



# Effects of environmental factors on the growth response of above- and below-ground parts of *Mankyua chejuense*, endangered endemic plant to Jeju province, in Korea

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## Abstract

*Mankyua chejuense* is a native endangered plant distributed only in Gotzawal, a forested wetland, in Jeju Province, Korea. In order to determine the optimal environmental conditions for the growth and development of *M. chejuense*, we investigated the above- and below-ground growth responses and survival rate to various soil texture (sand and clay), water regimes (flooding and non-flooding), and CO<sub>2</sub>+T (ambient and elevated) conditions. All of the treatments had significant effects on aboveground growth parameters, while only the water regime and CO<sub>2</sub>+T treatments influenced belowground growth. The survival rate of *M. chejuense* was about twice higher under the sand, non-flooding and elevated CO<sub>2</sub>+T conditions than clay, flooding and ambient CO<sub>2</sub>+T conditions. These results indicate that *M. chejuense* grows in well-drained sandy soil conditions and elevated CO<sub>2</sub> concentration and temperature situations. Thus, there is a need to maintain *M. chejuense* under constant non-flooding soil conditions by implementing appropriate soil drainage strategies.

**Key words :** fern, flooding, global warming, rhizome, soil texture, sporophyll

## INTRODUCTION

*Mankyua chejuense* is an endangered endemic fern to Jeju Island in Korea (Ministry of Environment of Korea 2009). The Korean peninsula has seven endemic genera; Pentactina, Hanabusaya, Echinosophora, Megaleranthis, Abeliophyllum, Coreanomecon, and Mankyua (Kim 2006). *Mankyua chejuense* is a monotypic fern genus belonging to the family Ophioglossaceae (Sun et al. 2001).

The distribution of *M. chejuense* is restricted to Gotzawal, a unique forested inland wetland, in the northeast region of Jeju Island that is fed by underground water in the rocky volcanic area (Hyeon et al. 2011, Lee et al. 2012). *Mankyua chejuense* is only found in small vernal pools with a variety of shapes and a small amount of soil and

nutrients. The fern grows within the lowland deciduous forest community at above 0°C during all the seasons (Lee et al. 2012).

*Mankyua chejuensis* currently has a small population size of 23 meta populations and is subject to minor changes in environmental conditions such as plant succession to the evergreen seral stage, litter accumulation and water drainage due to its small size (height < 5 cm), as well as its ecological and reproductive characteristics (Shin 2012).

Rare and endangered species with limited climatic ranges or restricted habitat requirements are generally less tolerant to environmental stresses and have lower

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phenotypic plasticity during acclimation to a broader range of environmental conditions than widely-distributed species (Sakai et al. 2001, Parmesan and Yohe 2003, Baruch and Jackson 2005). Thus, to understand any plant, it is important to obtain basic ecological information regarding the population response to diverse environmental factors such as soil, water and temperature by conducting detailed field- and laboratory-based ecological and physiological measurements (Jeffries 2006).

Recently, Kimball et al. (1993) demonstrated that elevated CO<sub>2</sub> concentrations and increased temperatures have had profound effects on plant life history. Native species specific to local regions may be negatively affected than widespread species under global warming (Song et al. 2009), and thus at greater risk of extinction. However, there has been attempt to elucidate the growth response or belowground dynamics of this plant to environmental gradient conditions, despite the fact that this information is essential to plant population conservation and habitat management (You and Kim 2010).

In the present study, we measured the survival rate, and aboveground- and belowground growth under various soil textures, water regimes, CO<sub>2</sub> concentration and temperature to verify the growth response of above- and below-ground parts of *M. chejuense* to different environmental conditions.

## MATERIALS AND METHODS

### Environmental treatment

The soil texture treatments were sand (1-2 mm particle size), or clay (particle size < 0.2 mm) which consisted of vermiculite. The water regimes were flooding (maintenance of water at 1-2 cm above the root), and non-flooding, which were achieved by pouring water into the growth pots and releasing it through holes in the bottom.

Global warming treatment was ambient CO<sub>2</sub> and ambient temperature (ACAT) and elevated CO<sub>2</sub> and elevated temperature (ECET). In the ACAT, the air is maintained at the ambient CO<sub>2</sub> concentration and temperature of the immediately surrounding air, which averages about 370-380 ppm on a 24-hours basis. The ECET was maintained by inputting a small quantity of pure CO<sub>2</sub> through two perforated plastic hoses so as to maintain the CO<sub>2</sub> concentration at approximately 742.30 ± 16.92 ppm, which is twice that of the ambient (360.38 ± 9.19 ppm) concentration. CO<sub>2</sub> concentration was monitored by a TEL-7001 CO<sub>2</sub> sensor (Onset Computer, Bourne, MA, USA) at 30-

min intervals, and data was stored on HOBO U12 datalogger (Onset Computer, Bourne, MA, USA) to evaluate the stability of the CO<sub>2</sub> concentration in the treatment. According to IPCC scenario (2007), the atmospheric CO<sub>2</sub> concentration is predicted to be double within the next 100 years.

The mean temperature in the treatment was about 2.5°C higher than the control. The air temperature was measured using a TR-71U thermo recorder (T&D Co., Matsumoto, Japan) at the same height in the control and treatment greenhouses during the study period. This study was conducted in glass greenhouse of 12 m × 7.8 m × 5 m (length × width × height). The floor surface area of each control and treatment is 46.8 m<sup>2</sup>.

Four individual ramets of *M. chejuensis* collected from natural habitat, Jeju Island, were transplanted in each plastic pot (30 cm × 20 cm × 10 cm) in November 2009. Four pots were assigned into soil texture, water states and global warming treatments condition, respectively. Pots were given additional organic fertilizer (Monsanto Korea Inc., Seoul, Korea) which contains an ammonium nitrogen content below 170 mg/L and nitrate nitrogen at a concentration of 150-330 mg/L two times, at each spring for two experimental years.

### Growth parameters

At the end of the study in November 2011, we counted the number of shoot, leaves, sporophyll and measured the length of shoot and sporophyll. Belowground growth of *M. chejuense* was calculated as the increment of number of rhizome, total rhizome length, rhizome diameter and total rhizome area from initial measured value in 2009 to the last measured value in 2011. Total length and area of rhizome were measured using an image analyser with the Root Measurement System software, ver. 1.11 (Skye Instruments, Llandrindod Wells, UK). Survival rate was determined from the changes in shoot number during two years.

### Statistical analyses

The effects of soil texture, water and elevated CO<sub>2</sub>+T on the aboveground and belowground growth parameters of *M. chejuense* measured in this study were confirmed via one-way ANOVA and then the statistical differences between environmental gradients were evaluated via Fisher's least significant difference test as post-hocs, with significance at  $P = 0.05$ .

The multivariate analysis of variance (MANOVA) is a

complex statistic similar to ANOVA but with multiple dependent variables analyzed together. That is, the MANOVA is a multivariate extension of ANOVA (Zar 2009). MANOVA was used to analyze the effects of soil texture, water and elevated CO<sub>2</sub>+T and their interaction on above- and below-ground growth of *M. chejuense*. All statistical analyses were performed at 0.05 levels with STATISTICA 8 software (Statsoft, Inc. Tulsa, OK, USA).

## RESULTS AND DISCUSSION

Plant growth responds to multiple environmental factors, light, water, temperature and soil properties, also two or more resources simultaneously limit plant development (Chapin et al. 1987). Unfortunately for most endangered species, little information is available on their ecological requirements. Thus, it is necessary to determine optimal environmental conditions of species for the conservation of rare and endangered plants (Smith et al. 1993). According to Lee et al. (2012), the habitats of *M. chejuense* have a high ratio of rock exposure, shallow and wet soil layer, but experimental studies on the optimal environmental conditions for growth is unknown. Thus, we determined the effects of environmental factors on above- and below-ground growth and development of *M. chejuense*.

As a result, the pooled data of the *M. chejuense* showed significant soil texture, water and CO<sub>2</sub>+T effects on all aboveground growth parameters, and the significant interaction effects of those were observed except for the length of sporophyll (Table 1).

The average aboveground growth response for *M. chejuense* grown under the three environmental treatments is shown in Fig. 1. Number and length of shoot of the *M. chejuense* were higher at sand and ECET gradient that at

clay and ACAT gradient, but those of *M. chejuense* were not significantly affected by water regimes (Fig. 1a and 1b). The number of sporophyll was higher at elevated CO<sub>2</sub> concentration and temperature than ambient ones, while those were not significantly affected by soil texture and water regimes (Fig. 1c), while sporophyll length of *M. chejuense* grown under the sand and ECET treatments were longer than those grown under the clay and ACAT treatments (Fig. 1d).

Growth and potential reproduction in pteridophyte are reflected in the number of leaves that are produced within a given time period (Sharpe and Mehlreter 2010). In the present study, there were significant water and CO<sub>2</sub>+T effects on number of leaves (Fig. 1e). The number of leaves of *M. chejuense* was higher at non-flooding (NF) than flooding (F) and *M. chejuense* grown at ECET had a lot more leaves than plants grown at ACAT.

Many pteridophytes supplement their sexual cycles with various forms of vegetative reproduction (Yatskiyevych 2003). The branching of the rhizome and formation of adventitious buds on rhizomes is ferns of Ophioglossaceae have been reported (McMaster 1996). According to Kim (2004), *M. chejuense* propagates vegetatively by occurrence of branches arising as adventitious buds on older portions of the rhizome only when the rhizome has attained a certain length. Thus, the rhizome development of *M. chejuense* may be directly related to reproductive success.

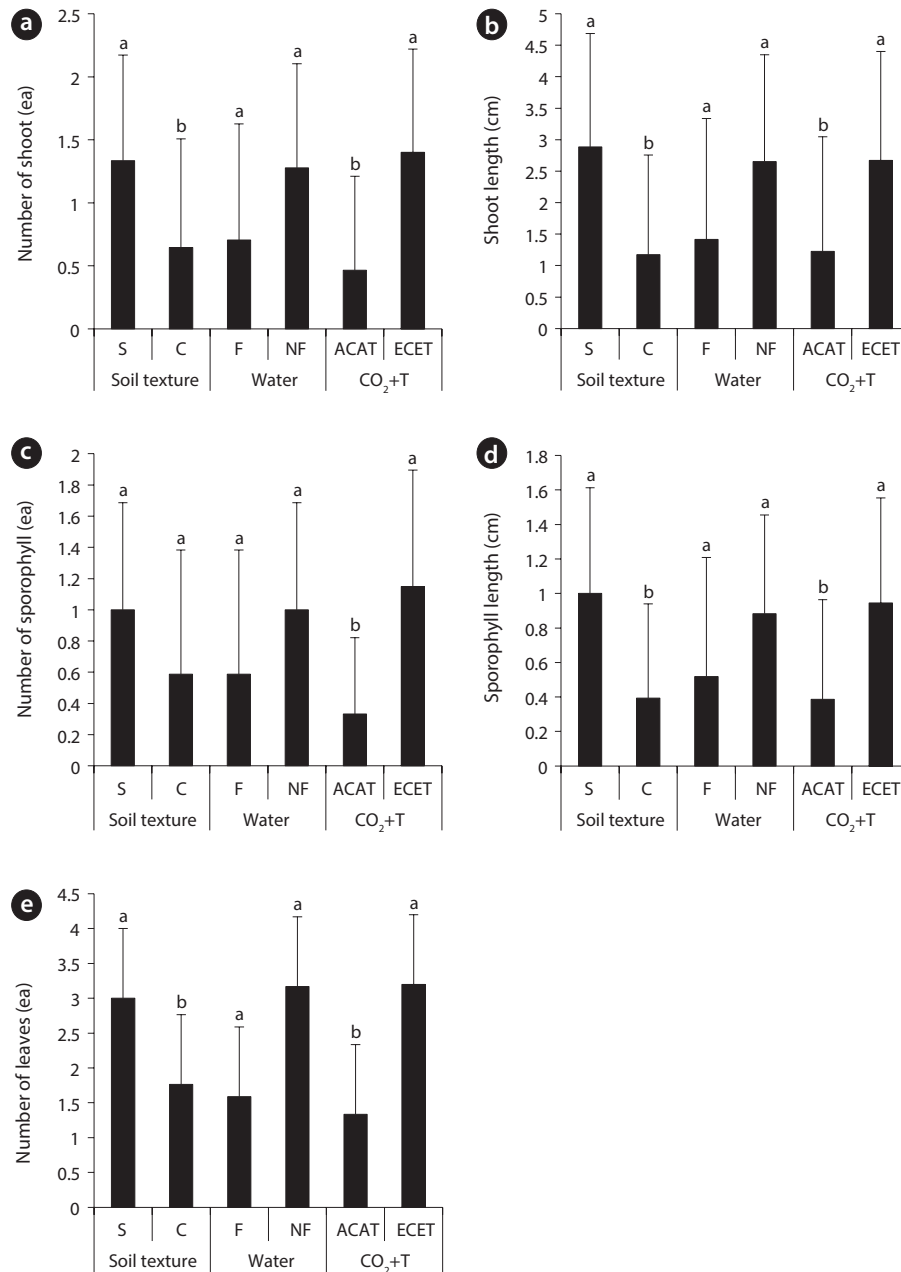
Unlike aboveground growth responses to three environmental factors, the development and growth of aboveground of *M. chejuense* were significantly affected by water and CO<sub>2</sub>+T treatments except for soil texture (Table 2).

The growth increment of the number and total area of rhizome showed similar trends (Fig. 2a and 2d) between 2009 and 2011. The mean number and total area of rhi-

**Table 1.** Effects of environmental factors and their interactions on aboveground growth of *Mankyua chejuense*

Factors	No. of shoot		No. of sporophyll		No. of leaves		Shoot length (cm)		Sporophyll length (cm)	
	F	P	F	P	F	P	F	P	F	P
Soil texture (S)	11.943	<0.002	6.141	<0.020	6.860	<0.028	15.839	<0.000	16.375	<0.000
Water (W)	9.561	<0.005	6.862	<0.014	11.967	<0.028	10.644	<0.003	8.313	<0.008
CO <sub>2</sub> +T (C)	21.795	<0.000	22.502	<0.000	14.859	<0.000	12.370	<0.002	15.433	<0.000
S × W	0.298	ns	0.847	ns	0.002	ns	0.283	ns	0.892	ns
S × C	0.180	ns	0.125	ns	0.022	ns	1.448	ns	2.843	ns
W × C	1.327	ns	0.847	ns	2.198	ns	4.241	<0.049	4.026	ns
S × W × C	6.179	<0.019	9.268	<0.005	5.864	<0.022	5.037	<0.033	2.006	ns

ns, non-significant ( $P > 0.05$ ). F and P values for the repeated measures ANOVA with soil texture, water, CO<sub>2</sub> concentration, temperature and their interactions as aboveground growth characteristics as response variables.

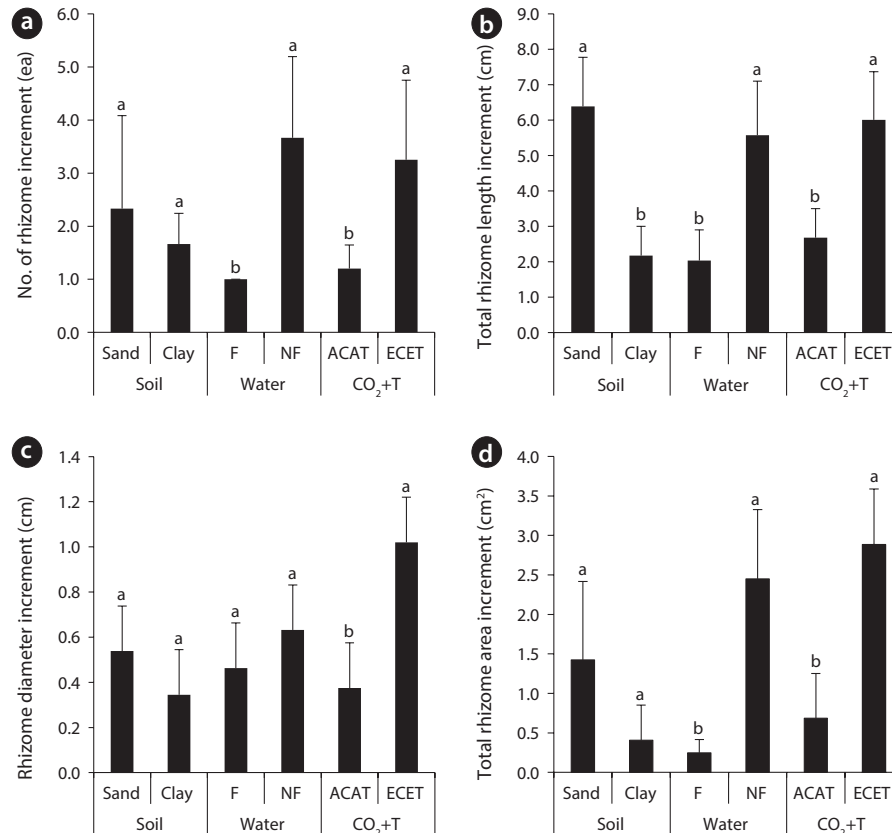


**Fig. 1.** Number of shoot (a), number of sporophyll (b), number of leaves (c), shoot length (d) and sporophyll length (e) of *Mankyua chejuense* grown under the soil texture (S, sand; C, clay), water regimes (F, flooding; NF, non-flooding) and CO<sub>2</sub>+T (ACAT, ambient CO<sub>2</sub> and ambient temperature; ECET, elevated CO<sub>2</sub> and elevated temperature) treatments. Different lower cases on the bars indicate significant differences within environmental factors (Fisher's least significant difference,  $P < 0.05$ ).

zome increment in the non-flooding treatment were 3.6- and 9.8-fold higher than in the flooding treatment respectively. In the CO<sub>2</sub> and temperature treatments, the growth increments in number and total area of rhizome were approximately 2.7- and 4.2-fold higher in the ACAT than in the ECET. However, soil texture effects were not detected in the growth increment of number and total area of rhi-

zome.

*Mankyua chejuense* grown in the sand treatments had significantly greater rhizome length increment than those in the clay treatments, and the mean rhizome length increment amounted to 2.7 cm in the ACAT but 6.0 cm in the ECET during two years (Fig. 2b). Rhizome diameter was not significantly affected by soil texture water regimes



**Fig. 2.** Variation of mean growth increment in number of rhizome (a), total rhizome length (b), rhizome diameter (c) and total rhizome area (d) of *Mankyua chejuense* grown under the soil texture (S, sand; C, clay), water regimes (F, flooding; NF, non-flooding) and CO<sub>2</sub>+T (ACAT, ambient CO<sub>2</sub> and ambient temperature; ECET, elevated CO<sub>2</sub> and elevated temperature) treatments from November 2009 to 2011. Different lower cases on the bars indicate significant differences within environmental factors (Fisher's least significant difference,  $P < 0.05$ ).

while the increment in rhizome diameter of *M. chejuense* grown in the ECET was 4.2-fold higher than those grown in the ACAT (Fig 2).

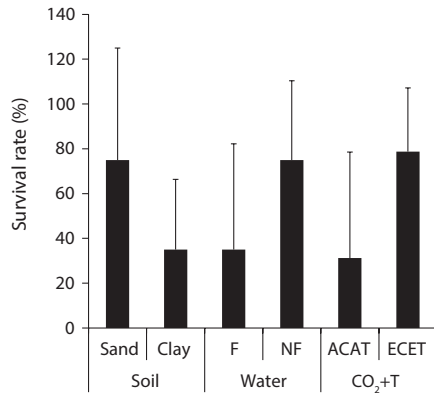
Elevated CO<sub>2</sub> concentration and temperature stimulated rhizome growth for *M. chejuense* (Fig. 2b). Many researchers determined that carbon allocation on the

root could be improved under elevated CO<sub>2</sub> and temperature, and then the ratio of belowground to aboveground increased (Rogers et al. 1996). Changes in belowground growth resulting from CO<sub>2</sub> enrichment and increased temperature will affect plant function and nutrient absorbability (Pritchard and Rogers 2000). Thus, elevated

**Table 2.** Effects of environmental factors and their interactions on belowground growth of *Mankyua chejuense*

Factor	No. of rhizome		Total rhizome length (cm)		Rhizome diameter (cm)		Total rhizome area (cm <sup>2</sup> )	
	F	P	F	P	F	P	F	P
Soil texture (S)	0.106	ns	0.015	ns	0.043	ns	0.027	ns
Water (W)	14.734	<0.018	5.222	<0.044	5.683	<0.049	7.169	<0.045
CO <sub>2</sub> +T (C)	3.086	ns	4.079	<0.047	8.206	<0.046	5.306	<0.033
S × W	0.038	ns	2.642	ns	0.175	ns	1.150	ns
S × C	0.038	ns	0.699	ns	0.003	ns	0.557	ns
W × C	1.528	ns	0.156	ns	0.013	ns	0.477	ns
S × W × C	0.106	ns	0.282	ns	0.594	ns	0.030	ns

ns, non-significant ( $P > 0.05$ ). F and P values for the repeated measures ANOVA with soil texture, water, CO<sub>2</sub> concentration, temperature and their interactions as belowground growth characteristics as response variables.



**Fig. 3.** The survival rate (%) of *Mankyua chejuense* in response to three environmental factors (soil texture, water regimes, and CO<sub>2</sub>+T). F, flooding; NF, non-flooding; ACAT, ambient CO<sub>2</sub> and ambient temperature; ECET, elevated CO<sub>2</sub> and elevated temperature. Error bars represent standard deviation.

CO<sub>2</sub> concentration and temperature may positively affect rhizome growth of *M. chejuense*.

*Mankyua chejuense* grown at sand, non-flooding and elevated CO<sub>2</sub>+T conditions had a higher rate of survival to the plant grown at sand, flooding and ambient CO<sub>2</sub>+T (Fig. 3).

We found that there was a significant effect of water regimes and elevated CO<sub>2</sub> and temperature on the above- and below-ground growth of *M. chejuense* (Table 1 and 2). In general, soil moisture is the most significant environmental factor affecting plant growth and population structure. Also, there is a positive relationship between pteridophyte species richness and water availability (Bickford and Laffan 2006). For example, in habitats of *M. chejuense*, water passes through soil relatively quickly because of high permeable soil originated volcanic rocks, and it is thus usually dry except during the summer rainy season, and Shin (2012) found that abundant habitats with the *M. chejuense* showed shorter flooding time than in rare one. In this study, we discovered that soil non-flooding conditions negatively affected on growth of *M. chejuense*. Thus, if flooding is prolonged in stages of plant development, the above- and below-ground growth of *M. chejuense* may be negatively affected.

Scenarios for future global and regional climate change will include elevated atmospheric CO<sub>2</sub> (Jones 2000), warmer average air temperatures and increasing the frequency of severe weather events, such as flooding (IPCC 2007). Under future climate change, the growth and survival of *M. chejuense* may positively be affected by elevated CO<sub>2</sub> and temperature, but not negatively be affected by flooding conditions. Thus, it can be concluded that the

optimal growth environmental conditions in habitats of *M. chejuense* is a well-drained and sand soil conditions and increasing CO<sub>2</sub> concentration and high temperature will greatly enhance growth of *M. chejuense* as long as drainage maintenance is performed in natural habitat in case of flash floods under future climate change situation.

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