Lee et al. Journal of Ecology and Environment (2017) 41:35
DOI 10.1186/s41610-017-0054-z

Journal of Ecology
and Environment

RESEARCH Open Access

Effect of nutrient and moisture on the growth and reproduction of *Epilobium hirsutum* L., an endangered plant

Eung-Pill Lee[1], Young-Sub Han[1,2], Soo-In Lee[1], Kyu-Tae Cho[1], Jae-Hoon Park[1] and Young-Han You[1*]

Abstract

Background: In this study, the growth and reproductive response of seedlings, grown in plastic pots with sand, to moisture and nutrients were analyzed in order to study the environmental conditions required to create an alternative habitat for *Epilobium hirsutum* L., an endangered plant.

Results: Vegetative and reproductive growths of *Epilobium hirsutum* L. are accelerated with increase in moisture and organic matter content in the soil. Among vegetative organs, the number of runners related to asexual reproduction was the highest when the moisture content was over 25% and nutrient content between 7 and 14% in the soil. But the number of flowers related to flowering responses, among reproductive organs, was the highest when the moisture content was maintained at 75% and when nutrient content was 21% in the soil. The number of seeds, related to sexual reproduction, was the highest when the moisture content was over 25% and nutrient content between 14 and 21%.

Conclusions: The study results show that a place with high moisture and nutrient content in the soil is advantageous to asexual and sexual reproduction of *Epilobium hirsutum* L. Therefore, we must serve periodically nutrient and seeds to sustain population in in situ conservation. Furthermore, it is advisable to create in riverside where abundant nutrient content have, making alternative habitat of *Epilobium hirsutum* L. Also, we must find species that have high contribution degree index through vegetation survey.

Keywords: Vegetative growth, Asexual reproduction, Population, Alternative habitat

Background

About 650 species within 17 families of *Onagraceae* are distributed around the world in temperate and subtropical regions where the majority of them are found in Western regions of North America (Levin et al. 2003). *Epilobium hirsutum* L., a perennial plant that belong to family *Onagraceae*, is known to be distributed in China, India, Europe, North America, and South Africa (National Institute of Biological Resources 2012). In South Korea, it has been reported that it is found only in Ulleong-do, Mt. Juwang, Samcheok, and Jeongseon. (National Institute of Biological Resources 2012; Yoon and You 2014).

Epilobium hirsutum L., which is known to be a northern plant around the world, is a common plant, but it is considered an endangered species in the central regions of Korea which is the southern limit line of its distribution (Ministry of Environment 2013). There are limited number of individuals and population of northern plants because they are scarcely distributed in Korea. And the increase in temperature caused by global warming has put them in the risk of extinction (Ministry of Environment 2013). Species that could not adapt to the environmental change, in terms of reproductive ecology, are less competitive than those that are widely distributed (Lavergne et al. 2004). In addition, the environmental change increases the risk of extinction for species in the region (Thomas and Williamson 2012).

Another reason for *Epilobium hirsutum* L. being endangered is because a lot of its habitat has been destroyed by numerous developments (National Institute of Biological Resources 2012). It is easy for population to become extinct if its habitat is only found in one or

* Correspondence: youeco21@kongju.ac.kr
[1]Life science, Kongju National University, Kongju, South Korea
Full list of author information is available at the end of the article

two places within a limited geographical area and if its habitat is affected by the people in the region (Lawler et al. 2010; Peery et al. 2004). Also, the habitat may not be suitable anymore for those species that live in that habitat if the habitat's surrounding environment is affected (Van Turnhout et al. 2010).

A root cause must be analyzed in order to restore an endangered species (Given 1994) and, in case of plants, a study on growth and reproductive traits should be carried out in advance (Song et al. 2009).

Soil is one of the important components for plants because it contains nutrients and moisture necessary for plant growth (Kim et al. 2003). Among nutrients, organic matter increases chemical, physical, microbiological features of the soil assisting plants in assimilation and in root elongation (Joergensen et al. 1990). But the plants cannot have a normal growth if there is excess or insufficient moisture in the soil (Hsiao and Jackson 1999). If there is a lack of oxygen supply from excess moisture, the plant growth is aggravated because the root cannot absorb nutrients and moisture (Hsiao and Jackson 1999). And this lack of moisture, a water stress, could cause abscission of leaves and reduction in leaf area and seed production (An et al. 2008).

Worldwide scholars have been studying how moisture and nutrients affect endangered plants (Hasselquist and Allen 2009; Lentz 1999). Found only in the oasis of Amargosa Valley, *Nitrophila mohavensis* and *Grindelia fraxinopratensis* face extinction as people used water in the oasis excessively for agriculture, cattle farming, and mining (Hasselquist and Allen 2009). A study on the effect of nutrient supply was conducted to restore *Scirpus ancistrochaetu*, an endangered plant, and it revealed that the plant's growth and biomass enlarged with increase in nutrient supply (Lentz 1999). Therefore, we need to know the ecological requirements of a plant species as it can react differently to various environmental conditions (Kim et al. 1995). Moreover, it is essential to search for an adequate environment of a plant species by experimenting with the environmental conditions (Larcher 2003).

Until now, the following studies related to endangered species have been conducted in Korea: "Distribution and Conservation (Lim et al. 2010; Lee et al. 2011)", "Habitat Characteristics Analysis (Kim et al. 2013; Choi et al. 2014)", "Climate Change (Han et al. 2012; Park et al. 2014)", "Growth and Reproduction Characteristics (So et al. 2008; Lee and Kim 2011)", and "Restoration and Evaluation (You and Kim 2010; Bae 2012)." A research on its taxonomy has been conducted (Lee et al. 2013), but basic data on its habitat, growth, and reproduction does not exist.

The moisture and organic matter in the soil, which are core factors for plant growth, were treated to obtain basic data on ecological characteristics of *Epilobium hirsutum* L., an endangered species, for the species conservation and restoration and to find adequate soil conditions based on the data.

Methods

Study material

The seeds of *Epilobium hirsutum* L. used in this study were bought from Key-chungsan botanical garden and were kept in the refrigerator at 4 °C. Then, they were germinated from mid-March to late-April before being used.

Habitat environmental factor analysis

The habitat of *Epilobium hirsutum* L. in Mt. Juwang National Park did not have species that block the light, and the illuminance was constantly measured at 280.87 ± 12.59 lx using digital lux meter (TES 1332A, TES, Taiwan). The soil from three places, within the habitat, was collected for the analysis of water content and organic matter. Five grams of fresh soil was dried in a hot air dryer over 48 h at 105 °C, and the difference in mass was expressed in percentage to measure the water content. After removing the foreign substance, 5 g of soil sample was dried for more than 48 h at 105 °C and weighed. It was then baked for 4 h in an electric furnace to calculate the organic matter by deducting ash content from dry weight. As a result, the water content was 17.6 ± 9.5% and organic matter was 3.0 ± 0.6%.

Cultivation and environmental factor treatment

The experiment was carried out outside, where environment conditions such as temperature, humidity, and precipitation are the same, from May 2015 to September 2015. In May, the seedlings of *Epilobium hirsutum* L. that have 2~3 leaves were individually transplanted into twenty small plastic pots (outer diameter 14.9 cm × height 13.2 cm × bottom diameter 15 cm) for each gradient. But from July, large plastic pots (outer diameter 24.5 cm × height 14.5 cm × bottom diameter 14.5 cm) were used to prevent impediment to the root growth. The seedlings were sufficiently watered before the transplant, and the empty spaces were covered with sand rather than pressing it with hand. During the transplant, we ensured that the amount of sand remain constant by maintaining the height of seedlings consistently because moisture or nutrient retention capacity could change depending on the amount of soil in the pots.

It is well known that moisture and nutrients are crucial for plant's growth (Barbour et al. 1999). Therefore, moisture and organic matter were treated to investigate the growth and reproductive response of *Epilobium hirsutum* according to increase in moisture and nutrients. Based on results about soil analysis of Juwang Mountain National Park, Korea , moisture gradient was divided into four gradients by 25% interval: low moisture condition (M0, 25%), low medium moisture condition (M1, 50%), high medium moisture condition (M2, 75%), and high moisture condition (M3, 100%).

As a result of soil analysis in Mt. Juwang National Park where inhabit *Epilobium hirsutum* L., organic matter content in soil is low as 3.0 ± 0.6%. However, we confirmed that their site is influenced by water erosion, so we referred to organic matter content of other plants (*Scrophularia takesimensis*; 9.1–19.1%, *Eleutherococcus senticosus*; 8.27–19.75%, *Viola websteri*; 8.1–22.8%) and then select maximum value. We referred report (Brady and Weil 1996) showed the good condition of plant growth is usually 5%, so we selected conveniently the first gradient as 7%. Therefore, considering nutrient contents of habitats of other endangered species, we treated four nutrient gradients: low nutrient condition (N0, 0%), low medium condition (N1, 7%), high medium condition (N2, 14%), and high nutrient condition (N3, 21%).

Moisture

The field capacity, which is the amount of moisture held in soil located in pots after excess, was drained away and was set as the maximum value for water treatment. The field capacities of small and large plastic pots were 200 mL (M3) and 1000 mL (M3) respectively. Dry sand with less than 2 mm sand grains were used as soil. When pots were filled with dry sand, the small and large plastic pots each weighed 1 and 2 kg.

Nutrients

Sand grains that are less than 2 mm in size were used as soil and organic matter was mixed proportionally by using dry sand as the standard (100%) for nutrient treatment. The composite (Heuksal Gold, KG Chemical Corp.) that has following properties was dried and used as nutrient in the study: 40~60% moisture, 60~80% water retention, 0.2~0.4 Mg m^{-3} bulk density, pH 5.5~7.5, EC ≤ 1.2dS m^{-1}, NH_4-*N* ≤ 600 mg L^{-1}, NO_3-*N* ≤ 300 mg L^{-1}, ≤ 500 mg L^{-1} soluble phosphate, and CEC 10–30 cmol L^{-1}.

Moisture and nutrient gradient were watered once in the morning and in the afternoon every day except during rainy season. During the rainy season (26 June; 7–9, 12–13, and 21–24 July), we provided water to the seedlings 3 days after the rain. The pots were rearranged every week to minimize the effect of pots' position.

Automatic weather system (AWS) of Korea Meteorological Administration was used to obtain values for the temperature (°C) and humidity (%) from the Gongju observatory. The daily values observed at 09:00, 12:00, and 17:00 were organized each month. The temperature was 21.38 ± 1.16 °C (Fig. 1), humidity was 55.93 ± 3.56% (Fig. 2), and precipitation was 1.62 ± 3.98 mm over the study period.

Data collection and measurement

The following variables were measured at the end of August 2015: shoot length (cm), leaf width length (ea), leaf lamina length (ea), and number of runners (ea) for growth; and number of flower buds (ea), number of flowers (ea), peduncle length (cm), number of seeds per peduncle (ea) and number of seeds per individual (ea) for reproduction.

The number of seeds per peduncle (ea) and the number of seeds per individual (ea) were counted every day from August, when the peduncle starts to mature, until end of September. The number of seeds per peduncle was calculated by dividing the total number of seeds by the total number of peduncle of an individual and the number of seeds per individual was calculated by adding all the seeds collected from all the peduncle of an individual.

Data analysis

Analysis of variance (ANOVA) and principal component analysis (PCA) were used to reveal the intraspecific variation and similarity of growth and reproductive responses of *Epilobium hirsutum* L. to each environmental gradient (No and Jeong 2002). For intraspecific variation, the significance of differences in gradients was verified by conducting analysis of variance for each trait. And the significance of differences in gradients was analyzed with post hoc analysis at 5% Fisher's LSD (No and Jeong 2002). The average value of trait was used as correlation coefficient and plotted using PCA to find the similarity (No and Jeong 2002).

The above statistical analysis was performed by using Statistica 8 (Statsoft Co. 2007).

Results and discussion

Vegetative growth

The shoot length (cm) tended to be long with increased in soil moisture (Fig. 3a). In nutrient gradient, the shoot length (cm) was longer high medium condition (N2) and high nutrient condition (N3) than low nutrient condition (N0) and low medium condition (N1) (Fig. 3e).

The number of leaves was greater in high medium moisture condition (M2) and high moisture condition (M3) than low moisture condition (M0) and low medium moisture condition (M1) (Fig. 3b). In nutrient gradient, the number of leaves was greater in low medium condition (N1) than low nutrient condition (N0). It tended to decline up to high medium condition (N2), but it was increased again in high nutrient condition (N3) (Fig. 3f).

The leaf width and leaf lamina length tended to be long with increased in soil moisture (Fig. 3c, d), and there was no significant difference in all nutrient condition (Fig. 3g, h).

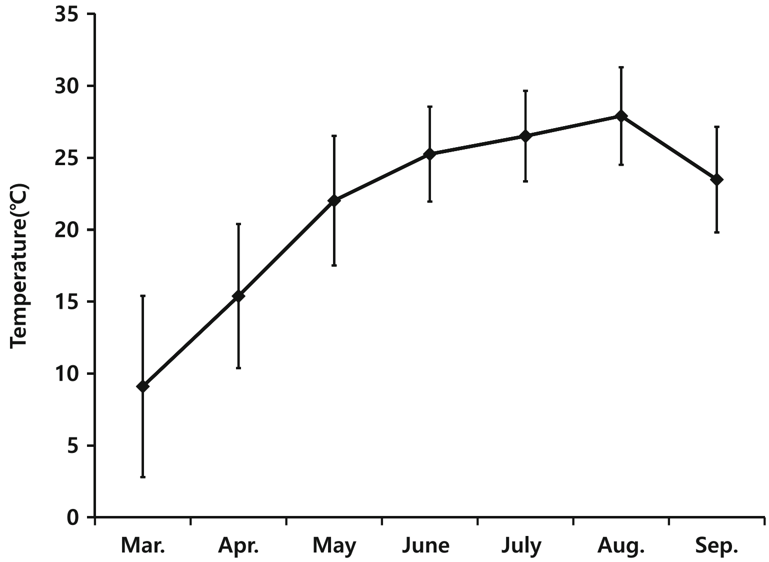

Fig. 1 Monthly average temperature from March to September 2015

Deducing from the increase in shoot length, number of leaves, leaf width length, and leaf lamina length in the moisture gradient (Fig. 3a–d), we think that the growth response got better because vegetative growth was great with increased in soil moisture because the morphological variation of leaves, which are directly related to the growth response, greatly affect physiological activities such as photosynthesis and respiration (Chim and Han 2003). The growth responses of perennial herbaceous plants were different as the moisture increased (Lee and Park 2005; Lee 1995; Jeong 2012). *Achyranthes japonica* increased in its shoot length (Lee and Park 2005), and *Valeriana fauriei* was on the rise in its leaf width length and leaf lamina length (Lee 1995). But *Bupleurum latissimum* showed no difference in the shoot length and the number of leaves across gradients (Jeong 2012).

In the nutrient gradient, it seems that the growth response was on the rise by increasing the number of leaves to obtain greater surface area for photosynthesis as organic matter increased (Lawlor and Mitchell 1991). The shoot length and number of leaves of *Bupleurum latissimum*, a perennial plant, was on the rise as organic matter increased (Jeong 2012). However, *Centella asiatica*, a perennial plant, showed no difference in the number of leaves across the gradients (Devkota and Jha 2009).

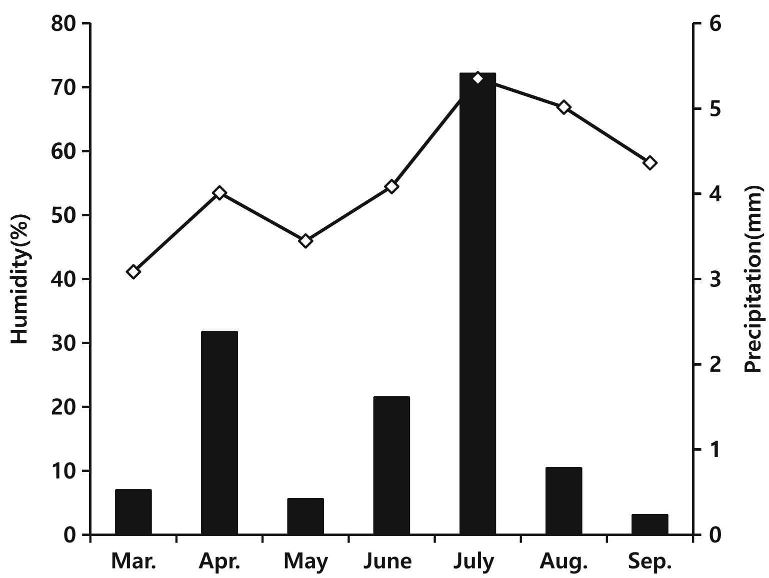

Fig. 2 Monthly average humidity and precipitation from March to September 2015

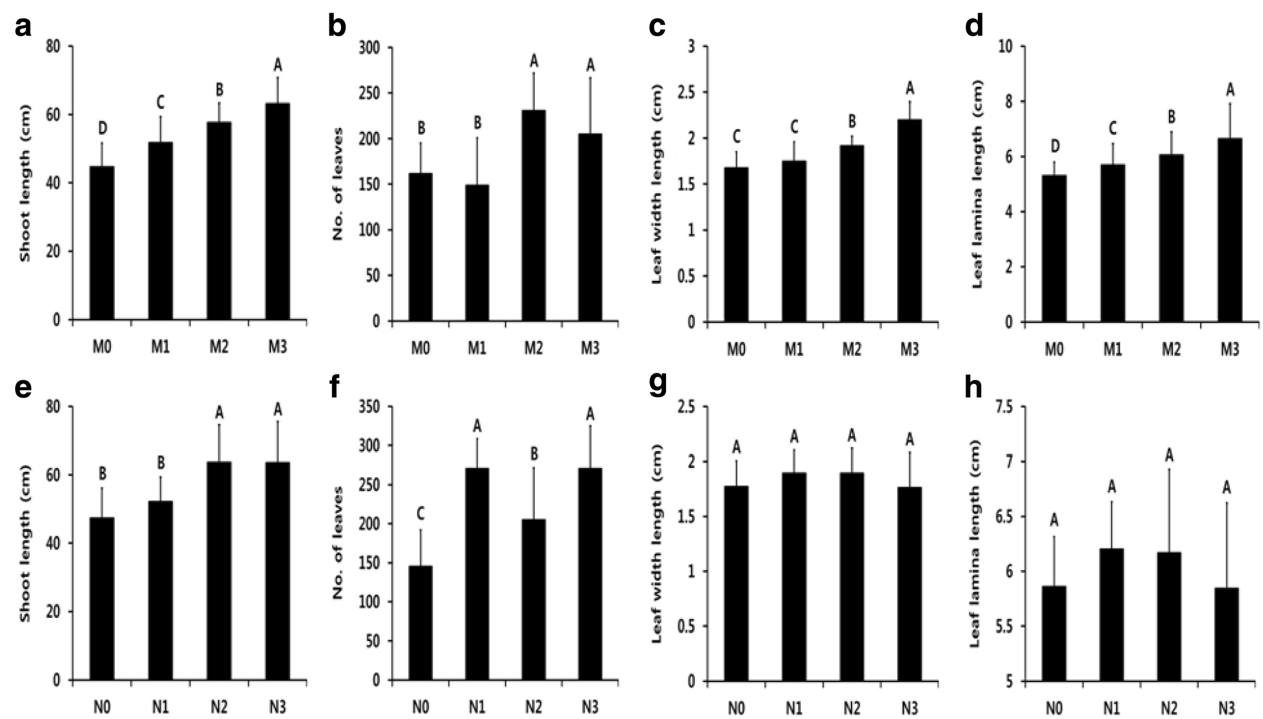

Fig. 3 Shoot length (**a**, **e**), no. of leaves (**b**, **f**), leaf width length (**c**, **g**), and leaf lamina length (**d**, **h**) of *Epilobium hirsutum* L. measured under moisture and nutrient gradients. Alphabets on the bars mean significant difference among environmental treatments (Fisher's least significant difference, $p < 0.05$). Above bars on the graph mean standard deviation. (*N = 20*)

Reproductive growth

The number of runners tended to rise with increased in soil moisture and the number of runners in low moisture condition (M0) was lower than the others (Fig. 4a). In nutrient gradients, the number of runners was higher in low medium condition (N1) than low nutrient condition (N0). It tended to increase up to high medium condition (N2), but it tended to decline again in high nutrient condition (N3) (Fig. 5a).

The number of flower buds and flowers tended to increase from low moisture condition (M0) to high medium moisture condition (M2), but it tended to decline from high moisture condition (M3) (Fig. 4b, c). In nutrient gradient, the number of flower buds and flowers tended to rise with increased in nutrient contents (Fig. 5b, c).

The peduncle length tended to be long with increased in soil moisture, and the peduncle length in low moisture condition (M0) was shorter than the others (Fig. 4d). In nutrient gradients, the peduncle length tended to be short from low nutrient condition (N0) to low medium condition (N1), but it tended to be long in high medium condition (N2) (Fig. 5d). However, the peduncle length tended to be short in high nutrient condition (N3) (Fig. 5d).

The number of seeds per peduncle was higher in low medium moisture condition (M1) than low moisture condition (M0), but it tended to decline from high moisture condition (M3) (Fig. 4e). In nutrient gradients, the number of seeds per peduncle tended to decline from low nutrient condition (N0) to low medium condition (N1), but it tended to increase from high nutrient condition (N3) (Fig. 5e).

The number of seeds per individual was lower in low moisture condition (M0) than the others (Fig. 4f). In high medium condition (N2) and high nutrient condition (N3), the number of seeds per individual was higher than low nutrient condition (N0) and low medium condition (N1) (Fig. 5f).

It seems that asexual reproduction of *Epilobium hirsutum* L. will become more active, leading to enlarge its population, with increase in moisture when the nutrient is 7~14% observing from the fact that the number of runners escalated with increase in moisture in the moisture gradient (Fig. 4a) and that the number runners are the greatest under medium nutrient condition (N1, N2) in the nutrient gradient (Fig. 5a). Similarly, *Polygonatum involucratum*, a perennial plant, and *Polygonatum humile* maintain their population by horizontally adding asexual reproductive individuals (Choung 1991).

Regarding the reproductive response, the number of flower buds and the number of flowers were the greatest under medium moisture condition (M2) in the moisture gradient (Figs. 4b, c and 5b, c). The peduncle length (Fig. 4d), the number of seeds per peduncle (Fig. 4e), and the number of seeds per individual (Fig. 4f) all

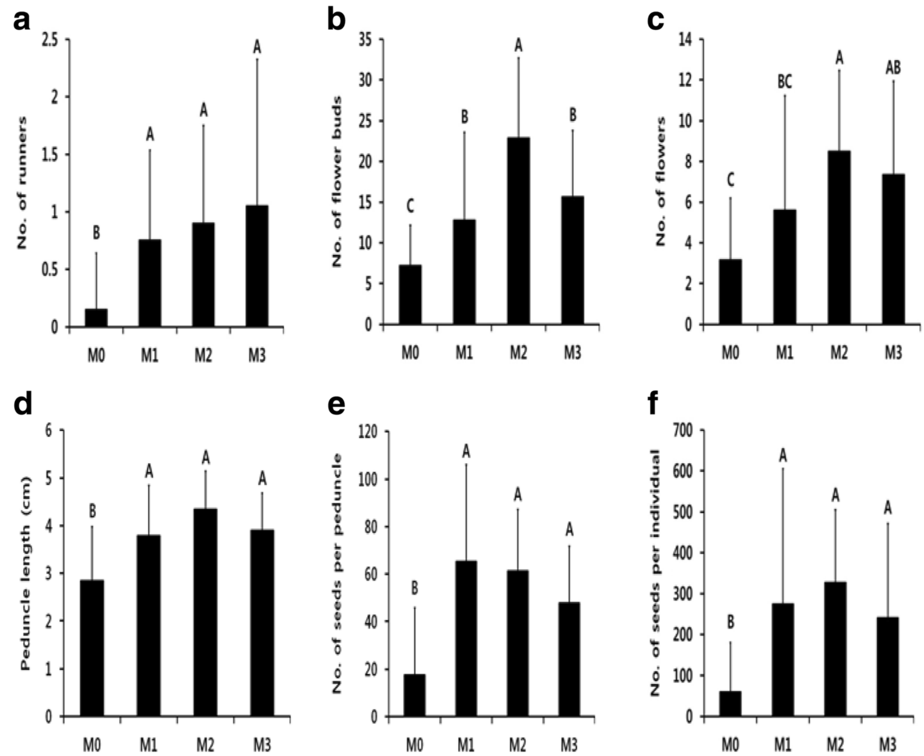

Fig. 4 No. of runners (**a**), no. of flower buds (**b**), no. of flowers (**c**), Peduncle length (**d**), no. of seeds per peduncle (**e**), and no. of seeds per individual (**f**) of *Epilobium hirsutum* L. measured under moisture gradients. Alphabets on the bars mean significant difference among environmental treatments (Fisher's least significant difference, $p < 0.05$). Above bars on the graph mean standard deviation. ($N = 20$)

escalated with increase in moisture and resulted in augmented reproductive response. The number of flower buds and the number of flowers were the greatest under high nutrient condition (N3) in the nutrient gradient (Fig. 5b, c). The peduncle length (Fig. 5d), the number of seeds per peduncle (Fig. 5e), and the number of seeds per individual (Fig. 5f) all escalated with increase in nutrient and resulted in augmented reproductive response. It is assumed that the result of reproductive response of *Epilobium hirsutum* L. in this study is due to change of plant phenology period according to optimal partitioning model (Beranacchi et al. 2000). The root of a plant, while absorbing moisture and nutrient from soil, changes its development stage and duration with the leaves and in the process it indirectly affects the reproductive response of the plants (Aizen 2003; Nord and Lynch 2009).

Principal component analysis

The distribution of individuals according to moisture and nutrient gradients was rather dispersed on the two-dimensional coordinates (Fig. 6). The lower left quadrant consisted of individuals from high nutrient condition (N3) whereas the center consisted of individuals from low moisture condition (M0), medium moisture condition (M1, M2), high moisture condition (M3), low nutrient condition (N0), and medium nutrient condition (N0) (Fig. 6). And the lower right quadrant consisted of individuals from low moisture condition (M0) and low nutrient condition (N0) (Fig. 6). Based on the plotted coordinates, it is evident that *Epilobium hirsutum* L. shows similar responses according to moisture and nutrients.

In the moisture gradient, the individuals from high moisture condition (M3) displayed similar responses as those from medium moisture condition (M1, M2) and the individuals from low moisture condition (M0) showed similar responses as those from medium moisture condition (M1) (Fig. 6). But the responses of individuals from medium moisture condition (M2) and high moisture condition (M3) were distinct from the responses of individuals from low moisture condition (M0) (Fig. 6). In the nutrient gradient, the responses of individuals from low nutrient condition (N0) and those from high nutrient condition (N3) were distinct although those from medium nutrient condition, N1 and N2, showed similar responses (Fig. 6).

There were several traits that affected the distribution pattern in the ordination. In case of factor 1 (37.80%), there were eight traits apart from leaf width length and leaf lamina length. In case of factor 2 (29.64%), there were five traits apart from shoot length, number of

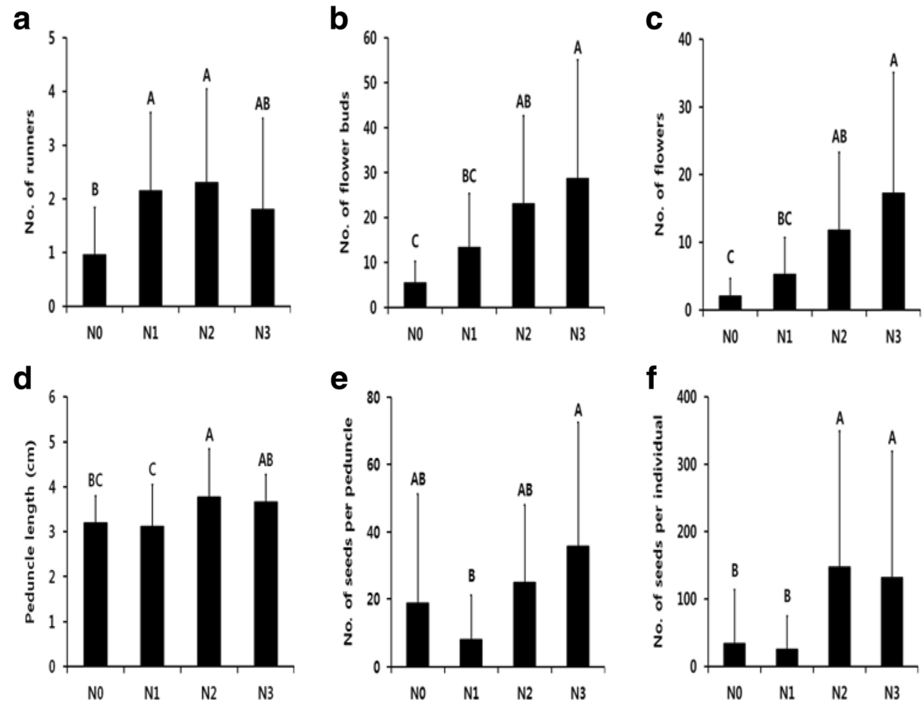

Fig. 5 No. of runners (**a**), no. of flower buds (**b**), no. of flowers (**c**), peduncle length (**d**), no. of seeds per peduncle (**e**), and no. of seeds per individual (**f**) of *Epilobium hirsutum* L. measured under nutrient gradients. Alphabets on the bars mean significant difference among environmental treatments (Fisher's least significant difference, $p < 0.05$). Above bars on the graph mean standard deviation. ($N = 20$)

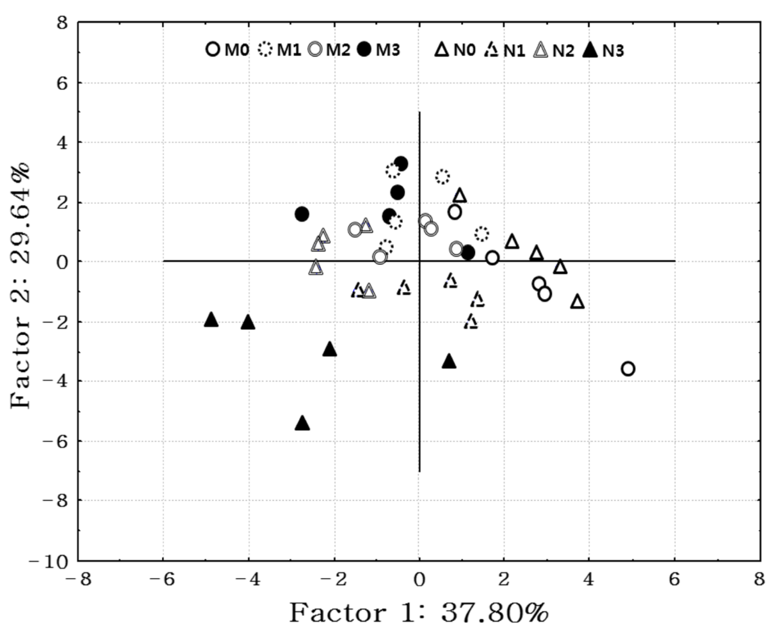

Fig. 6 Two dimensional of PCA ordination of 40 individuals of *Epilobium hirsutum* L. using 14 variables treated to moisture and nutrient gradients. (M moisture, N nutrient)

leaves, number of runners, number of flower buds, and number of flowers (Table 1).

The PCA result confirmed that the responses of individuals from high nutrient condition (N3) were different from responses of individuals from moisture conditions (M0, M1, M2, M3) and nutrient conditions (N0, N1, N2). Considering that ecological niche breadth include the range of environmental tolerance (Pianka 1983), the fact that vegetative and reproductive growth of *Epilobium hirsutum* L. were the highest (Figs. 3, 4, and 5) in high nutrient condition (N3) clearly shows that this plant adapts well to an environment such as N3. In addition, the distinction of responses displayed by *Epilobium hirsutum* L. into various groups according to moisture and nutrient conditions is not caused by one or two trait but rather it involves overall trait of the plant.

Conclusions

The endangered plants are weaker than other plants in competition, and they are greatly affected by the environmental conditions of their habitats (Kim et al. 1995). Therefore, basic studies on the growth and reproductive characteristics must precede to restore endangered plants (Song et al. 2009) and only then should the damaged habitat be restored and alternative habitat be provided considering the environmental conditions (Kim et al. 1995).

The soil is one of the essential components in the plant growth. All the plants grow and reproduce on soil and their distribution largely depends on the characteristic of soil (Kim et al. 2003). In this study, the moisture and nutrient were treated in soil—an essential element for plants—to examine the response of *Epilobium hirsutum* L. to environmental factors and the growth response was great with increased in moisture and organic matter. Also, sexual and asexual reproduction was increased by raising the number of seeds and runners.

Table 1 Correlation matrix of 14 variables with the first and two principal component scores of PCA analysis

Variables	Factors	
	I	II
Shoot length	− 0.895394	0.021109
No. of leaves	− 0.567502	− 0.207993
Leaf width length	− 0.176009	0.690317
Leaf lamina length	− 0.195294	0.543986
No. of runners	− 0.395305	− 0.171323
No. of flower buds	− 0.859450	− 0.266172
No. of flowers	− 0.872042	− 0.255074
Peduncle length	− 0.455668	0.630369
No. of seeds per peduncle	− 0.344601	0.652395
No. of seeds per individual	− 0.687888	0.529765
Variance explained (%)	37.80	29.64

Although in situ conservation like installation of pence and protective facility is carrying out for protecting habitat of endangered species, it is not reasonable because it is performed without sufficient information about the species (Byun et al. 2013). To succeed in in situ conservation of *Epilobium hirsutum*, sufficient nutrient that can recruit autonomously individual must be provided periodically. Also, it is necessary to multiply individuals in ex situ conservation institute and then supply seeds (Crawley and Ross 1990).

To carry out ex situ conservation with in situ conservation, we think that reservoirs near the riverside where have high moisture and nutrient content is suitable alternative habitat. However, it is necessary to figure out species that has high index. Because when habitat of *Epilobium hirsutum* is influenced by artificial and natural disturbance, the species invade first and then other plants are diffused. In other words, the species play a role bridgehead (Ren et al. 2008). We think that finding the species is important information to create new alternative habitat, to sustain population of rose and to manage habitat (Lortie and Turkington 2008).

Abbreviations
ANOVA: Analysis of variance; AWS: Automatic weather system; PCA: Principal component analysis

Funding
This study was supported by Mid-career Researcher Program (NRF-2016R1A2B1010709) through NRF grant funded by the MEST.

Availability of data and materials
Not applicable

Authors' contributions
All authors conducted a survey together during the study period. LEP wrote the manuscript. LSI and PJH collected the data. HYS and CKT analyzed the data. YYH participated in the design of the study and examined the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Life science, Kongju National University, Kongju, South Korea. [2]Division of Ecological Survey Research, National Institute of Ecology, Seocheon, South Korea.

Received: 15 June 2017 Accepted: 25 September 2017
Published online: 03 October 2017

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