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Effects of habitat differences on the genetic diversity of *Persicaria thunbergii*



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Abstract

To understand the effects of habitat characteristics on the genetic diversity of *Persicaria thunbergii*, three sites of different environmental conditions in a water system were surveyed. Site A was the closest to the source of the water system, and there was a dam between sites A and B. Site C is located on the lowest downstream in the water system. Vegetation survey of four quadrats at each site was performed, and soil samples were collected for physicochemical analysis. Random amplification of polymorphic DNA (RAPD) analysis of ten *P. thunbergii* individuals at each site was conducted to calculate population genetic diversity and genetic distance among populations. Soil was sterile sand at site A, whereas loamy soil at sites B and C. A pure stand of *P. thunbergii* appeared at site A, while other species occurred together (such as *Humulus japonicus* and *Phragmites australis*) at sites B (Shannon-Wiener index; $H_B = 0.309$) and C ($H_C = 0.299$). Similar to the species diversity, genetic diversity (Nei's gene diversity; h) within population of site A ($h_A = 0.2381$) was relatively lower than sites B ($h_B = 0.2761$) and C ($h_C = 0.2618$). However, site C was separated from sites A and B in genetic distance rather than the geographical distance (Nei's genetic distance; A~B, 0.0338; B~C, 0.0685; A~C, 0.0833).

Keywords: Cleistogamic flower, Genetic distance, Genetic diversity, RAPD-PCR

Introduction

Persicaria thunbergii (Siebold & Zucc.) H. Gross is an annual Polygonaceae herb that commonly appears at diverse wetland ecosystems such as streamlets, abandoned paddy fields, montane fens, and floating mats in Korea (Kim 2009; Kim et al. 2012; Park et al. 2013). *P. thunbergii* inhabits widely and sometimes dominates in wetland ecosystems where the water level is not much deep (Kawano 2008; Kim et al. 2013). In Korea, *P. thunbergii* is occasionally considered as a management object due to its rapid recovery through asexual reproduction after harsh flooding disturbances (Kim et al. 2012).

P. thunbergii has the amphicarpic trait that produces different types of seeds only shown in a few species (Cheplick 1987). *P. thunbergii* produces not only aerial chasmogamous flower but subterranean cleistogamic flower (Kawano et al. 1990; Choo et al. 2014). Two types of seeds from different types of flower (aerial and subterranean seed) have significant differences in seed and seedling stage

properties (Choo et al. 2015). In addition, the ratio of aerial and subterranean flower and seed number varies under different hydrological conditions and soil fertility in *P. thunbergii* (Choo et al. 2014; Kim et al. 2016).

There might be differences in genetic diversity between two seed types in *P. thunbergii* as well as physiological differences in seed and seedling stage as a result of differences in flower type (aerial and subterranean). However, studies about the genetic diversity of the wetland plants mainly have focused on clonal, perennial species (Piquot et al. 1996; Koppitz 1999; Honnay et al. 2010; Min et al. 2012; Raabová et al. 2015). Previous studies on the genetic diversity of *P. thunbergii* only focused on their spatiotemporal genetic distribution (Konuma and Terauchi 2001; Kim et al. 2008). Genetic diversity of *P. thunbergii* could be affected by phenotypic plasticity in the ratio of aerial and subterranean flower production, as a result of the plasticity caused by their environment.

The aim of this study is to assess the genetic diversity of *P. thunbergii* populations with different environmental characteristics. Some ecological characteristics of *P. thunbergii* also would be considered in population genetic diversity.

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Materials and methods

Study site

The study was conducted at the Han River and Gyeongan-cheon stream which is a tributary to the Han River, Seoul and Gyeonggi Province, Republic of Korea. Field study was conducted in July 2014. Three sites where the coverage of *P. thunbergii* was more than 50% with relatively few artificial disturbances were chosen for the field study and sampling (Fig. 1).

Site A (Yongin, Gyeonggi Province) is the upper region and adjacent to the source of Gyeongan-cheon (<2 km), where the width of the stream is 2~3 m. Before Gyeongan-cheon joins the Han River at Paldangho Lake, in site B (Gwangju, Gyeonggi Province), *P. thunbergii* has been inhabited at the floodplain of stream which width is about 80-m wide. Also, site C (Gangdong-gu, Seoul) is also a floodplain of the Han River with approximately 700-m width.

Field survey and sampling of *P. thunbergii*

Four 1 m × 1 m quadrats were installed at each site. Vegetation survey was conducted at each quadrat. Relative coverage of emerged plant species and species diversity were calculated (H' ; Shannon-Weiner index).

$$\text{Relative coverage} = \text{cover of species} / \text{total cover}$$

$$H' = -\sum_{i=1}^N p_i \log p_i \quad (N = \text{no. of emerged species}; p_i = \text{relative coverage of species } i)$$

Soil sampling was conducted closely by each quadrat. In case of site A, only one soil sample was collected because *P. thunbergii* population rooted on the rock with a little soil medium. For assessment on population genetic diversity of *P. thunbergii*, leaves of ten individuals in 2-m-interval nearby quadrats were sampled at each site. Leaf samples were stored at -20 °C until DNA extraction.

Soil analysis

Soil samples were passed through a 2-mm sieve (standard sieve #10) before analysis. Water content was determined after drying samples at 105 °C in an oven, and analysis of organic matter content followed by the loss-on-ignition method at 550 °C furnace (Boyle 2004). Soil pH (AP63, Fisher, Hampton, USA) and conductivity (EC; Corning Checkmate II, Corning, Lowell, MA) were measured from the soil solution which is a mixture of soil samples with distilled water at a mass ratio of 1 to 5. NO₃-N and NH₄-N were extracted with 2 M KCl solutions and measured by a hydrazine method and indophenol method, respectively (Kamphake et al. 1967; Kim et al.

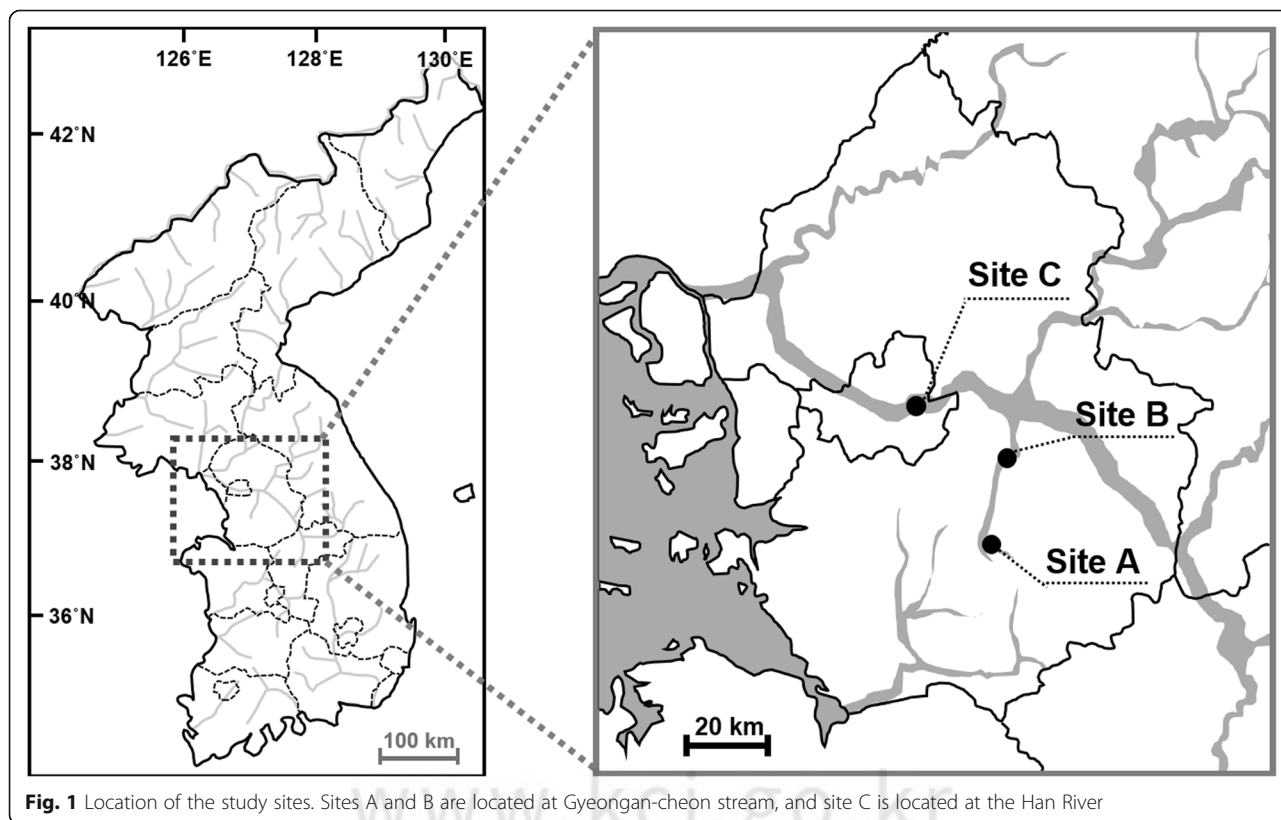


Fig. 1 Location of the study sites. Sites A and B are located at Gyeongan-cheon stream, and site C is located at the Han River

2004). PO₄-P was extracted and analyzed by a Bray No. 1 method (Bray and Kurtz 1945). Available K⁺, Ca²⁺, Na⁺, and Mg²⁺ were extracted with 1 N ammonium acetate solution (Allen et al. 1974) and measured with an atomic absorption spectrophotometer (AA240FS, Varian, Palo Alto, USA). Soil texture was determined using the hydrometer method and the soil texture triangle of USDA (Carter 1993).

RAPD-PCR

Extraction of individual genomic DNA was conducted by DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR was followed by using AccuPower PCR premix (Bioneer, Daejeon, Korea). The total reaction volume of PCR was 20 μL, composed of 1 μL of extracted DNA, 1 μL of primer (10 pmol) and 18 μL of deionized water. Four random primers which showed the polymorphism in electrophoresis were selected for random amplification of polymorphic DNA (RAPD) analysis (Table 1). Reaction cycle of PCR began with 2 min of denaturation at 94 °C. Forty cycles were followed with 45 s of denaturation at 94 °C, 45 s of annealing at the T_m of each primer, and 1 min and 30 s of elongation at 72 °C. Lastly, 2 min of the final extension step at 72 °C was followed.

Electrophoresis of amplified fragments was conducted in GelRed™ (Blotium, Hayward, CA) stained 1% agarose gel. Under the UV light, bands were observed and recorded by binary character matrix with 1 (presence) and 0 (absence).

Data analysis

Genetic diversity within the population (number of polymorphic loci; Nei's gene diversity, *h*; Shannon index, *i*) and genetic distance (Nei's genetic distance) among populations were calculated by Popgen32 (Nei 1973; Yeh and Boyle 1997). Principal coordinate analysis (PCoA) with the presence and absence of each band was conducted by R version 3.1.1.

Results

Habitat characteristics of *P. thunbergii* populations

Relative coverage of *P. thunbergii* at sites A, B, and C were 100, 52.5, and 77.9%, respectively. Five species emerged at site C, whereas three species emerged at site B (Table 2). At site A, only *P. thunbergii* emerged in the surveyed

Table 1 Random primer sequences and number of amplified RAPD fragments

Primer	Sequence	T _m (°C)	No. of observed bands
N-8002	CAATCGCCGT	32	13
N-8004	TCGGCGATAG	32	7
N-8007	GTGACGTAGG	32	9
N-8010	CTGAGACGGA	32	11

Table 2 Plant species, relative coverage (%), and species diversity

	Site A	Site B	Site C
Perennials			
<i>Phragmites australis</i>	-	-	4.1
<i>Equisetum arvense</i>	-	-	0.9
<i>Artemisia princeps</i> var. <i>orientalis</i>	-	-	0.4
Annuals			
<i>Persicaria thunbergii</i>	100	52.5	77.9
<i>Humulus japonicas</i>	-	47.2	16.7
<i>Persicaria perfoliata</i>	-	0.3	-
Shannon-Weiner index	-	0.309	0.299

quadrats although the other emergent macrophytes occurred nearby. Species diversity index (Shannon-Wiener index) at sites B and C were 0.309 and 0.299, respectively.

At field survey, *P. thunbergii* population at sites B and C had a distance from the surface of the stream or river. However, the average water depth was 2 cm at site A. Soil characteristics at site B and site C were similar except NH₄-N and NO₃-N content (Table 3). Soil textures were sandy loam~silt loam at site B, silt loam~loam at site C, whereas there was sand at site A. EC (A, 37.2 μS/cm; B, 52.5~102.3 μS/cm; C, 61.8~92.6 μS/cm) and organic matter content (A, 2.25%; B, 7.21~9.26%; C, 7.54~9.23%) at site A were considerably lower than sites B and C. Similarly, at site A, NO₃-N (A, 0.53 mg/kg; B, 2.01~3.51 mg/kg; C, 4.57~5.35 mg/kg) and PO₄-P content (A, 6.75 mg/kg; B, 8.08~15.74 mg/kg; C, 11.91~17.29 mg/kg) were lower than sites B and C. However, NH₄-N content at site A was relatively higher than sites B and C except one plot at site B (A, 12.5 mg/kg; B, 0.60~16.32 mg/kg; C, 0.49~1.63 mg/kg). K⁺, Ca²⁺, and Mg²⁺ content were relatively higher at sites B and C

Table 3 Physicochemical soil characteristics (Mean ± 1 SD)

	Site A (n = 1)	Site B (n = 4)	Site C (n = 4)
pH	6.86	6.62 (±0.06)	6.66 (±0.09)
EC (μS/cm)	37.2	75 (±20.9)	79.2 (±12.8)
Water content (%)	28.2	27.9 (±3.8)	30.1 (±1.4)
LOI (%)	2.25	8.24 (±1.12)	8.06 (±0.79)
NH ₄ -N (mg/kg)	12.5	6.56 (±6.95)	0.94 (±0.52)
NO ₃ -N (mg/kg)	0.53	2.76 (±0.69)	4.95 (±0.33)
PO ₄ -P (mg/kg)	6.75	11.9 (±3.9)	14.6 (±2.2)
K ⁺ (mg/kg)	64.1	543.8 (±109.5)	599.8 (±94.4)
Na ⁺ (mg/kg)	19.9	18.1 (±1.4)	16.8 (±1.7)
Ca ²⁺ (mg/kg)	928.4	2467 (±240)	2327 (±129)
Mg ²⁺ (mg/kg)	94.4	272.8 (±37.8)	322.1 (±40.6)
Soil texture	Sand	Sandy loam or silt loam	Loam or silt loam

than site A, but Na⁺ content was similar at the three sites (Table 3). Soil water content and pH showed just a little difference between sites A, B, and C.

Genetic diversity

A total of 40 band loci were observed in four primers, and 35 of them were polymorphic. Each number of polymorphic loci at sites A, B, and C were 29, 28, and 29, respectively. The total Nei's gene diversity (*h*) was 0.2969 and Shannon's information index (*i*) was 0.4472. The highest genetic diversity was observed at site B (*h* = 0.2761, *i* = 0.4100; Table 4).

In a 2-D plot of PCoA based on 35 RAPD bands, individuals from three sites were mixed (Fig. 2). However, individuals from site C were relatively separated from sites A and B on PCoA plot. Nei's genetic distance between sites A and B was 0.0338, between sites B and C was 0.0685, and between sites A and C was 0.0833.

Discussion

The total potential genetic diversity of *P. thunbergii* population seemed to decrease when harsh flooding occurs in a natural habitat. In addition, high soil fertility increases the number of aerial seed production despite the decrease of mass allocation in aerial seeds (Kim et al. 2016). Considering the mass allocation, genetic diversity of *P. thunbergii* population is expected to be relatively higher in relatively fertile habitat than sterile habitat.

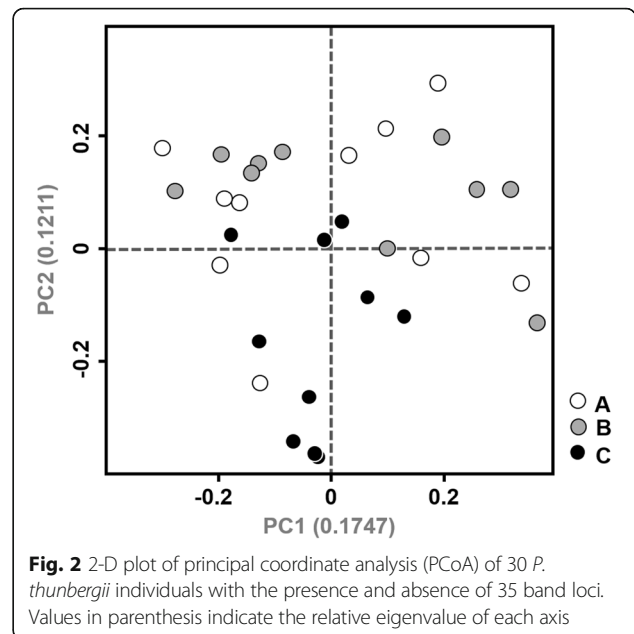
Both two sites with fertile soil are affected by annual harsh flooding in July and August (Han River Flood Control Office 2014). Although site A is located on the same water system as sites B and C, the flooding pattern is different being closer to the source of the stream. The frequency of flooding disturbance seemed to be relatively higher, but the intensity seemed to be less harsh in site A than sites B and C. Weathering and sediment deposition by flowing water frequently occurred in sites B and C than site A, where soil is relatively sterile. Number of aerial seed production might be higher in sites B and C, where the annual harsh flooding occurs with fertile soil (Choo et al. 2015; Kim et al. 2016). It also could contribute to the relatively higher genetic diversity than the habitat with oligotrophic soil.

While genetic diversity of sites B and C were similar, site C was genetically separated from sites A and B. It

Table 4 Number of polymorphic loci and percentage, Nei's gene diversity (*h*), and Shannon index (*i*) in the three sites

Site	No. of polymorphic loci (%)	<i>h</i>	<i>i</i>
Site A	29 (72.5)	0.2381	0.3592
Site B	28 (70.0)	0.2761	0.4100
Site C	29 (72.5)	0.2618	0.3925
Total	35 (87.5)	0.2969	0.4472

Total does not mean the sum of polymorphic loci numbers of three populations but the total loci number which considers overlapped loci among populations



could be inferred from the geographical barrier, a dam between two sites. In a previous study, *P. thunbergii* seemed to have a spatial genetic structure (isolation-by-distance) by reason of the limited seed dispersal (Konuma and Terauchi 2001). However, in this study, geographical barrier appeared to accelerate the genetic isolation between sites B and C.

Abbreviations

PCR: Polymerase chain reaction; RAPD: Random amplification of polymorphic DNA; USDA: United States Department of Agriculture

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Availability of data and materials

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

Authors' contributions

NBE participated in the design of the study, carried out the molecular studies, and wrote the manuscript draft. JGK conceived of the study, participated in the design of the study, edited the manuscript draft, and secured the funding. JMN participated in the field work. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval

Not applicable.

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