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Genetic Diversity of Natural and Cultivated Populations of *Oenanthe javanica* in Korea

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Enzyme electrophoresis was used to estimate genetic diversity and population structure in natural and cultivated *Oenanthe javanica* (Blume) DC. In the six natural populations, 8 of the 22 loci showed polymorphisms. Cultivated populations had fewer alleles per locus (1.84 vs. 1.91), fewer effective alleles per locus (1.47 vs. 1.52), a lower percentage of polymorphic loci (42.3 vs. 50.0), and lower diversity (0.210 vs. 0.228) than did natural populations. These parameters of genetic diversity indicate that the cultivated populations are genetically depauperate relative to their presumptive progenitor, and that the domestication process has partly eroded the level of genetic variation of this species. Nevertheless, the diversity of this species has higher-than-average values compared with other species having similar life-history traits.We propose that the mix-mating system; perennial, high gene flow; and large population sizes are possible factors contributing to this high diversity, which seemed to increase with distance from the coastlines.

Keywords: genetic diversity, *Oenanthe javanica*, population structure

Most plants, especially rhizomatous and stoloniferous species, have physical connections among their ramets, although the level of persistence is highly variable among species and habitats (Sobey and Barkhouse, 1977). The genetic structures of clonal plant populations have received increased attention over the past decade as a result of electrophoretic techniques that allow researchers to better assess their genotypic composition (Huh et al., 1999). Although asexually reproducing species are generally believed to be evolutionary dead-ends that lack genetic diversity, various studies have shown that they can be much more diverse than originally thought (Ellstrand and Roose, 1987). Clearly, descriptive genetic work on both sexual and asexual plant populations is needed. Knowledge and data concerning genetic variation are important for understanding ecological characteristics, conservation purposes, and population structures. However, the levels and distribution of genetic variation have not been studied for most species in Korea, for both sexual and asexually reproductive plants.

Natural populations of *Oenanthe javanica* (Blume) DC. (Umbelliferae) in Korea are diploid (2n = 20), and are typically distributed in wet fields. Only one species is found there (Lee, 1996). The wild type is called "Dolminari", and is usually used for medicine. The cultivated type, "Minari", is a major vegetable crop. These two types are not easily distinguishable; even quantitative characters such as leaf and petiole size cannot be used as criteria for their classification. Wild relatives of the cultivar offer an important system for both agricultural and evolutionary examinations, especially concerning the gene pool available for crop improvement (Doebley, 1989; Escalante et al., 1994). They also provide information regarding the effects of domestication (Doebley, 1989). Thus, it is very important to study natural populations of natural species from the viewpoint of crop evolution (Huh et al., 2000; Huh, 2001). The objectives of this study were to estimate the level of genetic diversity in this species, and to describe how its genetic variation is distributed within and among its populations. In addition, we investigated whether the domestication process had eroded the levels of genetic variation found in 'Minari', as has been reported with most other cultivated crop species (Doebley, 1989).

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MATERIALS AND METHODS

Sampling Procedure and Enzyme Electrophoresis

From 37 to 73 plants of *O. javanica* were collected

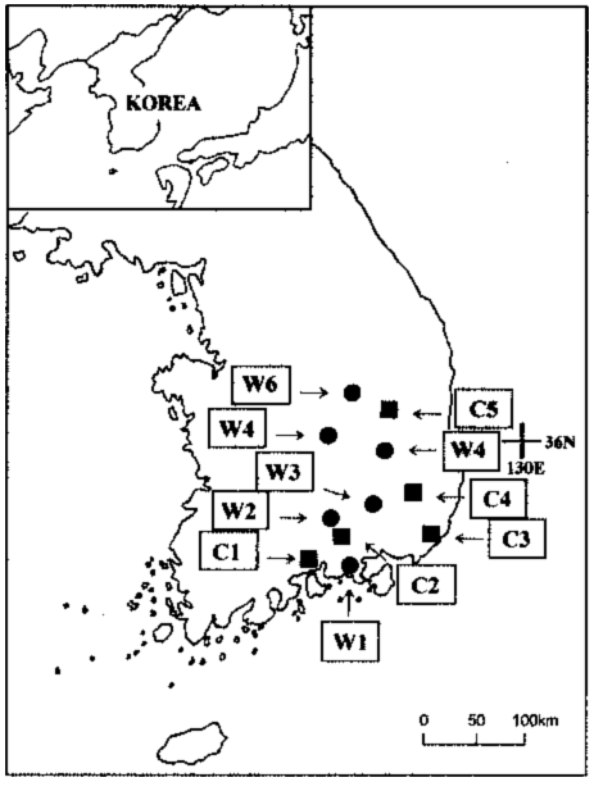

Figure 1. Collection sites for populations of *O. javanica* as sources for allozyme analysis. W1, Baedun-meon, Kosung-gun, Gyeongsangnam-do; W2, Konmeong-meon, Sacheon-ci, Gyeongsangnam-do; W3, Namgi-up, Changreng-gun, Gyeongsangnam-do; W4, Daechang-meon, Youngcheon-ci, Gyeongsangbuk-do; W5, Magok-meon, Youngdong-gun, Chungcheongbuk-do; W6, Buseck-meon, Youngju-ci, Gyeongsangbuk-do. C1, Ginko-meon, Hadong-gun, Gyeongsangnam-do; C2, Konmeong-meon, Sacheon-ci, Gyeongsangnam-do; C3, Ilkang-meon, Kijang-up, Pusan-ci; C4, Unyang-meon, Ulsan-ci; C5, Pungcheon-meon, Andong-ci, Gyeongsangbuk-do.

from each of six natural and five cultivated populations in Korea (Fig. 1). Enzyme extracts were made from the young leaves, following the procedures of Soltis et al. (1983). Approximately 1 to 2 g of the leaf tissues were ground with a cold mortar and pestle in 300 to 500 μL of extraction buffer (0.1% 2-mercaptoenthanol, 0.001 M EDTA, 0.01 M potassium chloride, 0.01 M magnesium chloride hexahydrate, 4% (w/v) PVP, and 0.10 M Tris-HCl buffer; pH 8.0).

Electrophoresis was performed using 12.0% starch gels, according to the methods of Soltis et al. (1983). Eleven enzyme systems were assayed in this study, including acid phosphatase (ACP), esterase (EST), fluorescent esterase (FE), leucine aminopeptidase (LAP), peroxidase (PER), phosphoglucose isomerase (PGI), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD). The first six were resolved on System 9; the second five on System 10 of Soltis et al. (1983). Genetic interpretations of the enzyme banding patterns were based on knowledge of the enzyme structures reported from most other allozyme studies of plants (Weeden and Wendel, 1989).

Data Analysis

Statistical analysis of the enzyme data was based on allele and genotype frequencies in each population. The following genetic parameters were calculated using a computer program developed by Loveless and Schnabel (see Edwards and Sharitz, 2000): the percentage of polymorphic loci (P_P for population level and P_S for species level), mean number of alleles per locus (A), effective number of alleles per locus (A_E), and gene diversity (H_E) (Hamrick et al., 1992). The levels of genetic diversity for species (indicated with the subscript $_S$) and mean population (subscript $_P$) were calculated according to the methods of Hartl and Clark (1989). We compared the observed heterozygosity (H_O) with Hardy-Weinberg expected values, using Wright's fixation index (F) for inbreeding coefficients (Wright, 1965).

To elucidate the organization of the variation in *O. javanica*, genetic variation was examined by partitioning the total genetic diversity (H_T) into within- (H_S) and among-population components, using Nei's genetic diversity statistics (Nei, 1973). A measure of differentiation among the populations, relative to the total diversity, was calculated at each locus. To gauge the extent of genetic departure of the populations from each other, we calculated Nei's genetic identity (I) and genetic distance (D) (Nei, 1972) for each pair-wise combination of populations. The PC-SAS program (SAS Institute, Inc., 1989) was used to conduct a cluster analysis on Nei's genetic distances via the unweighted pair-wise groups method arithmetic average (UPGMA). Bootstrapping was done via the PAUP 4.0 program (Swofford, 1999) to estimate the relative support for clades (Felsenstein, 1993).

The genetic structure within and among populations was also evaluated with Wright's (1965) F statistics: F_{IT}, F_{IS}, and F_{ST}. F_{IT} and F_{IS} measure excesses of homozygotes or heterozygotes (relative to panmictic expectations) within samples and within populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested with chi-square statistics (Li and Horvitz, 1953). We also calculated two indirect estimates of gene flow. Estimates of the number of migrants per generation

(*Nm*) were based on G_{ST} or the average frequency of private alleles found in only one population (Slatkin, 1985). Genetic diversity was tested against regions by Spearman rank to identify any correlations between genetic variation in the natural and the cultivated populations (Zar, 1984). We then used a modified Mantel's test to determine if any correlation existed between geographical and genetic distances (see Smouse et al., 1986).

RESULTS

Genetic Diversity

At the species level, 12 of the 22 loci (54.6%) showed detectable polymorphism in at least one population (Table 1). The remaining 10 loci (*Acp-2, Est-1, Idh-1, Lap, Mdh-3, Pgi, Per-3, Pgd, Pgm-2,* and *Skd-2*) were monomorphic in all populations.

In the natural populations, an average of 50.0% of the loci were polymorphic within populations, with individual population values ranging from 45.5 to 54.6% (Table 1). The average number of alleles per locus (A_P) was 2.82 across populations (2.67 to 2.91). The effective number of alleles per locus at the species level (A_{ES}) and at the population level (A_{EP}) was 1.57 and 1.52, respectively. Mean genetic diversity within populations was 0.23. Allele frequencies among populations differed significantly for 10 of the 12 polymorphic loci. Total genetic diversity values (H_T) ranged from 0.088 (*Per-1*) to 0.705 (*Mdh-1*), with an average of 0.443 over all polymorphic loci (Table 2). The interlocus mean variation of genetic diversity within populations (H_S) was 0.424.

In the cultivated populations, an average of 47.3% of the loci were polymorphic within populations, with individual population values ranging from 40.9 to 54.6% (Table 1). The average number of alleles per locus (A_P) was 1.84 across populations (1.73 to 1.95). The effective number of alleles per locus at the species level (A_{ES}) and at the population level (A_{EP}) was 1.51 and 1.47, respectively. Mean genetic diversity within populations was 0.210. We found significant differences in allele frequencies among populations for all 12 polymorphic loci. Total genetic diversity values (H_T) varied between 0.074 (*Per-1*) and 0.694 (*Mdh-1*), giving an average of 0.418 over all polymorphic loci (Table 2). The interlocus mean variation of genetic diversity within populations (H_S) was high (0.393).

Population Structure

F_{IS}, the measure of the deviation from random mating within the five populations, was 0.477 (Table 2). Values of genetic identity among pairs of populations ranged from 0.949 to 0.997. The majority of the genetic diversity observed at the polymorphic loci in natural *O. javanica* occurred within populations (G_{ST} = 0.053). Indirect estimates of *Nm* were based on a G_{ST} value of 4.52.

Table 1. Allozyme variation within 11 populations of *O. javanica*, as estimated by percentage of polymorphic loci (P_P), mean number of alleles per polymorphic locus (A_P), mean number of alleles per locus (A), effective number of alleles per locus (A_E), observed heterozygosity (H_{OP}), and Hardy-Weinberg expected heterozygosity or genetic diversity (H_{EP}).

Pop.	P_P	A_P	A	A_E	H_{OP} (SD)	H_{EP} (SD)
Natural populations						
W1	50.0	2.91	1.95	1.47	0.130 (0.009)	0.204 (0.056)
W2	45.5	2.90	1.86	1.48	0.107 (0.009)	0.214 (0.056)
W3	50.0	2.91	1.95	1.51	0.142 (0.010)	0.224 (0.058)
W4	54.6	2.67	1.91	1.54	0.132 (0.009)	0.245 (0.056)
W5	45.5	2.80	1.82	1.54	0.103 (0.008)	0.230 (0.059)
W6	54.5	2.75	1.95	1.59	0.125 (0.009)	0.253 (0.058)
Mean	50.0	2.82	1.91	1.52	0.123	0.228
SD	4.34	0.10	0.06	0.04	0.004	0.023
Cultivated populations						
C1	40.91	2.89	1.77	1.43	0.107 (0.008)	0.195 (0.056)
C2	40.91	2.78	1.73	1.43	0.076 (0.007)	0.191 (0.056)
C3	45.45	2.70	1.77	1.36	0.099 (0.008)	0.177 (0.052)
C4	54.55	2.75	1.95	1.58	0.133 (0.009)	0.249 (0.059)
C5	54.55	2.75	1.95	1.53	0.145 (0.010)	0.239 (0.055)
Mean	47.27	2.77	1.84	1.47	0.112	0.210
SD	4.71	0.07	0.11	0.09	0.004	0.025
Species	54.55	2.83	2.00	1.55		0.236

Table 2. Estimates of genetic diversity at 12 polymorphic loci in *O. javanica*, as estimated by total genetic diversity (H_T); genetic diversity within populations (H_S); deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}), within individual population (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST})

Locus	H_T	H_S	F_{IS}	F_{IT}	G_{ST}
Natural populations					
Est-2	0.430	0.418	0.481	0.495	0.026
Est-3	0.573	0.530	0.488	0.526	0.075
Mdh-1	0.705	0.661	0.111	0.167	0.063
Mdh-2	0.600	0.566	0.621	0.642	0.057
Skd-1	0.660	0.630	0.432	0.458	0.046
Per-1	0.088	0.085	0.576	0.578	0.026
Per-2	0.115	0.107	0.531	0.563	0.072
Pgm-1	0.285	0.261	0.574	0.610	0.086
Idh-2	0.500	0.447	0.577	0.580	0.007
Fe	0.256	0.251	0.435	0.446	0.020
Acp-1	0.637	0.622	0.379	0.394	0.024
Acp-3	0.513	0.510	0.581	0.584	0.007
Mean	0.443	0.424	0.481	0.505	0.042
Cultivated populations					
Est-2	0.440	0.416	0.541	0.566	0.054
Est-3	0.565	0.532	0.511	0.540	0.059
Mdh-1	0.694	0.659	0.326	0.360	0.051
Mdh-2	0.521	0.457	0.616	0.663	0.123
Skd-1	0.651	0.592	0.375	0.431	0.090
Per-1	0.074	0.069	0.471	0.503	0.060
Per-2	0.086	0.082	0.392	0.418	0.044
Pgm-1	0.262	0.230	0.521	0.580	0.123
Idh-2	0.373	0.358	0.569	0.587	0.042
Fe	0.237	0.231	0.336	0.353	0.025
Acp-1	0.604	0.591	0.360	0.374	0.022
Acp-3	0.505	0.496	0.587	0.494	0.018
Mean	0.418	0.393	0.467	0.497	0.059
Species	0.433	0.410	0.477	0.504	0.053

The similarity among the 11 *O. javanica* populations can be seen in the UPGMA dendrogram, where all populations clustered below a genetic distance of 0.25 (Fig. 2). Genetic relationships among these populations were recognized as belonging to two clades, one consisting of five northern populations, the other comprising six southern populations. In addition, the correlation between genetic distance and geographic distance was low and nonsignificant (r = 0. 477), indicating that geographically close populations tended to be genetically similar. Approximately 77% ($1 - r^2$) of the variation in genetic distance was due to unknown factors.

DISCUSSION

Genetic diversity in *O. javanica* is high compared with most other plant species. For example, diversity at the species level (H_{ES} = 0.236) was greater than what had been reported for selfing breeding systems (0.124), the mode of sexual or asexual reproduction (0.138), temperate-zone species (0.146), and sexual reproduction (0.151), as well as that found from the widespread geographic ranges of plant species (0.202) (Hamrick and Godt, 1989). The same trend was observed at the population level.

This relatively high level of genetic variation in *O. javanica* is consistent with several aspects of its biology, e.g., its breeding system. Species that predominantly reproduce sexually tend to have more genetic diversity overall, and maintain more variation within their populations than do species with higher frequencies of asexual reproduction (Hamrick and Godt, 1989; Huh, 1999). Factors that contribute to maintaining this variation in *O. javanica* may include the persistence of multiple generations within populations as well as large population sizes (Godt and Hamrick, 1993).

In addition, vegetative reproduction and spread can also affect the genetic structure of populations (Murawski and Hamrick, 1990). Cook (1983) has argued that clonal growth could act to retard the loss of genetic diversity within populations. That is, if a small amount of gene

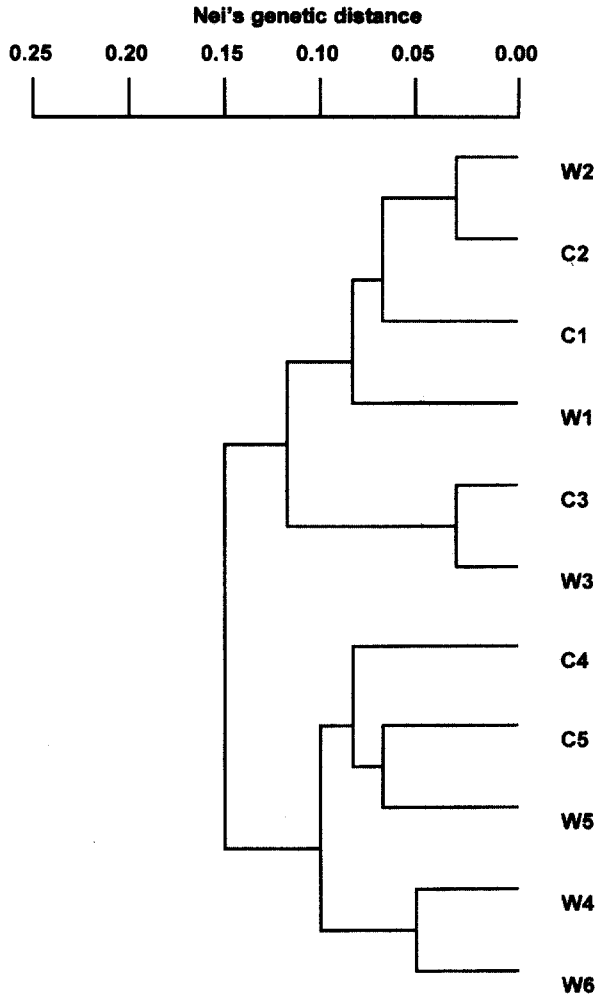

Figure 2. Neighbour-joining tree among 11 populations of *O. javanica*, based on genetic distance data.

flow and/or mutation should periodically add new clones to a population, clonal variation could be maintained. Thus, if clonalization were to occur in multiple genotypes, the ephemeral nature of populations might preclude a significant loss of genetic variation while those populations were extant (Ellstrand and Roose, 1987). Species with both sexual and asexual reproductive modes often are more highly diverse than those that are asexually reproductive only (Aspinwall and Christian, 1992). For example, the genetic diversity of *Filipendula rubra* (Hill) Robinson, a clonal species in the north central United States, is double that reported for 27 nearly obligate clonal plant species (Aspinwall and Christian, 1992).

An *Nm* value >1.0 is considered necessary to prevent the divergence that results from genetic drift (Wright, 1951). Although the level of gene flow found in *O. javanica* is sufficiently high to counterbalance genetic drift, these values are lower than those obtained for other species with similar traits, primarily because of its isolation by sea and topography (Hamrick, 1987). The mean *Nm* value of cultivated populations is higher than that of natural populations. Cultivation of *O. javanica* is very common in Korea. In agricultural areas, cultivated fields and natural populations of *O. javanica* may be juxtaposed. Hence, we can expect gene flow from cultivated populations to natural populations. During cultivation, the cutting of stems and seed dispersal by farmers to better fields may be one mechanism of gene flow. This occasional movement of plants may result in high flows, but little spatial genetic differentiation.

A substantial heterozygote deficiency occurred in some populations and at some loci (F_{IS} = 0.397, natural; F_{IS} = 0.376, cultivated). Because population structuring was not obvious, our selected pool for testing may have consisted of a group of heterogeneous subsamples from a population. If fairly large differences are seen in allelic frequencies among subsamples, when they are lumped together the result will be a net deficiency of heterozygotes and an excess of homozygotes, even if Hardy-Weinberg proportions exist within each subsample (Wahlund, 1928). Our sampling included individuals from several patches per population, thereby causing an overall deficiency of heterozygotes. This sampling method, therefore, created a Wahlund effect in our results (Hartl and Clark, 1989).

The percentage of polymorphism was 54.6% for natural populations and 33.3% for cultivated populations. The latter also had fewer alleles per locus (1.84 vs. 1.91), fewer effective alleles per locus (1.47 vs. 1.52), a lower percentage of polymorphic loci (42.3% vs. 50.0%), and lower diversity (0.210 vs. 0.228) than did the natural populations. Over the polymorphic loci, 44 alleles were detected in the natural populations; of the 41 alleles in the cultivated populations, none was unique. These parameters of genetic diversity indicate that the cultivated species had lower values than those of its presumptive progenitor, which may mean that the domestication process had eroded the level of genetic variation.

In contrast, the high levels of genetic variability in the natural population were expected because they were not subject to any of the selection pressures of domestication; maintenance of this high variability would have favored their survival under natural conditions (Doebley, 1989). Therefore, we conclude that domestication in *O. javanica* reduces genetic diversity. This generally agrees with the concept that most crops show reduced levels of polymorphism compared with their presumed

progenitors (Doebley, 1989).

It has been widely argued that environmental heterogosity is a major factor in maintaining and structuring genetic variation in natural populations. Nevo et al. (1988) have suggested that the amount of variation may be regarded as an adaptive strategy for increasing population fitness in a spatiotemporally heterogeneous and uncertain environment. Likewise, Nevo et al. (1988) and Charmet et al. (1993) proposed that the impact of climatic factors on genetic diversity is especially manifested in areas with numerous forests and cold climates. In our study, geographic distance explained no more than 23% of the variability with the interpopulation genetic distance ($r^2 = 0.23$). This implies that the geographically close populations tended to be genetically similar, and that about 77% of the variation in genetic distance may have been caused by factors other than distance.

In addition, we examined the correlations between genetic diversity per population and annual rainfall, temperature, solar radiation, elevation, latitude, and distance from the coastline. A pattern of somewhat greater genetic diversity was observed as distance from the coastline increased (Fig. 3). Unlike the coastal region, the inland areas displayed characteristics of a continental climate, such as severe winter conditions and snow cover, warm and relatively sunny summers, and aridity stress. When coupled with data related to the climatic effect on intra-populational differentiation, these results may indicate that the continental climate is conducive to sexual reproduction, thereby yielding highly diversified populations that better cope with the inherent climatic variability. In addition, a higher incidence of vegetative reproduction was found within the coastal populations compared with those in central Korea. Significant climatic variables had values that indicated milder conditions in the former region.

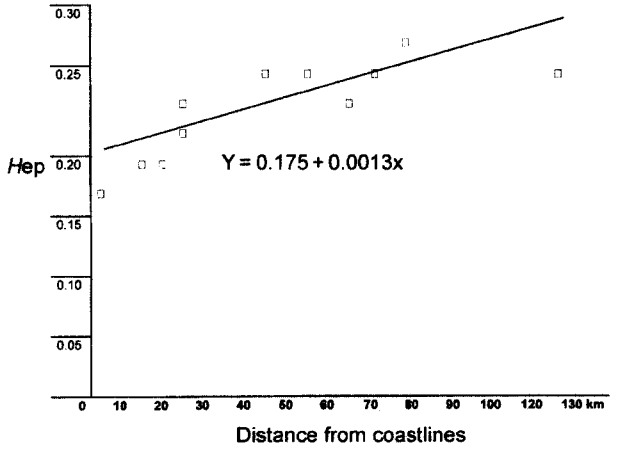

Figure 3. Relationship between genetic diversity and physical distance from coastline for 11 populations.

Our populations divided into two clades in Neighbour-joining -- one consisting of five northern populations (above 38°N), the other with six southern populations (below 38°N). However, because we did not find a longitudinal gradient in genetic diversity, we could not exclude the possibility that the two clades resulted from long-distance migration, by either water currents or human activity. No examples were found of a close connection between a cultivated population and a natural population of *O. javanica*, and there were no signs of the natural population contributing to the cultivated population in Korea. Though the allozyme markers were not greatly differentiated locally, they proved to be more effective than other molecular markers. Therefore, we believe that information about the phylogenetic relationships of *O. javanica* and its closely related species is very valuable for taxonomic studies of the genus *Oenanthe*, identifying the origin of cultivated plants, and its future breeding.

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