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Habitat Characteristics and Ecotypic Differentiation in *Cicuta virosa*, an Endangered Species in Korea

국내 멸종위기종인 독미나리의 서식지 환경 특성과 생태형적 차이

2013년 2월

서울대학교 대학원
과학교육과 생물전공
신 차 정
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ABSTRACT

Habitat Characteristics and Ecotypic Differentiation

in *Cicuta virosa*, an Endangered Species in Korea

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*Cicuta virosa* L. (water hemlock) is a perennial herb native to northern and central Europe, northern Asia, and northwestern North America. Its population size has decreased in Korea which is a southern marginal area of distribution and *C. virosa* listed as an endangered species in Korea. Only four habitats of *C. virosa* are known in South Korea. Pyeongchang (PC) was a fenced wet meadow with water shortage in spring and Hoengseng (HS) was about 12 year old abandoned paddy field and narrow streamlet holding shallow water level through the year. Gunsan (GS) was a 1m deep reservoir with floating mat composed of organic matter in 2° south of HS and PC and very low altitude. Last, Daegi-ri (DG) was an agricultural waterway recorded as a *C. virosa* habitat for the first time in South Korea but now, there are less than 10 individuals of *C. virosa*.

To conserve and restore habitats of this plant, I investigated water and soil environmental characteristics and vegetation at four habitats during the growing season. The *C. virosa* habitats differed in community
structure, water and substrate properties, and water regime. Although the total distribution ranges of the water and soil environments for *C. virosa* were wide and overlapped with the optimal environmental range of distribution of accompanying species, the optimal water level range for *C. virosa* was defined as $7 \pm 3.5$ cm. Water level was adjusted by substrate structure such as a mound of *P. japonica* and a floating mat comprised of accompanying species. A floating mat could be an aid to maintain an optimal and stable water level in deep or fluctuating water and to prevent strong competition with prolific macrophytes. The GS sampling site, which had floating mats, could be a good model for *C. virosa* conservation in a warm temperate region, whereas the PC sampling sites, which experienced a water shortage in spring, provided a clue about the decline in *C. virosa* population size.

Based on the first survey result, I surveyed seedling distribution in GS and HS habitats and examined growth increment in mesocosm by planting *C. virosa* on artificial mats and soil to investigate the function of floating mat in seedling establishment and population sustenance of *C. virosa*. Seedling density and coverage on floating mats and their edges, with high level of solar radiation and sufficient water level, were significantly higher than those on soil. In the mesocosm experiment, shoot and root dry-weight in soil was double to those in artificial mats, and these significant difference reflected nitrogen availability in the substrate. However, there was no significant difference in the numbers of flower stalks, or tiller or stem width, despite the difference in nutrients level of the three substrates. I also observed life history variation between GS and HS populations. These results showed that floating mat was advantageous to the establishment of seedlings and to the expansion of *C. virosa* population, and high allocation to reproduction of *C. virosa* sustained coexistence with prolific macrophytes on floating mat even though the overall growth on
floating mat was worse than that on soil. Using floating mat could be a way for *C. virosa* population sustenance and conservation.

Through the advanced two habitat surveys, I found out differences not only in habitat environments (e.g. altitudes, climates, water regimes and nutrient availability) but also in leaf morphology and flowering time among populations. I considered that those three populations (PC, HS and GS) could be classified as ecotypes and figured out intraspecific variations of *C. virosa* populations as ecotypic differentiations in morphological and physiological features of seed and seedling by tests of germination responses to light and temperature and the sensitivity of seedlings to dryness and shade. I also analyzed which traits were critical to separate three populations and interpreted relationships between discriminating traits and habitat environments.

The seed length of GS (2.04 ± 0.03 mm) was significantly shorter than that of PC (2.44 ± 0.05 mm) and HS (2.60 ± 0.03 mm). Seed weight of GS (0.83 ± 0.01 mg) was significantly lighter than that of the others (PC: 1.47 ± 0.02 mg, HS: 1.33 ± 0.02 mg). PC and HS seedlings had larger root: shoot ratio values and GS had significantly higher relative growth rate (RGR) through 8 weeks of growth. GS seedlings were more sensitive to dryness than PC and HS in leaf chlorophyll contents, specific leaf area (SLA) and RGR. The pattern of germination responses was similar among the three populations but germination rates were very different. Maximum germination rates were 63.0%, 23.9% and 96.9% in PC, HS, and GS, respectively, under 28/18°C/14h photoperiod. The nonviable seed rate of GS (18.05±0.70%) was significantly higher than that of the others (PC: 3.07±1.63%, HS: 3.44 ± 0.62%) in the tetrazolium tests result. Seed mass which was correlated to other plant traits (i.e., root: shoot ratio, RGR) and germination rate were the most discriminating variables in the discriminant analysis. Seed weight was negatively
correlated with temperature in January and water level and germination rate was significantly related with water level and water temperature in August. Based on these results, I suggest that these three *C. virosa* populations from different habitats are ecotypes.

**Keywords**: ecotype, floating mat, regeneration niche, seed size, substrate, water depth

**Student Number**: 2011-21592
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INTRODUCTION

*Cicuta virosa* L. (northern water hemlock) is a perennial herb native to northern and central Europe, northern Asia, and northwestern North America (Mulligan and Munro 1980, Lee 2003). The genus *Cicuta* is the most virulently poisonous group of flowering plants native to the northern temperate zone and was recognized four species - *C. bulbifera* L., *C. douglasii* (DC.) J.M Coult. & Rose, *C. maculate* L., and *C. virosa* L. (Lee and Downie 2006). In Korea, only *Cicuta virosa* distributed and its population size has decreased which is a southern marginal area of distribution. At last, it has been designated an endangered species by the Natural Environment Conservation Law and as a rare plant by the national arboretum in Korea (Korean Environment Institute 2005, National Arboretum 2009). *C. virosa* is also listed in 17 of 47 Japanese prefectural red lists (Japanese Wildlife Research Association 2012). In Hungary where almost all fens had been destroyed, *C. virosa* was a protected species (Tatár 2010). A few surveys of wild and rare plants in Korea have been carried out in the last decade due to urgency (e.g., Yoo et al. 2004, Jang et al. 2009, Song et al. 2010) and a series of studies on the conservation strategy for endangered and reserved plants based on the ecological and genetic characteristics has been performed by the National Institute of Environmental Research. However, *C. virosa* has not been a target species.

The water hemlock produces a lateral tuber before the root system dies in the fall and initiates a new plant the following spring (Kingsbury 1964). The plants persist by producing several new rootstocks from buds around the perimeter of the old rootstock (Mulligan and Munro 1980). The roots and tubers of *C. virosa* are toxic (Panter et al. 1988), and most studies on *C. virosa* have concentrated on its toxicology and pharmacology (e.g., Sarreveld 1975, Panter et al. 1988, Strauß et al. 1996). Water hemlock grows
in a very wet substrate and is usually found growing in streams, ditches, lakes, rivers, or marshy areas. This high water requirement limits its range (Panter et al. 1988). Other environmental characteristics have not been studied well, even though basic ecological information is necessary to conserve *C. virosa*.

Firstly, I tried to understand the environmental characteristics of *C. virosa* habitats. The specific objectives were to identify the major factors determining *C. virosa* distribution and the reason that the *C. virosa* population has decreased by comparing the water and soil environmental characteristics of *C. virosa* habitats. The water regime is a major determinant of plant community development and patterns in wetlands (Spence 1967, Casanova et al. 2000, Heegaard et al. 2001) and soil variable gradients are also important in determining plant community composition (Fitter 1982, Keddy 1984, Nilsson et al. 1989). An optimal environmental range of distribution for specific species has been proposed for several hydrophytes (Kwon et al. 2006, Lee et al. 2007, Yoon et al. 2011). My study could be a method to understand the reason of decrease in population size of *C. virosa* and to seek a management strategy for conservation and restoration of the *C. virosa* habitat, an endangered plant.

Among habitats, GS habitats developed floating mats along the bank and *C. virosa* was abundantly distributed on the mats and their edge. This wetland type has not been studied in Korea however there are various studies of floating mat in North America, North Europe and Japan (Vitt and Chee 1990; Swarzenski et al. 1991; Sasser et al. 1996; Haraguchi 2004; Somodi and Botta-Dukat 2004; Azza et al. 2006). According to the analyses in substrate, hydrology, nutrient cycle, vegetation and succession of such wetlands, there are various structure and succession process with distinctive physico-chemical properties and nutrient cycle under different climate, water regime and vegetation. Especially, *C. virosa* emerged as a
representative species with high germination and survival rates in the early successional stage of fen in the Netherlands (van den Broek and Beltman 2006). Naturally occurring floating mats are a general habitats type of *C. virosa* in Hungary (Tatár 2010), Finland (Pikanen, personal communication) and Japan (Nishihiro, personal communication). Additionally, artificial floating mats have been developed and applied to water purification, shoreline protection and landscape management (Hoeger 1988; Headley and Tanner 2006; Nakai et al. 2008; van de Moortel et al. 2010).

In my second study, the function of floating mat as a habitat of *C. virosa* was investigated in seedling establishment and population sustenance. I surveyed seedling distribution in the field and examined growth increment by planting *C. virosa* on artificial mats and soil in mesocosm. My questions were 1) how the floating mat is advantageous to seedling establishment of *C. virosa*, and 2) how much the floating mat is different from soil to support *C. virosa* growth. This would be helpful to understand regeneration niches of *C. virosa*. It is fundamental to the understanding of the distribution of adult wetland plants because there is a correlation among of species’ regeneration and adult niches (Grubb 1977, Seabloom et al. 1998). This would be also an elemental study of plant growth on floating mats in Korea and contribute to develop endangered plants conservation plan.

The habitat environment interacts with the developmental process and plays a role determining phenotype (Scheiner 1993, Rejmánková 2011). Various selective forces act on seed size, height, root and leaf morphology and root: shoot partitioning (Grime and Hunt 1975, Fenner 1985, Hunt and Nicholls 1986, Ludlow and Muchow 1990). Interspecific and intraspecific variations in those traits have been studied in relationship with habitat environments. Since ecotype or habitat-type was defined as the genotypic
response of a population to a definite habitat (Turesson 1922), there have been many examples of ecotype research regarding various responses to environmental gradients such as latitude, altitude, soil water content, and disturbance in various species (e.g. plant size and relative growth rate of Arabidopsis thaliana (Li et al. 1998), germination and seedling desiccation tolerance of Hordeum spontaneum local ecotypes in Israel (Gutterman and Gozlan 1998), flowering time in some A. thaliana ecotypes (Nordborg and Bergelson 1999), dormancy and freezing tolerance in Betula pendula Roth (Li et al. 2003), and physiological and growth responses in Populus davidiana ecotypes (Zhang et al. 2005). Those studies established the patterns of responses to the environment and adaptation traits to a given environment.

Generally, ecotype emerged in species distributed in geographically wide range. Although C. virosa is distributed from latitude of 35°N to 70°N in the Northern hemisphere, no research has been conducted on C. virosa ecotypes. Through the first and second study for C. virosa habitats, environmental differences in altitudes, climates, water regimes, and nutrient availability were figured out and population locality were observed in morphology and phenology. I considered that PC, HS and GS, these three populations, could be classified as ecotypes.

In the third study, I compared seed and seedling morphology and tested the sensitivity of seedlings to dryness and shade and the germination responses to light and temperature to identify ecotypic differences in C. virosa populations. I also analyzed which traits were critical to separate the three populations and interpreted the relationships between the discriminating traits and habitats. This kind of information will be helpful to conserve C. virosa populations in natural habitats or create man-made habitats.
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Chapter 1.

Comparison of environmental characteristics at *Cicuta virosa* habitats, an endangered species in Korea¹

Abstract

*Cicuta virosa* is an endangered species in Korea, which is a southern marginal area. To conserve and restore habitats of this plant, I investigated water and soil environmental characteristics and vegetation at four habitats during the growing season. The *C. virosa* habitats differed in community structure, water and substrate properties, and water regime. Although the total distribution ranges of the water and soil environments for *C. virosa* were wide and overlapped with the optimal environmental range of distribution of accompanying species, the optimal water level range for *C. virosa* was defined as 7 ± 3.5 cm. Water level was adjusted by substrate structure such as a mound of *P. japonica* and a floating mat comprised of accompanying species. A floating mat was an aid to maintain an optimal and stable water level in deep or fluctuating water and to prevent strong competition with prolific macrophytes. The Gunsan (GS) sampling site, which had floating mats, could be a good model for *C. virosa* conservation in a warm temperate region, whereas the Pyeongchang (PC) sampling sites, which experienced a water shortage in spring, provided a clue about the decline in *C. virosa* population size.

Keywords: water-hemlock, habitats, water depth, floating mat, endangered species

¹ This paper is submitted to *Journal of Ecology and Field Biology.*
1. Introduction

*Cicuta virosa* L. (water hemlock) is a perennial herb native to northern and central Europe, northern Asia, and northwestern North America (Mulligan and Munro 1980, Lee 2003). Its population size has decreased in Korea which is a southern marginal area of distribution. *C. virosa* has been designated an endangered species by the Natural Environment Conservation Law and as a rare plant by the national arboretum in Korea (Korean Environment Institute 2005, National Arboretum 2009). *C. virosa* is also listed in 17 of 47 Japanese prefectural red lists (Japanese Wildlife Research Association 2012). In Hungary where almost all fens had been destroyed, *C. virosa* was a protected species (Tatár 2010). Few studies have investigated the specific properties of endangered plants to plan counter-measures for conservation in Korea (Suh et al. 2001). A few surveys of wild and rare plants in Korea have been carried out in the last decade due to urgency (e.g., Yoo et al. 2004, Jang et al. 2009, Song et al. 2010) and a series of studies on the conservation strategy for endangered and reserved plants based on the ecological and genetic characteristics has been performed by the National Institute of Environmental Research. However, *C. virosa* has not been a target species.

The water hemlock produces a lateral tuber before the root system dies in the fall and initiates a new plant the following spring (Kingsbury 1964). The plants persist by producing several new rootstocks from buds around the perimeter of the old rootstock (Mulligan and Munro 1980). The roots and tubers of *C. virosa* are toxic (Panter et al. 1988), and most studies on *C. virosa* have concentrated on its toxicology and pharmacology (e.g., Sarreveld 1975, Panter et al. 1988, Strauß et al. 1996). Water hemlock grows in a very wet substrate and is usually found growing in streams, ditches, lakes, rivers, or marshy areas. This high water requirement limits its range.
(Panter et al. 1988). Other environmental characteristics have not been studied well, even though basic ecological information is necessary to conserve *C. virosa*.

The primary goal of this study was to understand the environmental characteristics of *C. virosa* habitats. The specific objectives were to identify the major factors determining *C. virosa* distribution and the reason that the *C. virosa* population has decreased by comparing the water and soil environmental characteristics of *C. virosa* habitats. The water regime is a major determinant of plant community development and patterns in wetlands (Spence 1967, Casanova et al. 2000, Heegaard et al. 2001) and soil variable gradients are also important in determining plant community composition (Fitter 1982, Keddy 1984, Nilsson et al. 1989). An optimal environmental range of distribution for specific species has been proposed for several hydrophytes (Kwon et al. 2006, Lee et al. 2007, Yoon et al. 2011). My study could be a method to understand the reason of decrease in population size of *C. virosa* and to seek a management strategy for conservation and restoration of the *C. virosa* habitat, an endangered plant.

2. Material and Method

2.1 Study Site Description

Only four habitats of *C. virosa* are known in South Korea, and I studied these four habitats (Table 1). The Hoengseong habitat (HS1 and HS2) was located between a grazing land and low hills at 520 m a.s.l. The distance between HS 1 and HS2 was about 700 m. HS1 was a 12 year old abandoned paddy field. HS2 was a narrow streamlet, and water depth and vegetation were different between them. The Gunsan habitat (GS) is an abandoned reservoir that supplied water to a rice paddy at 28 m a.s.l. and is 2° south of HS. Water flows into the reservoir from farming land
through an agricultural irrigation system in May and June and flows out during the rainy season in July. The Pyeongchang habitat (PC) was the highest at 793 m a.s.l. and the most northern among the four sites. This site has been protected by fences since 2008. The waterway has been altered by road construction and this resulted in drying at this site in the spring. I verified that there has been a marked decrease in the *C. virosa* population since an earlier informal survey was performed in 2008. The Gangneung habitat (DG) was recorded for the first time as a *C. virosa* habitat in South Korea. Although it is a private agricultural waterway, it has been fenced and protected since 2007 with agreement by the owner. Water at this site became dependent on an artificial supply in 2011, and the *C. virosa* population size decreased to < 10 individuals.

PC was the coldest with a heavy snowfall in winter (Fig. 1). HS and GS showed similar temperatures during the summer. However, HS was colder than GS during the winter. GS maintained above freezing temperatures except in January and HS received abundant rain in summer.

<table>
<thead>
<tr>
<th>Habitat name</th>
<th>GPS</th>
<th>Altitude (m)</th>
<th>Population size</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoengseong (HS1, HS2)</td>
<td>E128°10'05&quot; N37°29'28&quot;</td>
<td>520</td>
<td>170</td>
<td>HS1: Abandoned paddy field</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>70</td>
<td>HS2: Streamlet</td>
</tr>
<tr>
<td>Gunsan (GS)</td>
<td>E126°43'12&quot; N35°55'58&quot;</td>
<td>28</td>
<td>&gt;500</td>
<td>Reservoir</td>
</tr>
<tr>
<td>Daegwanryeong (PC)</td>
<td>E128°40'26&quot; N37°41'10&quot;</td>
<td>793</td>
<td>&lt;40</td>
<td>Wet meadow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(fenced since 2008)</td>
</tr>
<tr>
<td>Gangneung (DG)</td>
<td>E128°44'59&quot; N37°34'20&quot;</td>
<td>592</td>
<td>&lt;10</td>
<td>Agricultural waterway</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(fenced since 2007)</td>
</tr>
</tbody>
</table>
Fig. 1 Monthly mean temperature and precipitation at Hoengseong (HS), Gunsan (GS), and Daegwanryeong (PC) during 2007-2011 (data from National Weather Service 2011)

2.2 Vegetation survey

I established nine 1 m × 1 m permanent quadrats at HS1, five at HS2, 11 at GS, and three at PC according to *C. virosa* population size and investigated the cover, density, and height monthly during growing season in 2011, based on a modification of the Braun–Blanquet plant sociological method (Mueller-Dombois and Ellenberg 2003, Kim et al. 2004). A water gradient from the bank to the floating mat was observed at GS, where sizable *C. virosa* mono-populations were distributed. To determine the topography and the *C. virosa* community structure covering *C. virosa* mono-populations, I established five fixed line transects at representative locations at GS, and investigated vegetation distribution at each transect using the line intercept method (Canfield 1941). The 11 quadrats at GS were placed on those transects (Fig. 2).
2.3 Water environment analyses

I measured water level at each quadrat and fixed places comprised of major accompanying species mono-populations of every study site with a 1 m stick ruler. Water level was defined as the distance from substrate surface that *C. virosa* rooted in such as a floating mat or mound of plant roots. It was positive when the surface was below the water table and negative when the surface was above the water table. Water temperature and dissolved oxygen (DO) were measured with a DO meter (model PDO-520; UKAS, Taipei, Taiwan). Conductivity (EC) was measured with a Corning Checkmate II (model 311; Corning, Lowell, MA, USA), and pH was measured with a pH meter (model AP 63; Fisher, Pittsburgh, PA, USA) in the field. I collected water samples at each quadrat and fixed places comprised of major accompanying species mono-populations in 125 ml HDPE bottles once every month and brought them in a cool box to the laboratory and filtered them with a 0.45 μm membrane filter. I also took free water samples around the mat near permanent quadrats and interstitial mat water by squeezing the mat around *C. virosa* roots at a depth of 0–15 cm in each quadrat at GS. Those samples were collected twice before and after inundation of the mat on April 30 and June 20, 2012.
NO$_3$-N, NH$_4$-N, and PO$_4$-P were analyzed by the hydrazine method (Kamphake et al. 1967), indophenol method (Murphy and Riley 1962), and ascorbic acid reduction method (Solorzano 1969), respectively. The content of cations such as K$^+$, Ca$^{2+}$, Na$^+$, and Mg$^{2+}$ was measured using an atomic absorption spectrometer (Model AA240FS; Varian, Palo Alto, CA, USA). I used SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA), a one-way analysis of variance (ANOVA), and Duncan’s post hoc test or a $t$-test at a 5% significance level for analyses.

2.4 Substrate analyses

Soil was collected once at a depth of 0–10 cm in a 20 × 20 cm area at each quadrat and fixed locations of major accompanying species mono-populations at every study site with a soil hand auger, and gravel and organic debris were removed. Particularly at GS, the mat around the C. virosa roots at a depth of 0–15 cm was collected in April and June. The soil and mat were then sealed in plastic bags and transported to the laboratory. I passed the soil samples through a 2 mm sieve and determined soil texture using the hydrometer analysis method and a texture triangle (Sheldrick and Wang 1993). I measured pH and conductivity using a soil solution prepared by mixing wet soil and distilled water at a mass ratio of 1:5. The total volume of water in wet soil was measured as freshwater moisture immediately after passing it through a sieve, and the amount of soil particle moisture was measured as air-dried moisture in air dried soil by oven drying at 105°C for ≥ 24 hours. Oven dried mat samples were milled to pass through a 1 mm mesh screen (Sasser et al. 1991). Soil and mat organic matter contents were analyzed by the loss on ignition method (Boyle 2004). NO$_3$-N and NH$_4$-N were extracted with 2 M KCl solutions (Kim et al. 2004) and measured using the hydrazine (Kamphake et al. 1967) and indophenol methods (Murphy and Riley 1962), respectively.
PO₄-P was extracted with Bray No. 1 solution (Bray and Kurtz 1945) and measured using the ascorbic acid reduction method (Solorzano 1969). K⁺, Ca²⁺, Na⁺, and Mg²⁺ were extracted with 1 N ammonium acetate solution (Allen et al. 1974) and measured using an atomic absorption spectrometer. I used a one-way ANOVA and Duncan’s post hoc test at the 5% significance level for analyses.

3. Results
3.1 Plant community structure

Major accompanying species at HS1 (Fig. 3a), were P. thunbergii (coverage of 30–60%) and Sium suave (coverage of 1–5%). The coverage of P. thunbergii decreased with growth of C. virosa (coverage of 33–92%). Major accompanying species at HS2 (Fig. 3b) were Phragmites japonica (coverage of 0–36%) and P. thunbergii (coverage of 14–19%). Relative coverage of C. virosa (coverage of 29–69%) after the growing season increased due to wilt of companion species.

Major accompanying species at GS (Fig. 3c) were Zizania latifolia (coverage of 0–27.0%) and P. thunbergii (coverage of 0.5–15.5%). C. virosa (coverage of 37.1–92.2%) dominated on the mat in May and June, and Z. latifolia fell down by heavy rain in July, whereas the C. virosa population located on the floating mat was relatively safe. In the case of PC (Fig. 3d), Scirpus radicans (coverage of 51–96%) dominated a broad area inside the fence, and a few C. virosa (coverage of 2–18%) were distributed sparsely. C. virosa population size was very small at DG even though it has been protected and managed with an artificial water supply. S. radicans (coverage of > 90%), dropwort, and several C. virosa were found at the DG site in June.
Fig. 3 Species composition and relative coverage of *C. virosa* community at HS1(a), HS2(b), GS(c) and PC(d), CV: *Cicuta virosa*, PT: *Persicaria thunbergii*, SR: *Scirpus radicans*, SS: *Sium suave*, PJ: *Phragmites japonica*, ZL: *Zizania latifolia*, PD: *Paspalum distichum*, EK: *Eleocharis kuroguwai*

3.2 Height and coverage of *C. virosa*

*C. virosa* flowered in June at GS, in July at PC and in August at HS1, 2. During the blooming season, average height was $125 \pm 9.6$ cm at GS, $126 \pm 22$ cm at HS1, $128 \pm 21.8$ cm at HS2 and $70 \pm 7.1$ cm at PC (Fig. 4a). HS2, where tall *P. japonica* co-occurred, showed the greatest height.
Particularly at GS, flowering adults fell down in June and juveniles grew up to an average height of 90 cm and showed a second growth peak in August. Blooming and withering at PC, where temperature was low and altitude was high, occurred 1 month earlier than those at HS. A few flower stalks were broken in July at PC; thus, I could not exactly measure height and coverage.

Coverage increased with growth and decreased after flowering (Fig. 4b). GS, where the climate was mild, showed earlier shoot sprouting than that of other species, so coverage in spring was up to 90%.

**Fig. 4** Monthly change of height (a) and coverage (b) of *C. virosa* (Apr*: pilot survey result) Vertical bars indicate ± SE

### 3.3 Water environment characteristics

Seasonal changes and a significant difference among habitats in water level from substrate *C. virosa* rooted in were observed except in May (Fig. 5a) because of a high volume inflow at GS in June and heavy rain at HS in summer. I could not measure water level or collect water samples at PC in May and June due to the low subsurface water level. The water level at the *C. virosa* population was significantly different from that at the major
companion species mono-population sites (*P. thunbergii* at HS1, *P. japonica* at HS2, and *Z. latifolia* at GS) in each habitat (Fig. 5b). The water in all habitats was neutral or slightly acidic and mean pH was very similar (Fig. 6a). The EC range at HS1, 2 and GS was very wide from 23.4 to 250.0 μS/cm and higher in spring and fall (dry season) than that in summer (Fig. 6b). But the EC values at PC (279.0–764.0 μS/cm) were far higher than those at other sites. The dissolved oxygen concentration in water was highest at HS2 in lotic water with a mean value of 5.7 mg/kg (Fig. 6c). In contrast, water temperature was lowest at HS2, and its year-round range was narrow. The highest water temperature was recorded at GS with low DO values (Fig. 6d).

![Fig. 5](image)

**Fig. 5** Seasonal change in water level at HS1, 2, GS and PC (a) (no data at PC in May and June due to low subsurface water level) and comparison of water level at *C. virosa* populations and major companion species mono-populations (b) (HS1: *P. thunbergii* n = 55, HS2: *P. japonica* n = 83, GS: *Z. latifolia* n = 68) Letters on the graphs mean significant difference at the 5% level based on Duncan’s test. Meaningful t-test results at the 5% level are marked with *. Vertical bars indicate ± SE

The NO₃-N, NH₄-N, and PO₄-P contents were low at HS throughout the entire growing season (Fig. 6e–g). The seasonal pattern of change in NO₃-N, NH₄-N, and PO₄-P contents at GS was different from that at the
other sites. K\textsuperscript{+} and Na\textsuperscript{+} concentrations were highest at GS with wide seasonal variations (Fig. 6h, i). But, the concentration of divalent cations at PC was excessively high such as the EC values (Fig. 6j, k). NO\textsubscript{3}-N, NH\textsubscript{4}-N, and PO\textsubscript{4}-P contents at DG were similar to the levels at GS, and cation concentrations and EC values at DG were similar to those at HS in June. Water environmental properties at the major companion species mono-populations were within the C. virosa population range at each site except water level.

3.4 Soil characteristics

The common soil textures were silty loam and sandy loam. Soil was mostly sandy at HS2 and mostly silty at HS1 (Table 2). Average fresh moisture and air-dried moisture contents were 50.2 ± 13.9\% and 10.7 ± 8.5\%, respectively. Fresh moisture content was significantly higher at GS than that at the other sites. The soil was acidic with a mean pH of 4.7. Organic matter content, EC, and PO\textsubscript{4}-P concentration were similar at the four sites. However, soil at DG was less acidic and had a lower EC value and higher PO\textsubscript{4}-P concentrations than those at the other sites. Concentrations of NO\textsubscript{3}-N, NH\textsubscript{4}-N, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+} at GS were significantly higher than those at the other sites, but the concentration of Na\textsuperscript{+} at PC was ten times higher than that at GS. Soil properties at major companion species mono-populations were within the C. virosa population range at each site.
Fig. 6 Seasonal change in water environment characteristics at *C. virosa* habitats during the growing season in 2011. Letters on the graphs indicate significant difference at the 5% level based on Duncan’s test. HS1: n= 9, HS2: n=5, GS: n=7, PC: n=3 (no samples in May and June due to low subsurface water level)
Table 2. Means and ranges of soil environmental properties at C. virosa habitats in June

<table>
<thead>
<tr>
<th>Soil factor</th>
<th>HS1</th>
<th>HS2</th>
<th>GS</th>
<th>PC</th>
<th>DG</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>44.5a</td>
<td>37.9a</td>
<td>66.7b</td>
<td>36.9a</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>Air-dried moisture</td>
<td>11.2a</td>
<td>1.6a</td>
<td>11.8b</td>
<td>6.0a</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>LOI (%)</td>
<td>7.3a</td>
<td>4.1a</td>
<td>14.3b</td>
<td>5.9a</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4.9a</td>
<td>4.6a</td>
<td>4.4a</td>
<td>4.9a</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>EC(μS/cm)</td>
<td>34.0a</td>
<td>29.2a</td>
<td>246.7a</td>
<td>102.6a</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>NO₃-N</td>
<td>4.3ab</td>
<td>3.2a</td>
<td>6.5b</td>
<td>2.9a</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>NH₄-N</td>
<td>13.8ab</td>
<td>11.5a</td>
<td>32.5b</td>
<td>9.8a</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>PO₄-P</td>
<td>31.3a</td>
<td>12.5a</td>
<td>22.3a</td>
<td>13.8a</td>
<td>59.9</td>
<td></td>
</tr>
<tr>
<td>K⁺(mg/kg)</td>
<td>104.0ab</td>
<td>45.1a</td>
<td>162.7b</td>
<td>70.1a</td>
<td>66.0</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺(mg/kg)</td>
<td>43.1ab</td>
<td>28.3a</td>
<td>102.4c</td>
<td>60.7b</td>
<td>46.2</td>
<td></td>
</tr>
<tr>
<td>Na⁺(mg/kg)</td>
<td>837.5a</td>
<td>517.2a</td>
<td>1219.8a</td>
<td>12369.1b</td>
<td>902.7</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺(mg/kg)</td>
<td>131.1ab</td>
<td>50.2a</td>
<td>286.3c</td>
<td>168.8b</td>
<td>111.2</td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>39.4a</td>
<td>77.8b</td>
<td>50.7a</td>
<td>60.8ab</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>51.7c</td>
<td>21.0a</td>
<td>43.0b</td>
<td>32.3ab</td>
<td>73.3</td>
<td></td>
</tr>
<tr>
<td>Clay (%)</td>
<td>8.9b</td>
<td>1.3a</td>
<td>6.3b</td>
<td>6.9b</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>

Letters on the table indicate significant difference at the 5% level based on Duncan’s test. HS1 n=9, HS2 n=2, GS n=7, PC n=3, DG n=1.

3.5 A distinct habitat at Gunsan

Because GS is a deep reservoir and C. virosa was growing on floating mats, I made additional water depth profiles at intervals of 1 m on line transects from a start point near the bank to 1 m of water depth (Fig. 7). C. virosa was mainly distributed on the floating mat and extended to an inaccessible region that was deeper than 1 m, whereas Z. latifolia did not. There was loose and sunken mat on the fifth line; hence, water-hemlock was distributed only in the shallow water region near the bank.
Fig. 7 Water depth and distribution of dominant plants profiles on five lines transects in GS reservoir in September. Horizontal axis is distance (meter) from start point near the bank. Vertical axis is distance (cm) from water surface to soil and mat surface. For lines location, see Fig. 2. Dots indicate distribution of ZL and CV on the line. ZL: *Z. latifolia*, CV: *C. virosa*, SS: soil surface, MS: above border of mat, MT: mat thickness (below border of floating mat).

Organic matter content of the mat, as a *C. virosa* substrate at GS, was 86.8 ± 9.2% under a non-flooded condition on April 30 and 77.9 ± 5.1% under inundation on June 20. Interstitial mat water had a significantly higher EC value and concentrations of NO$_3$-N, NH$_4$-N, PO$_4$-P, K$^+$, Na$^+$, Ca$^{2+}$, and Mg$^{2+}$ than free water around the mat. All values in June were higher than those in April except NO$_3$-N content (Table 3). NO$_3$-N content was highest in the interstitial mat water in April. pH values were slightly lower in interstitial mat water, but the difference was not significant.
Table 3. Properties of free water around mat and interstitial mat water at GS

<table>
<thead>
<tr>
<th>factor</th>
<th>non-flooded (n=6)</th>
<th>Interstitial (n=6)</th>
<th>flooded (n=6)</th>
<th>Interstitial (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.2±0.07(^a)</td>
<td>6.7±1.20(^a)</td>
<td>6.7±0.28(^a)</td>
<td>6.5±0.71(^a)</td>
</tr>
<tr>
<td>EC(µS/cm)</td>
<td>123.6±8.52(^a)</td>
<td>390.5±187.59(^a)</td>
<td>356.2±8.04(^a)</td>
<td>958.8±569.73(^b)</td>
</tr>
<tr>
<td>NO(_3)-N(mg/l)</td>
<td>0.03±0.01(^a)</td>
<td>0.25±0.16(^b)</td>
<td>0.07±0.16(^a)</td>
<td>0.08±0.07(^a)</td>
</tr>
<tr>
<td>NH(_4)-N(mg/l)</td>
<td>0.02±0.03(^a)</td>
<td>2.25±1.65(^a)</td>
<td>0.08±0.10(^a)</td>
<td>14.98±16.62(^b)</td>
</tr>
<tr>
<td>PO(_4)-P(mg/l)</td>
<td>0.08±0.04(^a)</td>
<td>1.88±2.11(^a)</td>
<td>0.06±0.06(^a)</td>
<td>4.72±5.20(^b)</td>
</tr>
<tr>
<td>K(^+)(mg/l)</td>
<td>6.14±2.06(^a)</td>
<td>35.79±18.18(^b)</td>
<td>6.14±1.78(^a)</td>
<td>35.57±36.15(^b)</td>
</tr>
<tr>
<td>Ca(^2+)(mg/l)</td>
<td>7.33±0.99(^a)</td>
<td>37.86±24.82(^b)</td>
<td>15.67±1.40(^b)</td>
<td>49.30±31.18(^c)</td>
</tr>
<tr>
<td>Na(^+)(mg/l)</td>
<td>13.42±0.54(^a)</td>
<td>25.96±9.47(^a)</td>
<td>25.40±5.90(^b)</td>
<td>38.83±19.81(^b)</td>
</tr>
<tr>
<td>Mg(^2+)(mg/l)</td>
<td>2.65±0.23(^a)</td>
<td>11.44±7.42(^a)</td>
<td>4.46±1.03(^a)</td>
<td>15.18±13.07(^b)</td>
</tr>
</tbody>
</table>

Water samples were collected in non-flooded condition on April 30 and in flooded condition on June 20, 2012 at GS. Letters on the table indicate significant difference at the 5% level based on Duncan’s test.

4. DISCUSSION

4.1 Chemical characteristics of Cicuta virosa habitat environment

Based on the water chemistry of some typical natural wetlands (Kadlec and Knight 1996), water chemical properties in C. virosa habitats were general level except two factors. First, the range and seasonal change of NH\(_4\)-N and PO\(_4\)-P contents at GS were abnormal. This may have resulted from stream-flow input from a surrounding rice field and a rubbish heap and mat decomposition under high temperature. Second, concentration of divalent cations at PC also deviated from typical value. PC area included a pasture near a road and received a great amount of snowfall every winter. CaCl\(_2\) salt could have caused the high Ca\(^{2+}\) content in water at PC.

Soil was a little acidic and similar at four habitats except at DG. C. virosa primarily lives on grey-wooded and podzolic soils with peat in northern Canada (Mulligan and Munro 1980). My results support these observations, but an evaluation of the effect of the excessive Na\(^+\)
concentration in soil and the high Ca$^{2+}$ content in water at PC to *C. virosa* distribution is needed additionally.

In mat system of GS, almost all ion concentrations in the interstitial mat water were higher than those in the free surface water in July than those in April. But, NO$_3$-N content was higher in April under the non-flooded condition than that in July, because the redox potential decreased under the flooded condition (Haraguchi 1991). Water chemistry and redox potential of a floating mat is affected by water regime (Haraguchi 2004).

Synthetically, total distribution ranges of water and soil chemical environment for *C. virosa* were wide and overlapped with the optimal environmental range of distribution for typical hydrophytes such as *Z. latifolia*, *Typha augustifolia* (Kwon et al. 2006), *Scirpus tabernaemontani* (Lee et al. 2007), and *Juncus effuses* (Yoon et al. 2011). Water and soil chemical properties at *C. virosa* population were not different from those at major companion species mono-populations at each site.

### 4.2 Water level as a factor determining *C. virosa* distribution

The distribution of adult wetland plants can be determined by a single environmental gradient such as water depth (Spence 1982, Coops et al. 2004, Lee et al. 2005), because changes in water depth are associated with changes in a variety of environmental factors (e.g., light, soil nutrients, soil particle size, gas exchange rates) that physiologically constrain species distribution (Spence 1982, Keddy 1983). In addition, each species can be expected to have its own water depth tolerance (Spence 1967, Seabloom et al. 1998) and plant communities are more likely to respond to the history of water level than the water level at a particular time of a survey (Roberts 1994, Tabacchi 1995). This means that water level and competitive interactions with accompanying species under seasonal changes in water depth are important factors for the distribution and
I determined that there were meaningful differences between water levels of \textit{C. virosa} populations and major accompanying species monopopulations (\textit{P. thunbergii} at HS1, \textit{P. japonica} at HS2, \textit{Z. latifolia} at GS) (Fig. 5b). A mound shaped surface that was modified by \textit{P. japonica} may change its micro-environment and affect reproductive success of other species (Byun et al. 2008, Hong et al. 2012). The \textit{P. japonica} hummock offered a low water level for \textit{C. virosa} at HS2, which consisted of a 30 cm deep streamlet. Floating mats rise and fall with changes in local water level and support stands of emergent vascular vegetation (Sasser et al 1991). Mat made of live and dead roots and non-decomposing parts of plants like \textit{P. distichum} and \textit{Z. latifolia} provided shallow water level at GS, a 1m depth reservoir and shelter from strong competition with a prolific accompanying species, \textit{Z. latifolia} and disturbance by flooding in summer. Water depth for the distribution of adult \textit{C. virosa} was defined as $7 \pm 3.5$ cm, which was the mean water depth value at three habitats (HS1, HS2, and GS) through the entire growing season. A study reported that \textit{C. virosa} lives on sand in 0–20 cm water levels with light over 12,000 lux on Mt. Baekdu in China (Kim and Lee 2003).

The abundance of \textit{C. virosa} within its native range has been diminished where water courses and marshes have been diverted, filled, or drained (Mulligan and Munro 1980, this study). Vegetation studies have often revealed significant declines in area and/or species richness of shore vegetation after water regime alterations (Nilsson and Keddy 1988, Shay et al. 1999, Nishihiro et al. 2004, this study). In Japan, the major reason for the extinction of wild plants and decreases in their number is exploitation of wetlands (Jeon 1999). The population size of \textit{C. virosa} was diminished at PC and DG where the water regime was changed due to fragmentation by road construction and isolation by a protection fence.
There was a water shortage in May and June at PC and a need for an artificial water supply from the near mountain at DG. Monthly water levels from substrate *C. virosa* rooted in changed and were significantly different at the four sites because of different water regime. But, water levels in May were not different among habitats where *C. virosa* population sizes were large, and average water level was 7.2 ± 2.4 cm (Fig. 5a). The spring season is a very important period for regeneration and distribution of *C. virosa* considering its life history (Fig. 4). *C. virosa* sprouted earlier than other plants and flowered in July at PC.

Dispersal strategy of water hemlock is floating and spreading through water flooding in spring (Mulligan and Munro 1980, Panter et al. 1988); hence, sufficient water level could be a factor determining *C. virosa* distribution. Water hemlock forms overwintering rootstocks from buds around the base of the current year's rootstock in the fall. These may keep the plant afloat and serve to spread plants during spring flooding. The seeds, which are surrounded by a spongy fruit coat, are also buoyant (Mulligan and Munro 1980).

As a result, proper water level is a very important factor determining *C. virosa* distribution and dispersal, and the low water level in spring was considered a reason that the *C. virosa* population has decreased at PC and DG. I could propose connecting with corridor that *C. virosa* could use to disperse through and maintaining a high water level to preserve and restore *C. virosa* habitats. Here, the GS habitat could be a good model for *C. virosa* conservation in a warm temperate region.

5. Conclusion

*C. virosa* habitats were distinguished by comparison based on climate, wetland type, community structure, water and substrate properties and water regime. HS1 had a 0–5 cm water depth abandoned rice field in high
montane areas with emerging *P. thunbergii*, and its ion concentrations and EC were in the midrange and relatively constant. HS2 had a 10–15 cm water depth streamlet in high montane areas with *P. japonica* and its ion concentrations and EC were relatively poor and constant. GS was 0–10 cm water depth (to mat surface) a deep reservoir where floating mat developed at low altitudes and in a warm area. The main companion species was *Z. latifolia* and its ion concentrations and EC were relatively rich and seasonally highly variable. PC was dominated by *S. radicans* and has excessive high EC value in water and high Na⁺ contents in soil with the low water level in spring in high montane area.

The total distribution ranges of the water and soil chemical environments for *C. virosa* were wide and overlapped with the optimal environmental range of distribution for accompanying species. However, the optimal water level range for *C. virosa* distribution and dispersal could be defined. This optimum water level was adjusted by substrate structure such as a mound of *P. japonica* and a floating mat comprised of accompanying species. A floating mat at GS was an aid to maintain an optimal and stable water level in deep or fluctuating water and to prevent strong competition with prolific macrophytes. The GS, which had floating mats, could be a good model for *C. virosa* conservation in a warm temperate region, whereas the PC, which experienced a water shortage in spring, provided a clue about the decline in *C. virosa* population size.
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Characteristics (I). National Institute of Environmental Research (in Korean)
Chapter 2

Seedling establishment and growth of *Cicuta virosa* on the floating mat

Abstract

The population size of *C. virosa* has been decreased and designated as a domestic endangered species in Korea. The southernmost habitats of *C. virosa* in Korea are floating mats along the bank in reservoirs. To investigate the function of floating mat in seedling establishment and growth of *C. virosa*, I surveyed the seedling distribution in habitats, and examined growth increment in mesocosms, by planting *C. virosa* on artificial plant mats and soil in mesocosms. Seedling density and coverage on floating mats and their edge in Gunsan, with high level of solar radiation and sufficient water level, were significantly higher than those on soil. In the mesocosm experiment, shoot and root dry-weight in soil was double to those in artificial mats, and these significant difference reflected nitrogen availability in the substrate. However, there was no significant difference in the numbers of flower stalks, or tiller or stem width despite the difference in nutrients level of the three substrates. I also observed life history variation between Gunsan and Hoengsung populations. These results showed that floating mat which provided sufficient water level and light and mitigated competition with nutrient limitation under continuous disturbance was advantageous to the establishment of seedlings and to the population sustenance and high reproduction allocation of *C. virosa* sustained coexistence with prolific macrophytes on floating mat even though the overall growth on floating mat was worse than that on soil. Using floating mat could be a way for conservation of *C. virosa*. 
Keywords: nutrient availability, regeneration niche, substrate, water level

1. Introduction

The population size of *Cicuta virosa* has decreased in Korea, a southern marginal area of world distribution, and it has been designated as a domestic endangered species by the Natural Environment Conservation Law (Korean Environment Institute 2005). A few habitats are known, and the southernmost habitats in Korea are represented by 1 meter deep reservoirs surrounded by paddy fields in a low altitude. Floating mats were installed on the bank, and *C. virosa* was abundantly distributed on the mats and their edge (Shin et al. 2013). Those wetland types have not been studied in Korea but floating mats were destroyed by periodical dredging for the convenience of agricultural water supply.

There are various researches in wetlands which have developed floating mats in North America, North Europe and Japan (Vitt and Chee 1990; Swarzenski et al. 1991; Sasser et al. 1991; 1995; 1996; Haraguchi and Matsui 1990; Haraguchi 1991; 1995; 2004; Somodi and Botta-Dukat 2004; Azza et al. 2006). According to the analyses in substrate, hydrology, nutrient cycle, vegetation and succession of such wetlands, there are various structure and succession process with distinctive physico-chemical properties and nutrient cycle under different climate, water regime and vegetation. Especially, *C. virosa* emerged as a representative species with high germination and survival rates in the early successional stage of fen in the Netherlands (van den Broek and Beltman 2006). Naturally occurring floating mats are a general habitats type of *C. virosa* in Hungary (Tatar 2010), Finland (Pikanen, personal communication) and Japan (Nishihiro, personal communication). Additionally, artificial floating mats have been developed and applied to water purification, shoreline
protection and landscape management (Hoeger 1988; Headley and Tanner 2006; Nakai et al. 2008; van de Moortel et al. 2010).

In this study, the function of floating mat as a habitat of *C. virosa* was investigated in seedling establishment and population sustenance. I surveyed seedling distribution in the field and examined growth increment by planting *C. virosa* on artificial mats and soil in mesocosm. My questions were 1) how the floating mat is advantageous to seedling establishment of *C. virosa*, and 2) how much the floating mat is different from soil to support *C. virosa* growth. This would be helpful to understand regeneration niches of *C. virosa*. It is fundamental to the understanding of the distribution of adult wetland plants because there is a correlation among of species’ regeneration and adult niches (Grubb 1977, Seabloom et al. 1998). This would be also an elemental study of plant growth on floating mats in Korea and contribute to develop endangered plants conservation plan.

2. Material and method

2.1 Field survey

Gunsan (GS) habitats were two reservoirs with floating mats composed of similar species: GS1 (E126°43´12˝, N35°55´58˝) and GS2 (E126°44´51˝, N35°54´59˝). Heongseong (HS, E128°10´05˝, N37°29´28˝) habitat was abandoned paddy field with low water level, not having floating mat. I surveyed the density, coverage and height of *C. virosa* seedlings within 30 X 30 cm² grid at the GS edge of floating mat, on the GS floating mat and on GS and HS soil three times from April to May in 2012. Photon flux density was checked at each survey with light-meter (Li-250A, Li-COR, Lincoln, NE, USA) and water level was also measured with a stick ruler. In flowering season, I measured height and plant width of flowering individual in GS and HS and drew a vegetation map in GS1.
and GS2. I collected free water and mat interstitial water in GS, surface water and soil interstitial water in HS six times. I brought them in a cool box to the laboratory, and filtered them with a 0.45 µm membrane filter.

2.2 Experimental setting

*C. virosa* originated from two different populations were planted on the three substrates mesocosm with six replicates. Two origins of planted individuals were GS and HS and they were germinated in a growth chamber and had grown under the same condition for 1 year in Seoul. Three substrate treatments were soil, artificial floating mats with nutrient rich (r-mat) and nutrient poor (p-mat). Soil was the mixture of sand and bed soil (4: 1) and artificial mats were 50 X 50 X 7 cm³ mesh bags filled with rice-sheaf and coconut fiber. Water level was sustained 5cm above the soil surface in soil mesocosms and 0 cm over the mat surface in floating mat mesocosms. To make artificial floating mats with nutrient poor condition, I refilled water in half of mat subsets once each week.

I measured the height and weight of *C. virosa* with new shoots before planting and carried experiments in the greenhouse for 8 weeks until flowering. All plants were harvested at the end of May and measured plant width (cm), shoot and root length (cm), dry-weight (g), number of leaves, flower stalks and tillers, stem diameter (mm) and tuber length (mm). I collected surface water at the start and the end and soil from soil subset at the end.

2.3 Chemical analyses in water and substrates

NO₃-N and NH₄-N in soil were extracted with a 2 M KCl solution (Kim et al. 2004) and PO₄-P was extracted with Bray No. 1 solution (Bray and Kurtz 1945). NO₃-N, NH₄-N, and PO₄-P in water and extracted soil solution were analyzed by the hydrazine method (Kamphake et al. 1967),
indo-phenol method (Murphy and Riley 1962), and ascorbic acid reduction method (Solorzano 1969), respectively. Electronic conductivity (EC) was measured with a Corning Checkmate II (model 311; Corning, Lowell, MA, USA) and pH was measured with a pH meter (model AP 63; Fisher, Pittsburgh, PA, USA). Statistical analyses were performed using two-way analysis of variance and Tukey’s post hoc test or t-test at the 5% significance level using SPSS ver. 19.0 (SPSS, Inc., Chicago, IL, USA).

3. Result

3.1 Seedling survival rate at different substrates in original habitats

The average height of C. virosa seedlings wasn’t significantly different among locations, and was extended from 0.5 cm to 18 cm for six weeks (Table 1). But the density and coverage were significantly higher at GS edge and mat than GS soil and HS soil. Density decreased with coverage increase as time passed. Photon flux density was <10% at GS soil surface covered with litter at 13 April and was <40% at HS soil surface shaded by adult vegetation. GS soil was submerged when water level rose in reservoir at the 25 May. Water level averaged 0~5 cm at GS mat and its edge, but varied considerably at GS soil and HS soil.

There was zonation of three major species (Zizania latifolia, Paspalum distichum and C. virosa) on the floating mat in GS 1 and 2, from bank to the open water (Fig 1). C. virosa was abundantly distributed in the zone of P. distichum, and between open water and Z. latifolia zone, and more sparcely in the zone of Z. latifolia.
Fig 1 Vegetation map in GS1(a) and GS2(b) in June; ZL: *Zizania latifolia*; PD: *Paspalum distichum*; CV: *Cicuta virosa*
Table 1. *C. virosa* seedling distribution and water level at different locations

<table>
<thead>
<tr>
<th>Date</th>
<th>index</th>
<th>Height (cm)</th>
<th>Density(100no./m²)</th>
<th>Coverage (%)</th>
<th>Water level (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>13</td>
<td>0.5 ± 0.0ᵃ</td>
<td>45.7 ± 11.6ᵃ</td>
<td>33.3 ± 9.1ᵃ</td>
<td>1.1 ± 0.5ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.0ᵃ</td>
<td>63.3 ± 13.0ᵃ</td>
<td>57.9 ± 9.8ᵃ</td>
<td>0.0 ± 0.6ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.0ᵃ</td>
<td>21.1 ± 9.0ᵃ</td>
<td>30.0 ± 5.6ᵃ</td>
<td>-4.5 ± 2.0ᵃ</td>
</tr>
<tr>
<td>April</td>
<td>30</td>
<td>3.0 ± 0.4ᵇ</td>
<td>55.6 ± 13.1ᵇ</td>
<td>48.4 ± 9.1ᵇ</td>
<td>1.1 ± 0.8ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.3 ± 1.1ᵃ</td>
<td>41.4 ± 9.1ᵇ</td>
<td>64.0 ± 9.2ᵇ</td>
<td>1.0 ± 0.2ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.8 ± 0.6ᵃ</td>
<td>11.3 ± 5.0ᵇ</td>
<td>18.8 ± 10.2ᵃ</td>
<td>-3.0 ± 1.5ᵃ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8 ± 0.6ᵃ</td>
<td>24.6 ± 5.9ᵇ</td>
<td>57.7 ± 8.4ᵇ</td>
<td>0.4 ± 0.4ᵇ</td>
</tr>
<tr>
<td>May</td>
<td>25</td>
<td>12.2 ± 2.9ᵃ</td>
<td>30.3 ± 1.2₁ᵇ</td>
<td>55.0 ± 14.3ᵇ</td>
<td>5.2 ± 3.7ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.6 ± 1.5ᵃ</td>
<td>18.5 ± 5.6ᵇ</td>
<td>69.8 ± 5.5ᵇ</td>
<td>0.3 ± 1.7ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.0 ± 3.2ᵃ</td>
<td>3.6 ± 0.8ᵃ</td>
<td>16.1 ± 3.2ᵇ</td>
<td>11.0 ± 1.5ᵇ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.7 ± 1.5ᵃ</td>
<td>5.3 ± 1.2ᵃ</td>
<td>23.9 ± 6.6ᵇ</td>
<td>-0.6 ± 0.1ᵃ</td>
</tr>
</tbody>
</table>

Means that are significantly different using one-way ANOVA and Tukey’s test, are followed by the different letters (n=9).

3.2 Growth of *C. virosa* at different substrates in the mesocosm experiment

Root length, root: shoot length ratio, dry-weight, number of leaf and plant widths were significantly different at harvest among three substrate treatments (Table 2). Root length was 18.8 ± 1.6 cm in p-mat, 14.3 ± 0.8 cm in r-mat and 57.8 ± 2.1 cm in soil. Shoot dry-weight was 11.1 ± 1.8 g in the p-mat, 11.2 ± 2.3 g in r-mat and 25.4 ± 3.7 g in soil and root dry-weight was 10.3 ± 2.1 g in p-mat, 10.2 ± 2.7 g in r-mat and 23.3 ± 5.0 g in soil (Fig 2). Dry-weight of root and shoot in soil was double to that in mat, whereas shoot length, flower stalk number and stem diameter were not different.
Table 2. Two way ANOVA on different measures of plant growth parameters of *C. virosa*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Substrate</th>
<th>Origin</th>
<th>S X O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F₂,35 value</td>
<td>F₁,35 value</td>
<td>F₂,35 value</td>
</tr>
<tr>
<td>Start height</td>
<td>0.408 NS</td>
<td>0.104 NS</td>
<td>0.231 NS</td>
</tr>
<tr>
<td>Height growth</td>
<td>2.516 NS</td>
<td>6.194 *</td>
<td>0.011 NS</td>
</tr>
<tr>
<td>Shoot length</td>
<td>1.543 NS</td>
<td>3.634 NS</td>
<td>0.057 NS</td>
</tr>
<tr>
<td>Root length</td>
<td>225.279 ***</td>
<td>0.092 NS</td>
<td>2.426 NS</td>
</tr>
<tr>
<td>R/S length ratio</td>
<td>33.859 ***</td>
<td>2.117 NS</td>
<td>0.136 NS</td>
</tr>
<tr>
<td>Start fresh-weight</td>
<td>0.002 NS</td>
<td>0.044 NS</td>
<td>0.297 NS</td>
</tr>
<tr>
<td>Weight growth</td>
<td>11.524 ***</td>
<td>5.947 *</td>
<td>0.858 NS</td>
</tr>
<tr>
<td>Shoot dry-weight</td>
<td>8.210 **</td>
<td>0.388 NS</td>
<td>0.092 NS</td>
</tr>
<tr>
<td>Root dry-weight</td>
<td>4.090 *</td>
<td>0.193 NS</td>
<td>0.331 NS</td>
</tr>
<tr>
<td>R/S dry-weight ratio</td>
<td>0.203 NS</td>
<td>0.775 NS</td>
<td>0.879 NS</td>
</tr>
<tr>
<td>Growing leaf no.</td>
<td>5.569 **</td>
<td>0.021 NS</td>
<td>0.102 NS</td>
</tr>
<tr>
<td>Plant width</td>
<td>8.918 **</td>
<td>3.722 NS</td>
<td>0.094 NS</td>
</tr>
<tr>
<td>Flower stalk no.</td>
<td>1.781 NS</td>
<td>3.769 NS</td>
<td>0.399 NS</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>2.067 NS</td>
<td>0.823 NS</td>
<td>0.117 NS</td>
</tr>
<tr>
<td>Tiller no.</td>
<td>0.982 NS</td>
<td>0.163 NS</td>
<td>0.105 NS</td>
</tr>
<tr>
<td>Tuber length</td>
<td>1.843 NS</td>
<td>0.173 NS</td>
<td>0.001 NS</td>
</tr>
</tbody>
</table>

*: p≤0.05; **: p≤0.001 ***; p≤0.0001; ns: non-significant; R/S=root/shoot

Fig 2 Shoot and root length (a) and dry-weight (b) of *C. virosa* in different substrates; p-mat: nutrient poor mat; r-mat: nutrient rich mat; Means that are significantly different using one-way ANOVA and Tukey’s test, are followed by the different letters. Vertical bars show standard errors (n=12)
Between origin, the height growth (HS: 52.47 ± 6.95 cm, GS: 71.50 ± 3.90 cm) and weight growth (HS: 202.05 ± 31.36 g, GS: 117.29 ± 30.37 g) were different without interaction effect between substrate and origin (Fig 3). Shoot length of GS was bigger than that of HS, but dry-mass was smaller in GS. Every individual of GS flowered with similar number of nodes in stem and flower stalks, but about 45% of HS individual didn’t make stem for flowering despite of significantly more weight growth and similar dry-weight to flowered GS ones under same substrate condition. Non-flowered HS individuals were 2 on soil, 3 on p-mat and 3 on r-mat. The height of *C. virosa* at flowering stage in the field was average 110.8 ± 5.1 cm in GS and 132.15 ± 6.1 cm in HS and they were significantly different. Plant width of flowering individual was similar each other (GS: 72.9 ± 7.1 cm, HS: 79.5 ± 5.1 cm).

**Fig 3** Height growth (a) and weight growth (b) of *C.virosa* from different origin at flowering stage; p-mat: nutrient poor mat; r-mat: nutrient rich mat; Means that are significantly different using ANOVA and Tukey’s test, are followed by the different letters among substrates and by * between the origin. Vertical bars show standard errors (n=6)

### 3.3 Water properties of field and mesocosm

EC, NH₄-N and PO₄-P contents in GS water were higher than that in
HS water and water pH was similar each other (Table 3). NO$_3$-N content of submerged mat water was lower than soil water in August and free water showed lower values in N and P contents than interstitial water.

In mesocosm experiments, N contents in surface water was lower in mat than in soil at start, and the decrease of N level was also less in mat than in soil (Table 4). PO$_4$-P contents in surface water were higher in mat than in soil, but soil itself contained 7.52 ± 0.54 mg kg$^{-1}$ of PO$_4$-P, 2.14±0.19 mg kg$^{-1}$ of NO$_3$-N and 13.40±2.77 mg kg$^{-1}$ of NH$_4$-N.

Table 3. Habitat water properties in August (mean ± SE, n=6)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mat</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>free</td>
<td>interstitial</td>
</tr>
<tr>
<td>pH</td>
<td>6.7±0.03</td>
<td>6.8±0.06</td>
</tr>
<tr>
<td>EC (µScm$^{-1}$)</td>
<td>162.5±7.4</td>
<td>160.8±2.3</td>
</tr>
<tr>
<td>NO$_3$-N (mg kg$^{-1}$)</td>
<td>&lt;0.005</td>
<td>0.01±0.002</td>
</tr>
<tr>
<td>NH$_4$-N (mg kg$^{-1}$)</td>
<td>0.22±0.07</td>
<td>0.92±0.26</td>
</tr>
<tr>
<td>PO$_4$-P (mg kg$^{-1}$)</td>
<td>0.50±0.16</td>
<td>2.03±0.58</td>
</tr>
</tbody>
</table>

Table 4. Mesocosm water properties at the start and the end (mean ± SE, n=6)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Poor mat</th>
<th>Rich mat</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
<td>Start</td>
</tr>
<tr>
<td>pH</td>
<td>7.17±0.08</td>
<td>7.61±0.05</td>
<td>7.10±0.04</td>
</tr>
<tr>
<td>EC (µScm$^{-1}$)</td>
<td>1194±8</td>
<td>1254±43</td>
<td>1200±9</td>
</tr>
<tr>
<td>NO$_3$-N (mg kg$^{-1}$)</td>
<td>0.057±0.004</td>
<td>0.006±0.002</td>
<td>0.061±0.002</td>
</tr>
<tr>
<td>NH$_4$-N (mg kg$^{-1}$)</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>PO$_4$-P (mg kg$^{-1}$)</td>
<td>0.500±0.107</td>
<td>0.016±0.003</td>
<td>0.605±0.147</td>
</tr>
</tbody>
</table>

4. Discussion

4.1 Floating mats for seedling establishment

Physical parameters, e.g. water depth, primarily determine plant distribution and composition (Spence 1982; Chambers 1987; Coops et al.
2004), and exposure to light affects a gap-detection for regeneration and germination (Schütz and Rave 1999). The mat and its edge provided an opportunity for regeneration and establishment of *C. virosa*, with stable water level and sufficient radiation. Fallen litter negatively influences seedling survival through reducing light penetration, soil temperature and space availability (Vaccaro et al. 2009; Larkin et al. 2012). Plant mats are essentially great heaps of litter, but floating mats with bumpy surfaces could provide sufficient water levels of 0~5cm and exposure to light in patches, even under conditions of water fluctuation (Table 1). Increased shade due to the presence of litter in early spring was prevented the establishment of *C. virosa*. Although they emerged on soil in GS reservoir, inundation by massive water inflow in May was fatal to the seedlings. A significantly negative correlation between inundation period and abundance of seedlings that emerged in the spring were revealed in analysis of the patterns of seedling emergence, and the establishment at microsites within various water level elevations (Nishihiro et al. 2004). *C. virosa* seeds were floating and spreading through the watercourse, settling and germinating readily, even on a small piece of polystyrene (personal observation), and a water level of 7 ± 3.5 cm was sufficient for adult *C. virosa* (Shin et al. 2013). *C. virosa* as an invasive plant to areas of flooded peat excavation (van der Valk and Verhoeven 1988) engages in development of a mat itself.

After all, floating mat system was more advantageous to the dispersal, germination, establishment and onward growth for successful regeneration; that was falling in a regeneration niche, environmental effects on germination and seedling mortality (Grubb 1977) of *C. virosa*.

### 4.2 Floating mats for population sustenance

Total nutrient availability is reflected in the aboveground plant
biomass (Best et al. 1996), and macrophytes contribute to the nutrient cycle (Rejmánková 2011). Significant increases in net annual aboveground production occurred in *Sparganium* and *Typha* after fertilization with nitrogen and phosphorous (Neely and Davis 1985), and the nutrient (N, P, K) contents of rooting sediments were reflected in dry-weight, shoot length and leaf number of *Sparganium emersum* in the field and in the experiment both (Ságová-Marečková and Květ 2002; Ságová-Marečková et al. 2009). Basically, higher productivity on soil in mesocosm experiments (Fig 2) can also be interpreted to reflect nitrogen availability in the substrate (Table 4). The floating mat system shows seasonal changes in redox potential, nutrients and phenology of plant distribution because of water table oscillation (Haraguchi 1991, 2004). Actually, there was a fluctuation of nutrients during the growing season in GS and the NO$_3$-N content in rich mat water at the start was similar to that in the field in June (Shin et al. 2013), but a difference of NO$_3$-N level between the start and the end in the rich mat was less than that in the soil. With non-significant differences in growth between rich mats and poor mats, it was apparent that nitrogen uptake in mats seemed to be less than that in soil, indicating the need for more investigation of *C. virosa* nitrogen uptake in floating mat system.

Phosphorous also limits plant productivity due to its low mobility in soil, and phosphate availability regulates root architecture in the *Arabidopsis* root system (López-Bucio et al. 2002). Low phosphate availability favors lateral root growth and reduced primary root growth (Williamson et al. 2001). Although low PO$_4$-P contents were detected in surface water of the mesocosm, the decrease in PO$_4$-P level was little, and the primary root length in soil was longer than that in mats, which had higher phosphate contents in water due to the high PO$_4$-P contents in soil.

Even though nutrients levels in three substrates of mesocosm
experiments were different, there was no significant difference in numbers of flowers, stalks, tiller and stem width, as determinants of generative and propagative capacity. Larger plants are usually better able to survive and more fecund, but the relationship of plant size-fecundity can vary among species and among genotypes, and under different environmental stresses (Aarssen and Taylor 1992; Aarssen and Clauss 1922). *C. virosa* showed a strategy of high reproduction allocation under nutrient limitation. High allocation to of resources towards reproduction of *C. virosa* sustained coexistence with prolific macrophytes on floating mats, although growth on floating mats was worse than that on soil.

My mesocosm test was carried out without water fluctuation stress or competition with other species. The zonation of *C. virosa* along the floating mat margin in the habitats showed its low competitiveness and narrow niche breadth, and *Zizania latifolia* expanded its occupational space on the mat (Fig 1). Dominant macrophytes on floating mats, such as *Phragmites* Trin. and *Typha* L., shared key traits of most problematic wetland invasive species: large stature, dense growth, nearly monotypic stands, and abundant litter (Zedler and Kercher 2004). However, vegetation on floating mats, through water level fluctuation and wind wave action, was damaged and suppressed horizontal distribution (Azza et al. 2006). It resulted in floating mats with continuous disturbance mitigated competition from prolific macrophytes. *C. virosa* could finish its life history with high fecundity both in the mesocosm with nutrient limitation and in the field with water fluctuation and competition. Even periodical dredging of thick mats may be a benefit to *C. virosa* population sustenance. Using floating mats which provide a stable water level and gaps for regeneration could enhance population sustenance and help to conserve *C. virosa*.
5. Conclusion

My results showed that floating mat which provided sufficient water level and light and mitigated competition with nutrient limitation under continuous disturbance was advantageous to establishment of seedling and sustenance of *C. virosa* population. High allocation to reproduction of *C. virosa* sustained coexistence with prolific macrophytes on floating mat even though the overall growth on floating mat were worse than that on soil. Planting *C. virosa* on organic floating mat could be a way for conservation. The differences between GS and HS populations in growth and flowering were interpreted as life historical variation of two populations under interaction between genotype and environment.
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Chapter 3

Ecotypic differentiation in seed and seedling morphology and physiology among *Cicuta virosa* populations

Abstract

There are three different *Cicuta virosa* habitats in Korea: Pyeongchang (PC), a fenced wet meadow with a water shortage in spring; Hoengseng (HS), a 12 year old abandoned paddy field and narrow streamlet holding shallow water throughout the year; Gunsan (GS), a 1 m deep reservoir with a floating mat composed of organic matter located 2° south of HS and PC and at very low altitude. These three populations with different altitudes, climates, water regimes and nutrient availability showed differences in leaf morphology and flowering time. I compared seed and seedling morphology and tested the sensitivity of seedlings to dryness and shade and the germination responses to light and temperature to identify intraspecific variation as ecotypic differences in *C. virosa* populations. The seed length of GS (2.04 ± 0.03 mm) was significantly shorter than that of PC (2.44 ± 0.05 mm) and HS (2.60 ± 0.03 mm). Seed weight of GS (0.83 ± 0.01 mg) was significantly lighter than that of the others (PC: 1.47 ± 0.02 mg, HS: 1.33 ± 0.02 mg). PC and HS seedlings had larger root: shoot ratio values and GS had significantly higher relative growth rate (RGR) through 8 weeks of growth. GS seedlings were more sensitive to dryness than PC and HS in leaf chlorophyll contents, specific leaf area (SLA) and RGR. The pattern of germination responses was similar among the three populations but germination rates were very different. Maximum germination rates were

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2 This paper is submitted to Aquatic Botany.
63.0%, 23.9% and 96.9% in PC, HS, and GS, respectively, under 28/18°C/14h photoperiod. The nonviable seed rate of GS (18.05±0.70%) was significantly higher than that of the others (PC: 3.07±1.63%, HS: 3.44 ± 0.62%) in the tetrazolium tests result. Seed mass which was correlated to other plant traits (i.e., root: shoot ratio, RGR) and germination rate were the most discriminating variables in the discriminant analysis. Seed weight was negatively correlated with temperature in January and water level and germination rate was significantly related with water level and water temperature in August. Based on these results, I suggest that these three *C. virosa* populations from different habitats are ecotypes.

Keywords: ecotype, germination response, growth process, seed size, stress sensitivity

1. Introduction

The habitat environment interacts with the developmental process and plays a role determining phenotype (Scheiner 1993, Rejmánková 2011). Various selective forces act on seed size, height, root and leaf morphology and root: shoot partitioning (Grime and Hunt 1975, Fenner 1985, Hunt and Nicholls 1986, Ludlow and Muchow 1990). Interspecific and intraspecific variations in those traits have been studied in relationship with habitat environments. Since ecotype or habitat-type was defined as the genotypic response of a population to a definite habitat (Turesson 1922), there have been many examples of ecotype research regarding various responses to environmental gradients such as latitude, altitude, soil water content, and disturbance in various species (e.g. plant size and relative growth rate of *Arabidopsis thaliana* (Li et al. 1998), germination and seedling desiccation tolerance of *Hordeum spontaneum* local ecotypes in Israel.
(Gutterman and Gozlan 1998), flowering time in some *A. thaliana* ecotypes (Nordborg and Bergelson 1999), dormancy and freezing tolerance in *Betula pendula* Roth (Li et al. 2003), and physiological and growth responses in *Populus davidiana* ecotypes (Zhang et al. 2005). Those studies established the patterns of responses to the environment and adaptation traits to a given environment.

*Cicuta virosa* L. (water hemlock) is distributed in northern and central Europe, northern Asia, and northwestern North America (from 51°N to 71°N) (Mulligan and Munro 1980, Lee 2003). About 35°N latitude seems to be a southern boundary for *C. virosa* distribution in Asia (Japanese Wildlife Research Association 2012, Shin et al. 2013). Although *C. virosa* is distributed from a latitude of 35°N to 70°N in the Northern hemisphere, no research has been conducted on *C. virosa* ecotypes. *C. virosa* populations in southernmost Korea and Japan have decreased and seed longevity and germination characteristics of *C. virosa* have been investigated for restoration purposes in Hungary (Tatár 2010).

In Korea, a few *C. virosa* habitats are known and *C. virosa* is listed as a domestic endangered species. Those habitats are located at sites with different altitudes, climates, water regimes, and nutrient availability (Shin et al. 2013). Shin et al. (2013) found differences in leaf morphology and flowering time among populations and considered that these three populations could be classified as ecotypes. Thus, I compared seed and seedling morphology and tested the sensitivity of seedlings to dryness and shade and the germination responses to light and temperature to identify ecotypic differences in *C. virosa* populations. I also analyzed which traits were critical to separate the three populations and interpreted the relationships between the discriminating traits and habitats. This kind of information will be helpful to conserve *C. virosa* populations in natural habitats or create man-made habitats.
2. Materials and methods

2.1 Seed preparation

Experiments were conducted with seeds collected from Pyeongchang (PC), Hoengseng (HS), and Gunsan (GS) in 2011 (Table 1). Pyeongchang (PC) was a fenced wet meadow dominated by *Scirpus radicans* and the coldest during the winter among the three locations. A water shortage occurred in spring because a waterway was diverted by road construction. Hoengseng (HS) was a 12 year old abandoned paddy field (HS1) and had a narrow streamlet (HS2) that held shallow water throughout the year. HS1 was just 700 m away from HS2 on the same hill; thus, individuals could cross-pollinate freely. Here, I treated them as one population. Gunsan (GS) was located 2° south of HS and PC at a very low altitude with a 1 m deep reservoir and a floating mat composed of *Zizania latifolia*, *Paspalum distichum* and *C. virosa*. Several below-zero temperature days were noted, and nutrient rich water was flowing in (Shin et al. 2012). The populations were named after their origin. Ripe seeds were cleaned and stored in sealed plastic bag at 4°C in the dark until germination tests.

### Table 1. Seed collection sites and dates.

<table>
<thead>
<tr>
<th>Origin (code)</th>
<th>GPS (Altitude a.s.l.)</th>
<th>Habitat type (companion species)</th>
<th>Mean Temp(°C)</th>
<th>Seed collection date (flowering month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyeongchang (PC)</td>
<td>E128° 40' 26&quot; N37°41'10&quot; (793m)</td>
<td>Fenced wet meadow (<em>Scirpus radicans</em>)</td>
<td>Jan: -8.3 Aug: 20.4</td>
<td>24 Sept 2011 (July)</td>
</tr>
<tr>
<td>Hoengseng (HS)</td>
<td>E128°10' 05&quot; N37°29'28&quot; (520m)</td>
<td>Abandoned paddy field (<em>Persicaria thunbergii</em>) &amp; Narrow streamlet (<em>Phragmites japonica</em>)</td>
<td>Jan: -4.5 Aug: 25.8</td>
<td>22 Oct 2011 (August)</td>
</tr>
<tr>
<td>Gunsan (GS)</td>
<td>E126°43' 12&quot; N35°55'58&quot; (28m)</td>
<td>Floating mat in a reservoir (<em>Zizania latifolia</em>)</td>
<td>Jan: -1.4 Aug: 25.7</td>
<td>22 Jul 2011 (May-June)</td>
</tr>
</tbody>
</table>

Mean temperature in January and August at Daegwanryeong (PC), Hoengseong (HS), and Gunsan (GS) during 2008–2012 (data from National Weather Service 2012)
2.2 Experimental design

Germination tests were conducted under constant (16, 20, 24, 28 and 32°C) and alternating temperature regime at 12 h interval (16/6, 20/10, 24/14, 28/18 and 32/22°C), under different photoperiods (0 h, 14 h and 24 h) and at two water levels (0 cm-contact and 2 cm-floating condition of seed) for 3 weeks by sowing three replicates of 50 seeds each on 120-mm diameter Petri dishes containing three pieces of filter paper (Baskin and Baskin 1998). Germination test under 28/18 °C/14h photoperiod was repeated three times with the storage time intervals. Treatments were carried out in temperature and light-controlled incubators providing a photon flux density of approximately 45 μmolm$^{-2}$s$^{-1}$ (Li-250A, Li-COR, Lincoln, NE, USA). Each day I scored germination as positive if the radicle was visible and transferred it to a new dish. TZ tests with 0.5% tetrazolium solution were carried out to check for viability of un-germinated seeds after the germination tests.

For the stress sensitivity test, three 14 day old seedlings growing in Petri dishes at 25°C were planted in 15 cm diameter pots filled with sand and top soil (5:1) into nine replicates from each origin. Stress treatments were 25% light with shade netting and < 5% soil water content with 30 ml watering once per week. Soil water content was checked with a moisture meter (W1 1000N, Mirae sensor, Seoul, Korea). The control group was maintained in a greenhouse with 100% light and > 30% soil water content with everyday watering. Harvest and measurements of nine seedlings from each origin were taken 4 weeks later in the dryness treatment group and 6 weeks later in the shade treated group.

2.3 Measurement and data analyses

I measured length and width of 30 seeds with Vernier calipers and weighed 100 seeds 20 times. Root and shoot length and dry mass of
seedlings after drying at 80°C, over 48 h were measured at every harvest. I also detected leaf area with a portable area meter (Li-3000C, Li-COR). Leaf chlorophyll was checked with a chlorophyll meter (SPAD-502 plus, Konica Minolta sensing, Inc., Tokyo, Japan). I calculated specific leaf area (SLA), relative growth rate (RGR) and the root: shoot ratio in length and dry-weight to compare the growing process among the three populations.

\[ R_{ij} = \Delta \ln y_{ij} / \Delta t \]  
\[ S_{ij} = (R_{imax} - R_{ij}) / (C_{imax} - C_j) \]

RGR \((R_{ij})\) of a species origin \(i\) in environment \(j\) is defined in equation (1) (Hunt 1982), here, \(y\) is length (cm) or dry-weight (g) on time (week). Stress sensitivity \((S_{ij})\) is the ratio of strain \((R_{imax} - R_{ij})\) to stress \((C_{imax} - C_j)\) and defined in equation (2) (Shipley and Keddy 1988). Here, a gap of \(R_{imax}\) in the optimal environment and \(R_{ij}\) under the stressed condition was used as \(S_{ij}\), because I controlled and fixed the stress level (light and moisture) and treated at only two levels (optimal and distinct stress level). I also compared the gap in leaf chlorophyll contents and SLA between the optimal group’s mean values and the stressed group’s values as stress sensitivity in leaf trait.

Statistical analyses were performed using one-way analysis of variance without transformation and Duncan’s post hoc test at the 5% significance level using SPSS ver. 17.0 (SPSS, Inc., Chicago, IL, USA). I used canonical discriminant analysis (Hair et al. 2005) to separate the three populations and to identify the most discriminating variables. The three populations were treated as dependent variables, whereas the trait variables (seed size, SLA, root: shoot ratio, RGR, stress sensitivity and germination rate) were independent variables. Relationships between the traits and environment factors from Shin et al. (2013) were described with linear multiple regression analyses \((p<0.05)\).

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3. Results

3.1 Seed size and growth process

Seed length of GS (2.04 ± 0.03 mm) was significantly shorter than that of PC (2.44 ± 0.05 mm) and HS (2.60 ± 0.03 mm), and seed weight of GS (0.83 ± 0.01 mg) was significantly lighter than the others (PC: 1.47 ± 0.02 mg, HS: 1.33 ± 0.02 mg) (Fig. 1). Root length of 14 day old GS seedlings (27.22 ± 1.40 mm) was significantly shorter than that of HS (63.87 ± 3.04 mm) and PC (86.98 ± 4.00 mm) and the dry weight of 20 PC seedling roots (13.33 ± 0.67 mg) and shoots (7.67 ± 1.20 mg) was significantly heavier than that of the others (Fig. 2). Leaf chlorophyll contents and SLA values were not significantly different among the three populations even as time passed (Table 2). PC and HS had bigger values for root: shoot allocation ratio in length and dry-weight throughout the 8 week growing process. Length and dry-weight RGR were significantly higher in GS.

Fig. 1. Seed size and weight of *C. virosa*. Means that are significantly different (*p* ≤ 0.05) by one-way analysis of variance and Duncan’s test are followed by different letters. Vertical bars show standard errors (length and width: n = 30, weight: n = 20).
Fig. 2. Length of 14 day old seedlings (A) and dry weight of 20 seedlings (B) of *C. virosa*. Means that are significantly different (*p* ≤ 0.05) by one-way analysis of variance and Duncan’s test are followed by different letters. Vertical bars show standard errors (length: n = 60, dry weight: n = 3).

Table 2. Growth parameters of *C. virosa* seedlings (mean ± standard error)

<table>
<thead>
<tr>
<th>Time</th>
<th>Origin</th>
<th>Week 6</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>HS</td>
<td>GS</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>28.91 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.47 ± 0.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.58 ± 3.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SLA (cm&lt;sup&gt;2&lt;/sup&gt; g&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>211.7 ± 40.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303.4 ± 73.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>260.1 ± 58.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>R:S ratio</td>
<td>1.05 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.12 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGR (L)</td>
<td>0.50 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RGR (DW)</td>
<td>0.26 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SLA: specific leaf area; L, length (cm); DW, dry weight (g); RGR (L: cm cm<sup>−1</sup> week<sup>−1</sup>, DW: g g<sup>−1</sup> week<sup>−1</sup>). Means (n = 9) that are significantly different (*p* ≤ 0.05) by one-way analysis of variance and Duncan’s test are followed by different letters.

3.2 Stress sensitivity and germination rate

GS was significantly sensitive to dryness stress in chlorophyll content (7.60 ± 1.72 µg cm<sup>−2</sup>), SLA (191.1 ± 9.8 cm<sup>2</sup> g<sup>−1</sup>) and RGR (DW) (0.43 ± 0.06 g g<sup>−1</sup> week<sup>−1</sup>) than the others (Table 3). Under a 25% light treatment, PC showed a larger increase in SLA (490.3 ± 185.1 cm<sup>2</sup> g<sup>−1</sup>) and a decrease in RGR (DW) (0.36 ± 0.04 g g<sup>−1</sup> week<sup>−1</sup>). HS showed ambiguous responses to the two stressors.
The general pattern of the germination responses was similar in the three populations but germination rates were significantly different (Fig. 3). *C. virosa* was more sensitive to an alternating temperature regime than to constant temperature, and a higher germination rate was recorded during the 24 h photoperiod treatment than that at 14 h and 0 h. All three populations showed higher germination rates in a floating condition (under 24/14°C, 14h photoperiod) than under just a wet condition. Maximum germination rates were 63.03 ± 1.88%, 23.89 ± 3.99%, and 96.93 ± 0.90% in order of PC, HS, and GS under 28/18°C/14h photoperiod. GS had the highest germination rate and that of HS was the lowest, whereas the nonviable seed rate of GS (18.05±0.70%) was significantly higher than that of the others (PC: 3.07±1.63%, HS: 3.44 ± 0.62%) in the TZ test results. The PC and GS populations showed consistent germination rates even after the storage time intervals but HS seeds had fluctuating responses( 15.81 ± 1.12% in March, 40.37 ± 1.95% in August, 15.49 ± 2.33% in October)(Fig. 4).

### Table 3. Stress sensitivity to dryness and shade (mean ± standard error)

<table>
<thead>
<tr>
<th>Stress</th>
<th>Dryness (4 weeks)</th>
<th>Shade (6 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>HS</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>3.76 ± 1.43 a</td>
<td>2.04 ± 0.64 a</td>
</tr>
<tr>
<td>SLA (cm²g⁻¹)</td>
<td>34.1 ± 7.1 a</td>
<td>145.4 ± 10.6 b</td>
</tr>
<tr>
<td>RGR (L)</td>
<td>0.17 ± 0.01 a</td>
<td>0.16 ± 0.01 a</td>
</tr>
<tr>
<td>RGR (DW)</td>
<td>0.21 ± 0.04 a</td>
<td>0.32 ± 0.03 b</td>
</tr>
</tbody>
</table>

Dry condition with 5% water content in soil for 4 weeks, dark condition with 25% light for 6 weeks, control with everyday watering and 100% light, SLA: specific leaf area; L: length (cm), DW: dry-weight (g), RGR (L:cmcm⁻¹week⁻¹, DW: gg⁻¹week⁻¹). Means (n = 9) that are significantly different (p ≤ 0.05) by one-way analysis of variance and Duncan’s test are followed by the different letters.
Fig. 3. Germination responses of C. virosa to alternating temperature regime, photoperiod and water level. Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan’s test are followed by different letters. Vertical bars show standard errors (n = 3, 28/18°C/14h: n=9).

Fig. 4 Germination rates of C. virosa under 28/18°C/14h with storage time intervals. Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan’s test are followed by different letters. Vertical bars show standard error (n = 3).
3.3 Discriminant analysis and relationship with habitats

The three populations were separated in a discriminant analysis based on variables related to plant morphology and physiology (Fig. 5, Table 4). Seed mass and germination rate were most important variables to discriminate between HS, PC, and GS on the first function ($p<0.0001$). The GS centroid (mean of discriminant functions) located farther away from that of PC or HS. In a multiple regression analysis between traits and site-of-origin environments, seed weight was negatively correlated with temperature in January and water level and germination rate was significantly related with water level and water temperature in August (Table 5). Average water level through growing season was significantly related with various traits.

![Fig. 5](image)

**Fig. 5** Plot of individual cases and centroids values (means of discriminant functions) on discriminant functions separating the three *Cicuta virosa* populations.
Table 4. Standardized coefficients for the canonical discriminant functions, Eigen values, cumulative percent of variance and the canonical correlation.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Function</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Seed weight</td>
<td>0.96</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>SLA</td>
<td>-0.08</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>R: S ratio</td>
<td>0.09</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>RGR</td>
<td>-0.05</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Dryness sensitivity</td>
<td>0.04</td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>Shade sensitivity</td>
<td>-0.13</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Germination rate</td>
<td>-0.82</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Eigen value</td>
<td>55.93</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>Cumulative (%)</td>
<td>93.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Canonical correlation</td>
<td>0.99</td>
<td>0.89</td>
<td></td>
</tr>
</tbody>
</table>

SLA, R: S ratio (DW) and RGR (DW) at week 8, stress sensitivity: gap in RGR (DW), germination rate under 18/28°C/14h photoperiod.

Table 5. Summary of multiple regression between discriminating traits and site-of-origin environment factors of the three C. virosa populations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Temperature</th>
<th>Water level</th>
<th>Water temperature</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed weight</td>
<td>-</td>
<td>--</td>
<td>0</td>
<td>0.951</td>
</tr>
<tr>
<td>SLA</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0.350</td>
</tr>
<tr>
<td>R:S ratio</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0.355</td>
</tr>
<tr>
<td>RGR</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0.395</td>
</tr>
<tr>
<td>Dryness</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.356</td>
</tr>
<tr>
<td>Shade sensitivity</td>
<td>0</td>
<td>++</td>
<td>-</td>
<td>0.342</td>
</tr>
<tr>
<td>Germination rate</td>
<td>0</td>
<td>++</td>
<td>--</td>
<td>0.825</td>
</tr>
</tbody>
</table>

+: positive correlation, -: negative correlation, 0: no significant correlation at p < 0.05, ++, -- at p < 0.01

4. Discussion

4.1 Variation in seed size and growth process

Seed weight was a key trait to discriminate the GS population from the other populations in Cicuta virosa, which was similar to Hordeum
Ecotypes of Arabidopsis also exhibit variations in seed mass and seeds of the Cape Verde Island ecotype are almost double the weight of those of Ler (Landsberg erecta) (Alonso-Blanco et al. 1999). It is generally agreed that species exposed to more stressful environments such as shade or drought preferentially produce larger seeds (Leishman and Westoby 1994). This was in agreement with the negative correlation between seed weight and temperature in January and average water level in my result (Table 5). However, seed size is not a neutral trait and is generally negatively related to RGR (Maranon and Grubb 1993, Poorter et al. 2008). I found that the RGRs of PC and HS seedlings from larger seeds were lower than GS from smaller seeds and the correlation between RGR and habitats contrasted to that of seed weight. Seed size is also related to resource allocation and larger-seeded species might be able to allocate a larger proportion of mass to roots rather than shoots during early growth (Jurado and Westoby 1992, Swanborough and Westoby 1996). In my result, PC and HS seedling root was significantly longer and heavier than GS seedlings.

According to study of latitudinal variation in 40 A. thaliana ecotypes, variation in RGR among ecotypes is relatively small compared to that of other traits or interspecific variations (Li et al. 1998). But, the variation in mean RGR over a period of 4 weeks was significant in C. virosa populations (Table 2) and similar to the intraspecific variation in A. thaliana and other herbaceous plants (Meerts and Garnier 1996, Li et al. 1998, van Rijn et al. 2000). The instantaneous root: shoot ratio in any individual plant is subjected to genetic, ontogenetic, and environmental control (Gedroc et al. 1996). The root systems of plants show highly plastic development because root systems develop by responding to the availability of phosphate and nitrate (Zhang et al. 1999). But, root systems
originate from a primary root that develops during embryogenesis; that is, an \textit{intrinsic pathway} (Malamy 2005). The typical root: shoot ratio of tundra plants is high (Chapin and Chapin 1981) and species at dry sites tend to have higher root: shoot ratios than those in mesic and hydric areas (Penka 1965, Gibson et al. 1995). 20 \textit{Aegilops} (Poaceae) species from higher annual rainfall regions had higher RGR and low R: S ratio (Villa et al. 1998). Although these patterns are more obvious in extreme environments, they corresponded with my result that PC and HS root: shoot ratios were higher than those of GS over the 8 weeks and R: S ratio had negative correlation with water level.

To test the hypothesis of a trade-off between capacity for a high potential RGR and optimal growth and the degree of reduction of RGR under suboptimal conditions, Shipley and Keddy (1988) defined stress sensitivity as the decrease in RGR of a species relative to a decline in resource supply and suggested the positive relationship between \( R_{ij} \) and \( S_{ij} \) to nutrient stress. I simplified and applied this concept to investigate ecotypic differences in \textit{C. virosa} populations and found the positive relationship between \( R_{ij} \) and \( S_{ij} \) to dryness, but not to shade (Shipley and Keddy 1988, Table 2, 3). Stress sensitivity, the decrease in RGR under stress, was significantly different among the three \textit{C. virosa} populations in my tests. Zhang et al. (2005) suggested that a wet climate ecotype possesses a wasteful water use strategy and quick growth, whereas dry climate ecotypes exhibit a conservative water use strategy and slow growth. These strategy could also be why GS seedlings originated from deep reservoir had a higher RGR and high sensitivity to dryness in leaf chlorophyll, SLA, and RGR (DW) (Table 3).

\textbf{4.2 Variation in dormancy and germination response}

Dormancy level and germination responses are related to mother plant,
population and years of seed collection (Anderson and Milberg 1998), cold treatment (Norborg and Bergelson 1999), seed weight and site moisture (Schütz and Rave 1999) and specie’s habitat preferences (Vandelook et al. 2008). The number of genes involved in dormancy and germination is increasing rapidly (Bradford and Nonogaki 2007). Due to these complex effects at the environmental and gene levels, there was difficulty in interpreting germination differences as habitat-related differences. But different germination rates among C. virosa populations could be identified as ecotypic differences by considering the consistency of differences in germination rates (Fig. 4) and similarity to advanced test results. According to 5 years of germination test results in Hungary (N 47°38'; E 19°16') (Tatár 2010), C. virosa germinated an average 43.3% higher rate in spring and fall, similar to the PC population. In the case of Japan (Ajima et al. 1999), the seed germination rate in a marsh at a 36°N region was 88.8 ± 1.3% under 25/15°C after 1 week of chilling, similar to GS. The higher germination rate under a floating condition (Fig. 3) was reasonable because the C. virosa dispersal strategy is floating and spreading through water flooding (Mulligan and Munro 1980). But, the interpretation of positive correlation between germination rate and water level was complicated due to the influence of seed weight on germination and a correlation between seed weight and site moisture (Schütz and Rave 1999, Elwell et al. 2011).

4.3 Variation in flowering time

The flowering times of the three populations were different in the field (Table 1). The GS population flowered about 7 weeks earlier (April 25) than that of HS (June 12) at a greenhouse in Seoul and about 20% of the GS C. virosa flowered in the first year at the end of June. However, none of the HS and PC plants flowered during the first year (unpublished
Flowering timing in *A. thaliana* has a genetic basis and is associated with fitness under field conditions (Korves et al. 2007) and is an important plant phenotype associated with ecological differentiation (Banta et al. 2012). *A. thaliana* has variable flowering time within and among populations; later flowering is associated with greater plant height, number of leaves, plant reproductive ability, and higher plasticity and fecundity (Westerman and Lawrence 1970, Aarssen and Clauss 1992, Zhang and Lechowicz 1994). It corresponded with my result that the later flowering population, HS showed lower sensitivity to stress and lower failure rate on the TZ test compared to that of earlier flowering population, GS.

5. Conclusion

I identified significant differences in seed size and weight, root: shoot partitioning of seedlings, RGR, and stress sensitivity as characters to distinguish among three ecotypic *C. virosa* populations in South Korea. The three populations were separated in a discriminant analysis based on those traits and the most discriminating trait was seed weight, which correlated with other plant traits (i.e., R: S ratio and RGR). Germination responses and flowering time were also critical to distinguish the populations. Those morphological and physiological traits were correlated with the environmental gradient at the sites of origin. Based on these results, I suggest that these three populations from different habitats are ecotypes of *Cicuta virosa*. 
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CONCLUSION

To conserve and restore habitats of *Cicuta virosa*, an endangered species in Korea, I investigated habitat and life-historical characteristics of *C. virosa*. The *C. virosa* habitats differed in community structure, water and substrate properties, and water regime. Although the total distribution ranges of the water and soil environments for *C. virosa* were wide, the optimal water level range for *C. virosa* was defined as $7 \pm 3.5$ cm. Water level was adjusted by substrate structure such as a mound of *P. japonica* and a floating mat comprised of accompanying species.

Especially, a floating mat in Gunsan was a good model for *C. virosa* conservation in a warm temperate region. It was an aid to maintain an optimal and stable water level in deep or fluctuating water and to prevent strong competition with prolific macrophytes. Seedling density and coverage were significantly higher at floating mats and its edge with high irradiation than soil. Growth of *C. virosa* was worse on floating mat than on soil in mesocosm experiments, but high allocation to reproduction of *C. virosa* sustained coexistence with other hydrophytes on floating mats. Planting *C. virosa* on floating mat could be a way for population sustenance and conservation.

Three populations, Pyungchang (PC), Hoengsun (HS) and Gunsan (GS), under those habitat environmental differences (e.g. altitudes, climates, water regimes and nutrient availability) showed intraspecific variations in seed and seedling morphology and physiology. There were significant differences in seed size, seedling growth process (relative growth rate and root: shoot ratio), the sensitivity of seedlings to dryness and shade, germination responses to light and temperature and flowering time among them. Seed mass was the most discriminating trait and negatively correlated with temperature in January and water level in
habits. I suggested differences among populations from different environments as ecotypic differentiation under interaction between genotype and habitat environment.

This study indicates that there are three ecotypes in Korea and all of them should be conserved. I suggest artificial floating mat system to conserve this species in warm temperate region.

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국문초록

독미나리 (*Cicuta virosa* L.)는 다년생 습지성 초본으로 유라시아와 북미 전반에 걸쳐 분포하나 국내에서 개체수가 줄어들고 있고 서식지도 제한적이어서 멸종위기식물 II급으로 지정 보호되고 있다. 알려진 국내 서식지는 총 4곳으로 평창(PC)은 목초지 형태이며 펜스로 보호되고 있으나 물길의 변화에 따른 봄철 물부족 현상을 보이고 있다. 황성(HS)은 묵눈과 산기슭의 작은 시내 형태로 연중 낮은 수위를 유지하며 700m 거리를 두고 작은 개체군이 여러 분포한다. 군산(GS)은 수심 1m 이상의 작은 저수지로 부유성 식물 매트가 발달되어 있으며 가장 남쪽의 낮은 고도에 위치한 서식지이다. 마지막으로 가장 멀어진 강릉 대기리(DG)의 경우 개인의 농수로였으나 2011년 현재 독미나리 개체군 크기는 10개체 이하로 파악되고 인공적인 물공급에 의존하고 있다.

이러한 독미나리의 보존과 서식지 복원을 위해 서식지 환경 특성을 파악하고자 식물 생장 기간 동안 수환경, 토양환경, 식생환경을 매달 조사 분석하였다. 결과 각 서식지에서의 군집구조, 수질, 토양의 성질과 수리적 특성이 달랐고 독미나리가 서식하는 물과 토양 환경의 전체 범위는 매우 넓었는데 주된 수반종의 최적 생육조건과 겹쳤다. 그러나 특이할 점은 7±3.5cm의 좁은 수위 범위로 이는 달蕞리풀의 뿌리기둥이나 군산의 부유성 매트라는 미지형에 의해 조정됨을 확인하였다. 부유성 매트는 연중 큰 폭의 수위 변화와 주변의 왕성한 대형수생식물과의 경쟁으로부터 독미나리를 안전하게 보호해주는데 구조로 온난한 지역에서의 보존 방안의 모델을 제시해주었다. 반면 수위가 분포의 중요한 요인인 평창의 봄철 물부족은 개체수 감소의 원인으로 파악되었다.

서식지 특성 연구 결과에 바탕하여 부유성 매트가 독미나리
유묘 정착과 지속적인 생장에 있어 어떤 기능을 하는지 파악하고 자 서식지에서의 유묘 분포 조사와 토양과 인공 매트에의 식재를 통한 생장 비교 실험을 수행하였다. 유묘의 밀도와 피도는 토양보다 수위가 0~5cm 범위에서 유지되고 광도가 높은 매트 위와 그 가장자리에서 높게 나타났고 시간이 지남에 따라 더욱 뚜렷해졌다. 메조코즘 실험에서는 토양에 식재한 경우가 매트에 식재한 것보다 뿌리와 줄기의 건중량에 있어 두 배 이상 큰 값을 보였고 이는 매트와 토양의 양분 가용성을 반영하는 결과였다. 반면 꽃대의 길이와 굵기, 소화경과 절눈의 개수, 즉 생식 기관에서는 매질에 따른 유의미한 차이를 보이지 않았다. 추가적으로 군산과 횡성의 개체군은 생장과 개화에 있어 유의미한 차이를 보였다. 결국 부유성 매트와 그 가장자리 공간은 종자의 산포, 유묘의 정착, 개체군의 확장에 유리하며 독미나리의 생장은 토양에서보다 불량하나 생식기관으로의 양분 분배를 늘리므로써 주변 종의 경쟁에서도 생존 가능성을 확인할 수 있었다. 부유성 매트를 이용한 식재방식은 실현가능한 복원 방안으로 판단된다.

앞선 서식지 특성 조사를 통해 서식지간의 환경 차이와 각 개체군의 형태적, 생활사적 차이를 파악할 수 있었다. 이는 세 개체군 즉, 평창, 횡성, 군산의 서식지 환경 차이에 의한 생태형 분화 가능성을 보여주었고 이를 확인하고자 생태형적 차이를 조사 실험해보았다. 종자의 크기, 무게, 유묘의 형태적·생리적 특성 (지하부 지상부의 길이 및 무게비(R:S ratio), 상대생장률 (RGR), 단위 무게당 잔여량(SLA), 엽록소량), 빛과 온도, 수위에 대한 발아율, 건조와 그늘에 대한 스트레스 민감도를 조사하고 이들 특성으로 세 개체군이 구별되는지 알아가 개체군을 구별하는 핵심적인 특성이 무엇인지 판별분석을 실시하였다. 또한 이들 특성과 서식지 환경요인들과의 관계를 회귀분석을 통해 해석해 보았다. 종자는 군산이 가장 작고 가벼웠으며 상대생장률도 유의미하게 높았다. 평창과 횡성의 유묘는 지하부의 비율이 8주간
지속적으로 큰 값을 보였다. 발아패턴은 세 개체군이 비슷하였으나 발아율은 평창이 63.0%, 횡성이 23.9%, 군산은 96.9%로 큰 차이를 보였고 이는 횡성 개체군을 제외하고 재현성이 있었다. 이들 차이 중 종자크기는 상대생장률이나 지하부지상부 비와 같은 다른 특성들에 영향을 주는 특성으로서 개체군을 구별하는 가장 큰 특성으로 확인되었고 발아율과 개화시기도 중요한 차이였다. 종자의 크기는 서식지의 1월 평균 기온과 수위와 음의 상관관계를 보였다. 이를 종합하여 서로 다른 서식지로부터의 세 개체군, 평창, 횡성, 군산이 생태형임을 제안한다.

**Keywords:** 생태형, 부유성 매트, 세대교체 지위(regeneration niche), 종자 크기, 매질, 수위

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