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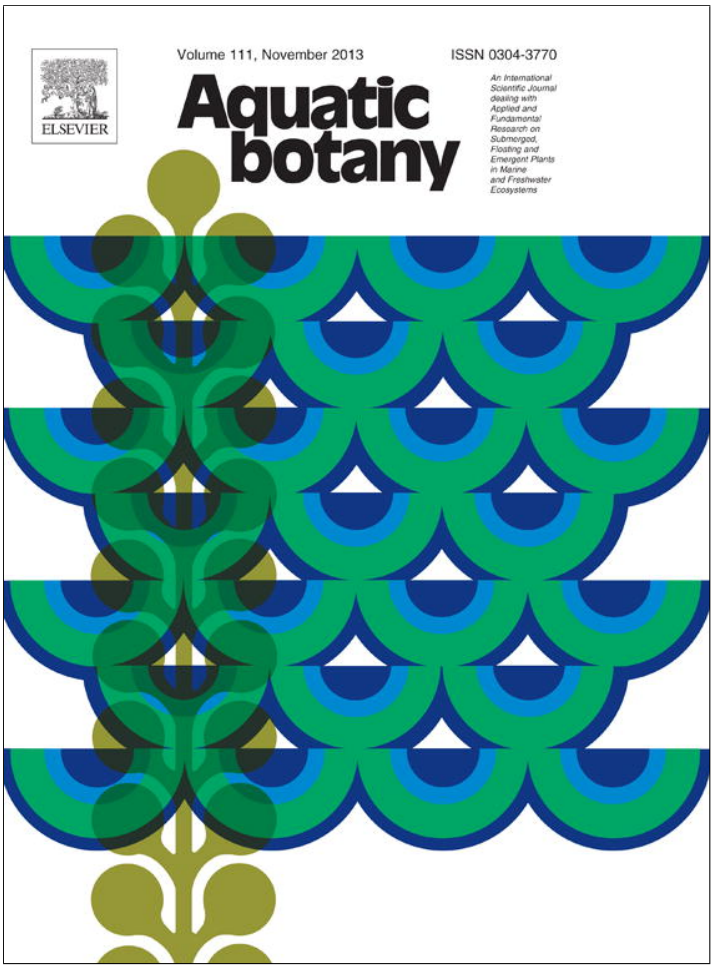

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Ecotypic differentiation in seed and seedling morphology and physiology among *Cicuta virosa* populations

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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. We 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 ($p \leq 0.05$). 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 rate (%) was very different. Maximum germination percentages were 63.0%, 23.9% and 96.9% in PC, HS, and GS, respectively, under 28/18 °C/14 h photoperiod. The nonviable seed (%) of GS ($18.1 \pm 0.7\%$) was significantly higher than that of the others (PC: $3.1 \pm 1.6\%$, HS: $3.4 \pm 0.6\%$) 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 under uniform conditions, we suggest that these three *C. virosa* populations from different habitats are ecotypes.

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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, 2000; 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, 1981; Lee, 2003). About 35° N latitude seems to be a southern boundary for *C. virosa* distribution in Asia. Although *C. virosa* is distributed widely from a latitude of 35° N to 70° N in the Northern hemisphere, *C. virosa* populations in southernmost Korea and Japan have decreased (Japanese Wildlife Research Association, 2012; Shin et al., 2013) and in Hungary also, seed longevity and germination characteristics

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of *C. virosa* have been investigated for restoration purposes (Tatár, 2010). However, no research has been conducted so far comparing the life history of different *C. virosa* populations as ecotypes.

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. To further explore differences among populations, we 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. We also analyzed which traits were critical to separate the three populations and interpreted the relationships between the discriminating traits and habitats. Although the intraspecific variation is less than the interspecific variation, selection acts also within species and understanding of trait variation within species helps understanding of the evolution of life history strategies (Leck et al., 2008). Our results about intraspecific variation as ecotypic differences in *C. virosa* populations from seed to seedling and adult 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, we 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., 2013). 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.

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), at different photoperiods (0 h, 14 h and 24 h) under 24/14 °C and at two water levels (0 cm-contact and 2 cm-floating condition of seed) under 24/14 °C/14 h for 3 weeks (21days) by sowing three replicates of 50 seeds each on 120-mm diameter Petri dishes containing three pieces of filter paper (Baskin and Baskin, 1998) in March. Germination test under 28/18 °C/14 h photoperiod was repeated two times more with the storage time intervals in August and October to double check germination percentage within population. Treatments were carried out in temperature and light-controlled incubators providing a photon flux density of approximately 45 μmol m^{-2} s^{-1} (Li-250A, Li-COR, Lincoln, NE, USA). Each day we scored germination as positive if the radicle was visible and transferred it to a new dish. Viability of un-germinated seeds was checked by TZ tests with 0.5% tetrazolium solution after every germination tests. Here, TZ test result means a percentage of non-viable seeds of all seeds.

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% greenhouse 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 1000 N, Mirae sensor, Seoul, Korea). The control group was maintained in a greenhouse without shade 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. Seed and seedling measurement

We measured length and width of 30 seeds with Vernier calipers and weighed 20 samples of 100 seeds. Root and shoot length and dry mass of seedlings after drying at 80 °C, over 48 h were measured at every harvest. Leaf area containing cotyledon was detected with a portable area meter (Li-3000 C, Li-COR). Leaf chlorophyll was checked with a chlorophyll meter (SPAD-502 plus, Konica Minolta sensing, Inc., Tokyo, Japan). We 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.

$$R_{ij} = \frac{\Delta \ln y_{ij}}{\Delta t} \quad (1)$$

$$S_{ij} = \frac{R_{i\max} - R_{ij}}{C_{i\max} - C_j} \quad (2)$$

RGR (R_{ij}) of a species origin i in environment j is defined in Eq. (1) (Hunt, 1982), here, y is length (cm) or dry-weight (g) on time (week). Stress sensitivity (S_{ij}) is the ratio of strain ($R_{i\max} - R_{ij}$) to stress ($C_{i\max} - C_j$) and defined in Eq. (2) (Shipley and Keddy, 1988). Here, a gap of $R_{i\max}$ in the optimal environment and R_{ij} under the stressed condition was used as S_{ij}, because we controlled and fixed the stress level (light and moisture) and treated at only two levels (optimal and distinct stress level). We 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.

2.4. Data analysis

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). We used canonical discriminant analysis (Hair et al., 2010) 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 ($p \leq 0.05$).

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

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Table 1
Seed collection sites and dates.

Origin (code)	GPS (Altitude a.s.l.)	Habitat type (companion species)	Mean Temp. (°C)		Seed collection date (flowering time)
			January	August	
Pyeongchang (PC)	E128° 40′ 26″ N37° 41′ 10″ (793 m)	Fenced wet meadow (*Scirpus radicans*)	−8.3	20.4	24 September 2011 (90 days)
Hoengseng (HS)	E128° 10′ 05″ N37° 29′ 28″ (520 m)	Abandoned paddy field & Narrow streamlet (*Persicaria thunbergii Phragmites japonica*)	−4.5	25.8	22 October 2011 (120 days)
Gunsan (GS)	E126° 43′ 12″ N35° 55′ 58″ (28 m)	Floating mat in a reservoir (*Zizania latifolia*)	−1.4	25.7	22 July 2011 (60 days)

Mean temperature in January and August at Daegwanryeong (PC), Hoengseong (HS), and Gunsan (GS) during 2008–2012 (data from National Weather Services, 2012), flowering time (day) is the average time from sprouting to flowering.

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

Time	Week 6			Week 8		
Origin	PC	HS	GS	PC	HS	GS
Chlorophyll (μg cm^{-2})	28.91 ± 0.78 a	27.47 ± 0.67 a	27.58 ± 3.17 a	27.56 ± 0.41 a	27.54 ± 0.70 a	29.04 ± 0.7 a
SLA (cm^2 g^{-1})	211.7 ± 40.9 a	303.4 ± 73.2 a	260.1 ± 58.2 a	246.4 ± 9.4 a	263.9 ± 11.5 a	236.79 ± 17.6 a
R:S ratio (L)	1.05 ± 0.12 b	1.12 ± 0.06 b	0.75 ± 0.08 a	1.36 ± 0.10 b	1.60 ± 0.10 b	0.82 ± 0.04 a
R:S ratio (DW)	0.50 ± 0.04 a	0.52 ± 0.04 a	0.42 ± 0.02 a	0.97 ± 0.06 b	1.08 ± 0.09 b	0.74 ± 0.04[a]
RGR (L)	0.26 ± 0.02 a	0.36 ± 0.02 b	0.48 ± 0.03 c	0.33 ± 0.01 a	0.38 ± 0.001 b	0.43 ± 0.01 c
RGR (DW)	0.32 ± 0.03 a	0.46 ± 0.04 b	0.58 ± 0.05 c	0.62 ± 0.01 ab	0.58 ± 0.03 a	0.67 ± 0.03 b

SLA: specific leaf area; R: root; S: shoot; L, length (cm); DW: dry-weight (g); RGR: relative growth rate (L: cm cm^{-1} week^{-1}, DW: gg^{-1} week^{-1}). Means (n = 9) that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test are followed by different letters.

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

Stress (period)	Dryness (week 6)			Shade (week 8)		
Origin	PC	HS	GS	PC	HS	GS
Chlorophyll (μg cm^{-2})	3.76 ± 1.43 a	2.04 ± 0.64 a	7.60 ± 1.72 b	1.29 ± 0.48 a	1.24 ± 0.72 a	2.90 ± 0.42 a
SLA (cm^2 g^{-1})	34.1 ± 7.1 a	145.4 ± 10.6 b	191.1 ± 9.8 c	490.3 ± 185.1 a	444.1 ± 16.8 a	401 ± 31.2 a
RGR (L)	0.17 ± 0.01 a	0.16 ± 0.01 a	0.21 ± 0.02 a	0.09 ± 0.01 b	0.08 ± 0.01 ab	0.05 ± 0.02 a
RGR (DW)	0.21 ± 0.04 a	0.32 ± 0.03 ab	0.43 ± 0.06 b	0.36 ± 0.04 b	0.26 ± 0.02 a	0.35 ± 0.03 ab

Stress sensitivity means average gap values between control and treatment groups, control with everyday watering and no shade in greenhouse (see Table 2 for data of the control group), dry treatment with 5% water content in soil for 4 weeks and shade treatment with 25% of greenhouse light for 6 weeks, SLA: specific leaf area; RGR (L: cm cm^{-1} week^{-1}, DW: gg^{-1} week^{-1}). Means (n = 9) that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test are followed by the different letters.

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.

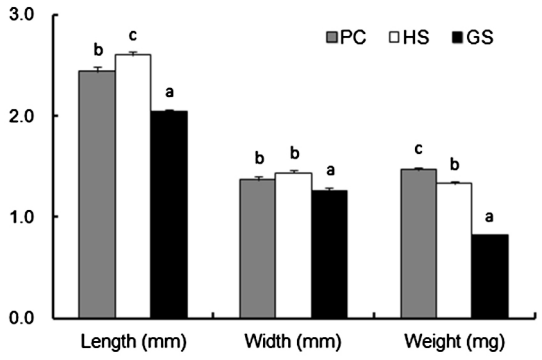

Fig. 1. Seed size and weight of *C. virosa*. Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test among populations at each factor are followed by different letters. Vertical bars show standard errors (length and width: n = 30, weight: n = 20).

PC and HS had bigger values for root: shoot allocation ratio in length and dry-weight throughout the 8 week growing process under control condition with everyday watering and no shade in greenhouse (Table 2). Length and dry-weight RGR were significantly higher in GS. GS was significantly sensitive to dryness stress in chlorophyll content (7.60 ± 1.72 μg cm^{-2}), SLA (191.1 ± 9.8 cm^2 g^{-1}) and RGR (DW) (0.43 ± 0.06 gg^{-1} week^{-1}) than the others (Table 3). PC showed a larger increase in SLA (490.3 ± 185.1 cm^2 g^{-1}) and a decrease in RGR (DW) (0.36 ± 0.04 gg^{-1} week^{-1}) under shade treatment with 25% light. HS showed ambiguous responses to the two stressors.

3.2. Germination response

The general pattern of the germination responses was similar in the three populations but germination rate (%) was significantly different (Fig. 3). *C. virosa* was more sensitive to an alternating temperature regime than to constant temperature, and a higher germination (%) was recorded during the 24 h photoperiod treatment than that at 14 h and 0 h (under 24/14 °C). All three populations showed higher germination (%) in a floating condition (under 24/14 °C, 14 h photoperiod) than under just a wet condition (Fig. 4). Maximum germination percentages 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/14 h photoperiod. GS had the highest germination (%)

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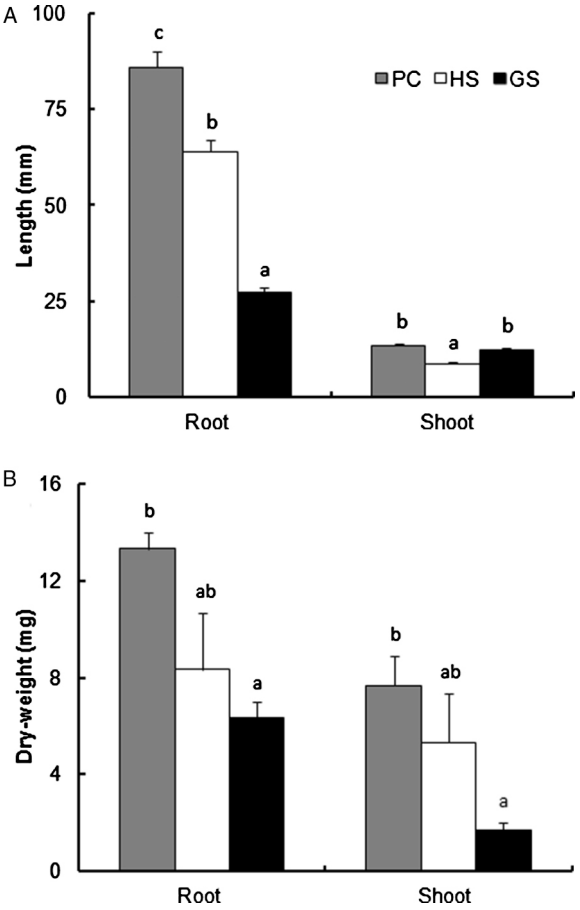

Fig. 2. The length of 14 day old seedlings (A) and dry-weight of 20 seedlings (B) of *C. virosa*. Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test among populations at each category are followed by different letters. Vertical bars show standard errors (length: $n = 60$, dry-weight: $n = 3$).

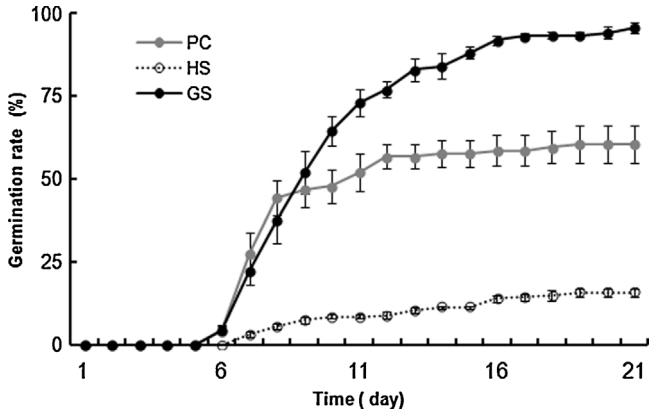

Fig. 3. Germination rate (%) of *C. virosa* under 28/18 °C/14 h in March. Vertical bars show standard error ($n = 3$).

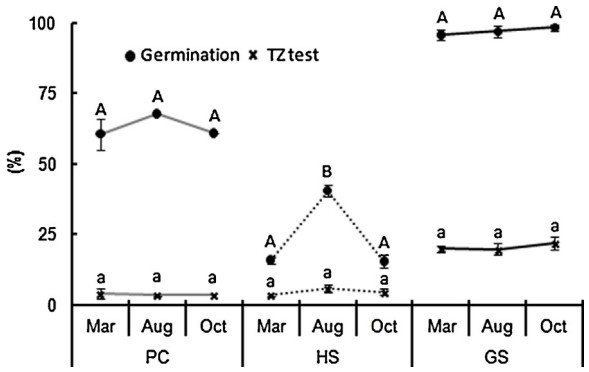

Fig. 5. Consistency of germination (%) and tetrazolium (TZ) test result (nonviable seed (%)) of *C. virosa* under 28/18 °C/14 h with storage time intervals. Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test among test times at each population are followed by different letters. Vertical bars show standard error ($n = 3$).

and that of HS was the lowest, whereas the nonviable seed (%) 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 (%) even with 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. 5).

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. 6 and Table 4). Seed mass and germination (%) 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

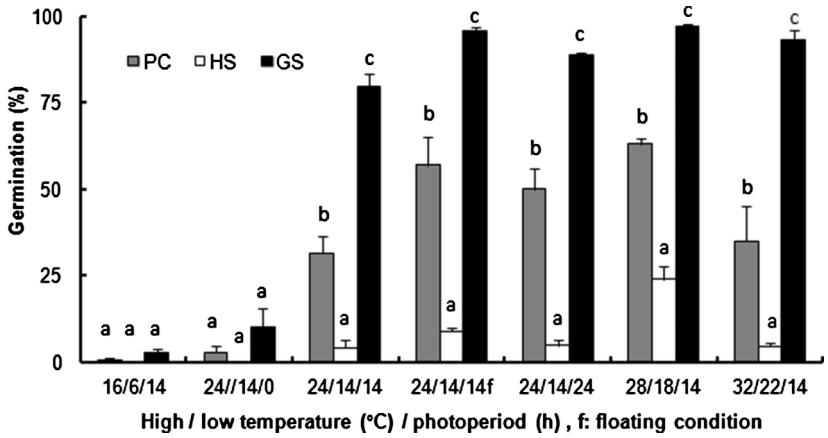

Fig. 4. Germination responses of *C. virosa* to alternating temperature regime, photoperiod (under 24/14 °C) and water level (under 24/14 °C/14 h). Means that are significantly different ($p \leq 0.05$) by one-way analysis of variance and Duncan's test among populations at each setting are followed by different letters. Vertical bars show standard errors ($n = 3$, 28/18 °C/14 h: $n = 9$).

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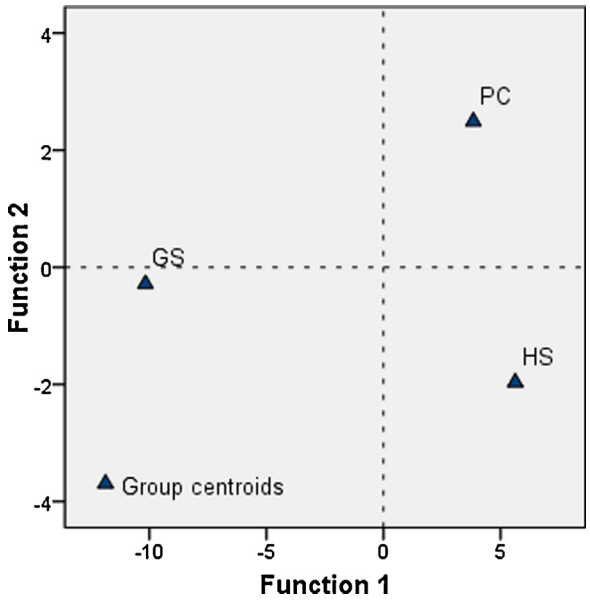

Fig. 6. Plot of group centroids (means of discriminant functions) on discriminant functions separating the three *C. virosa* populations.

Table 4
Standardized coefficients for the canonical discriminant functions, Eigen values, cumulative percent of variance and the canonical correlation.

Trait	Function	
	1	2
Seed weight	0.96	0.49
SLA	−0.08	0.21
R:S ratio	0.09	−0.17
RGR	−0.05	0.08
Dryness sensitivity	0.04	−0.16
Shade sensitivity	−0.13	0.21
Germination (%)	−0.82	0.66
Eigen value	55.93	3.87
Cumulative (%)	93.5	100
Canonical correlation	0.99	0.89

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

regression analysis between traits and site-of-origin environments, seed weight was negatively correlated with temperature in January and water level and germination (%) 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.

4. Discussion

We explored differences among *C. virosa* in seed, seedling and adult stage of life history and verified those intraspecific variation as ecotypic differences in morphological and physiological traits.

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* (Table 4). 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 our 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). We 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 our result, PC and HS seedling root was significantly longer and heavier than GS seedling root.

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 *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 *Aegilops* (Poaceae) species from higher annual rainfall regions had higher RGR and low R:S ratio (Villar et al., 1998). Although these patterns are more obvious in extreme environments, they corresponded with our 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. We simplified and applied this concept to investigate ecotypic differences in *C. virosa* populations and found the positive relationship between R_{ij} and S_{ij} to dryness, but not to shade (Shipley and Keddy, 1988; Tables 2 and 3). Stress sensitivity, the decrease in RGR under stress, was significantly different among the three *C. virosa* populations in our tests. Zhang et al. (2005) suggested that a wet climate ecotype

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

Trait	Temperature (January)	Water level	Water temperature (August)	R^2
Seed weight	–	–	0	0.951
SLA	+	0	0	0.350
R:S ratio	0	–	0	0.355
RGR	0	++	0	0.395
Dryness sensitivity	0	0	0	0.356
Shade sensitivity	0	++	–	0.342
Germination (%)	0	++	–	0.825

+: Positive correlation, –: Negative correlation, 0: No significant correlation at $p < 0.05$, ++, - - at $p < 0.01$.

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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).

4.2. Variation in dormancy and germination response

Dormancy level and germination responses are related to mother plant, population and years of seed collection (Andersson 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 (%) among *C.virosa* populations could be identified as ecotypic differences by considering the consistent differences in total germination (%) (Fig. 5) 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 (%) 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. 4) was reasonable because the *C. virosa* dispersal strategy is floating and spreading through water flooding (Mulligan and Munro, 1981). But, the interpretation of positive correlation between germination (%) 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 two year old GS population flowered about 7 weeks earlier (April 25) than HS population (June 12) at a greenhouse in Seoul and about 20% of the GS which planted in April flowered at the end of June. However, none of the HS and PC plants flowered during the first year (unpublished data). 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 our result that the later flowering population, HS showed lower sensitivity to stress and lower failure (%) on the TZ test compared to that of earlier flowering population, GS.

5. Conclusion

We 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 (%) and flowering time were also critical to distinguish the populations. Those morphological and physiological traits in every stage of life history were correlated with the environmental gradient (e.g. water level, temperature in Jan) at the sites of origin. Based on these differences under uniform conditions, we suggest that these three populations from different habitats are ecotypes of *Cicuta virosa* and these three populations should be conserved all together for the genetic diversity.

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