was high, with a slight increase observed at 12:00 hour. The lack of moisture, oligotrophic soils, and high temperature conditions in coastal sand dunes hinder the photosynthetic activity of *V. rotundifolia*, leading to decreased photosynthesis due to temperature changes. Furthermore, *V. rotundifolia* growing in coastal sand dunes had higher WUE, Y(NPQ), chlorophyll *a*/*b* ratio, and carotenoid content in August and September 2022 than those grown in pots. The results indicate the survival strategies of *V. rotundifolia* in coastal sand dunes associated with environmental stress. *Vitex rotundifolia* exerts a positive effect on the biodiversity of the coastal sand dunes by adapting to the barren environment, facilitating the maintenance, stability, and formation of coastal sand dunes. The findings of this study can guide the conservation of coastal sand dunes and restoration of damaged sand dunes in the future.

Abbreviations

WC: Water content
WUE: Water use efficiency
CE: Carboxylation efficiency
FW: Fresh weight
DW: Dry weight
PAR: Photosynthetically active radiation
LHC: Light harvesting complex
LWC: Leaf water content
Y(II): The quantum yield of photochemical energy conversion in PSII
Y(NPQ): The quantum yield of regulated non-photochemical energy loss in PSII
Y(NO): The quantum yield of non-regulated, non-photochemical energy loss in PSII

Acknowledgements

All the authors are deeply grateful to Plant ecophysiology lab in Kyungpook National University, South Korea.

Authors' contributions

BJK did data curation, investigation, and writing-original draft. SH did data curation, funding acquisition, writing-review and editing. YS did data analysis, writing-review and editing. YS did conceptualization, supervision, writing-original draft, and writing-review and editing. All the authors approved the manuscript.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1A6A1A05011910 & 2016R1D-1A1B03934927), and by grants from the National Institute of Ecology (NIE-A-2023-20 & NIE-B-2023-02).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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