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Population analysis of eelgrass, *Zostera marina* L. in Geojedo, Gaedo, and Jedo on the southern coastal water of Korea using RAPD-PCR

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Assessments of population genetic structure and diversity can be of value in formulating management plans for threatened eelgrass (*Zostera marina*). Using randomly amplified polymorphic DNA markers, we found evidence of significant genetic structure among the populations of eelgrass sampled at three areas (Geojedo, Gaedo, and Jedo). A highly isolated (>100 km apart) population from the Geojedo had a long genetic distance (0.16), whereas the populations from the Gaedo and Jedo (<10 km apart) exhibited far less distance (0.08). The analysis of similarity within population showed that Geojedo was over 70%, which was of lower value than of Gaedo and Jedo. Based on these results, we realized that heterogeneous population was in accordance with geographic separation. This is caused by limited seed dispersal and interrupted gene flow, although the sample size is small.

Key words – eelgrass, gene flow, geographic scale, population structure, RAPD

Introduction

Seagrass beds are one of the most productive of plant communities which provide living space and shelter for marine animals [16,18,21,42,49]. In particular, seagrass habitats have higher availability of uptake inorganic materials, which are associated with extensive removal of enriched water nutrients caused by coastal eutrophication [23,55]. The ecological significance of seagrass is clarified, but considerable deterioration of seagrass coverage in the world including Korea has reported with the increased impact of industries and increasing population [3,11,41]. To cope with habitat recovery, some researcher have much studied on the distribution [27-30,33], reproduction [25,32], and ecology of seagrass [5,6,22,24,60].

It is understood that the number of leaf vein, shape of leaf apex, and striation pattern of seed coat play an important role in taxonomic tools to identify the species of *Zostera* [43]. At present, five species of *Zostera* have been reported in Korea [52]. Among the family of Zosteraceae, *Z. marina* Linnaeus (eelgrass) is one of the most abundant seagrass species in the world [10] and also common in Korea [36,37]. In this fact, the extensively ecological characteristics including morphological variations of *Z. marina* in the Korean peninsula were much investigated by Lee et al. [25,27,29-33]. Lee et al. [28-30] suggested that the habitats of *Z. marina* in Korea were recognized in morphological variations. Likewise, some researchers have already reported considerable morphological variations [10,50,51,56]. In order to avoid problems associated with phenotypic plasticity of *Z. marina*, genetic sequencing data now provide an important solution in solving taxonomic difficulties and discriminating between morphologically similar species [20,26,34,35].

Data on the genetic structure of populations are of particular interest in that they may reveal evidence of restricted gene flow or genetic isolation that is undetectable. Such knowledge can be used to identify genetically based management, enabling conservation practices to be focused appropriately. All seagrasses reproduce vegetatively, both by branching of rhizomes and sexually through seeds. This leads to imitations in sexual reproduction and restricted gene flow, resulting in further limited genetic diversity of local populations. Some researchers have examined the analysis of population structure in seagrasses using allozyme data [9,13,58], but such research is not enough to estimate a precise gene flow and effective population size because of the low level of genetic variation. At present, the use of RAPD (Random Amplified Polymorphic DNA) and DNA microsatellites have a higher resolution in terms of detecting more genotypes in seagrasses [19,44,45]. However, *Z. marina* in Korea is poorly represented in studies of population genetic structure for conservation and management perspective based on genesis. Consequently,

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our goal is to quantify genetic population structure and estimate levels of genetic diversity within populations and gene flow among populations. Our results are expected to help clarifying the genetic status and composition of Korean threatened populations of *Z. marina*, thus aiding these charged with preparing plans for the management of the species in Korea.

Materials and Methods

Sampling collection

Eelgrass (*Z. marina*) samples were collected from three localities in Korea as submerged stock in nature (Fig. 1). We used a total of 16 specimens from Geojedo (5 individuals), Gaedo (5 individuals), and Jedo (6 individuals) on the southern coast. The samples were collected between February and March on 2006.

Total RNA extraction

Total RNA was extracted from 0.05 g of blotted wet weight of leaf materials using TRI Reagent kit (Molecular Research Center) after homogenizing the pellets. The RNAs were frozen at -80℃ until required.

RT-PCR amplification

Synthesis of complementary DNA (cDNA) from total RNA and PCR amplification using the synthesized cDNA as a template were carried out using random hexamer (Bioneer) and *AccuPower* RT/PCR premix kit (Bioneer) according to manufacturer's instructions.

RAPD

DNA concentrations were adjusted to 50 ng μl^{-1}. PCR reactions were performed in a final volume of a 20 μl mixture: 1.25 unit *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Roche Co.); 10 × PCR reaction buffer (Roche Co.); 0.5 mM dNTPs; and 100 pmol of RAPD primer. Amplifications were performed with the MyCycler thermal cycler (Bio-Rad). The thermocycling profile included an initial denaturation step of 94℃ for 4 min, followed by 35 cycles of 1 min at 94℃, primer annealing for 1 min at 55℃, and extension for 2 min at 72℃. Amplification products were separated for 3 h at 100V on 1.5% agarose TBE gels. These were stained with 10 μl ethidium bromide added to the gel and 0.25 μl ethidium bromide ml^{-1} TBE buffer to the TBE running buffer. Twelve random primers (Seoulin UniPrimer™ kit I) were screened (Seolulin Scientific Co.,

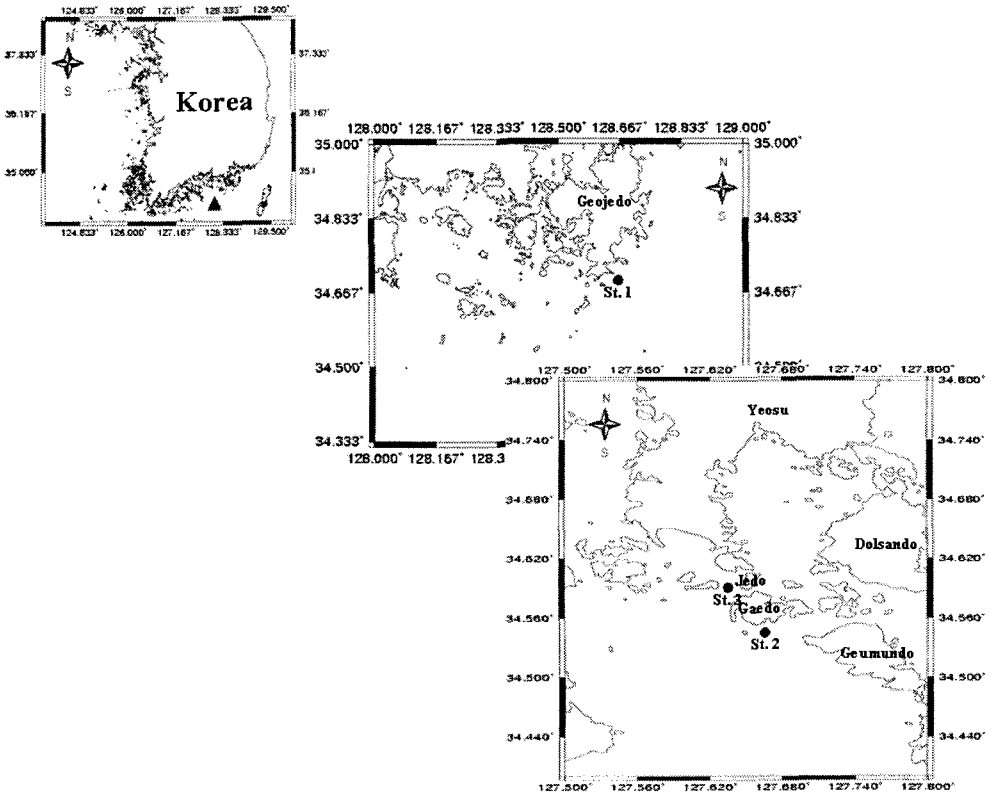

Fig. 1. Map of sampling sites for eelgrass (*Zostera marina*).

Table 1. Primers used in this study

Primer	Oligonucleotide sequence (5'-3')
URP 02	5'-CCCAGCAACTGATCGCACAC-3'
URP 04	5'-AGGACTCGATAACAGGCTCC-3'
URP 05	5'-GGCAAGCTGGTGGGAGGTAC-3'
URP 09	5'-AATGTGTGGCAAGCTGGTGG-3'
URP 11	5'-GGACAAGAAGAGGATGTGGA-3'

Seoul), five of which, URP 02, URP 04, URP 05, URP 09, and URP 11 were used for analysis (Table 1). RAPD bands were scored as present/absent (p/a = 1/0) and only well-resolved profiles were showed.

Data analysis

Individual data set from each primer were combined to make data matrix. Statistical analyses were performed on a personal computer using the MVSP Windows program ver. 3.1 (Kovach Computing Service) for phylogenetic relationships and constructing genetic tree using unweighted pair-group method with arithmetic averages (UPGMA, Sneath and Soka [53]).

Results

RAPD patterns were assessed in the populations of eelgrass (*Z. marina*) from Geojedo, Gaedo, and Jedo using five universal primer sets selected from 12 primers (Fig. 2). RAPD patterns of triplicate PCR reaction under the same PCR condition showed identical patterns, which ascertained the reproducibility of RAPD patterns (data not shown). On the basis of amplification product patterns, the population from Geojedo was clearly discriminated from Gaedo and Jedo using the URP 02 and 05 universal primers.

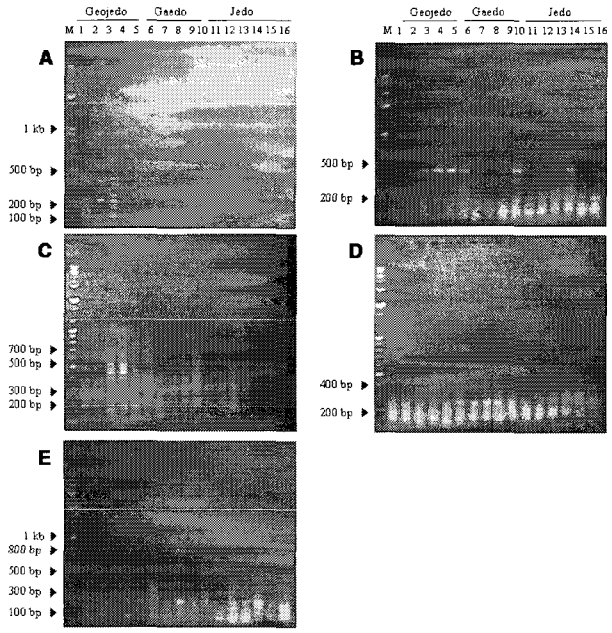

Fig. 2. Random amplification of polymorphic DNA of the populations of *Zostera marina* from Geojedo, Gaedo, and Jedo using five universal primers URP 02 (A), URP 04 (B), URP 05 (C), URP 09 (D), and URP 11 (E). Individual numbers are present above each lane. Molecular weight size markers are indicated in base pairs to the left.

Interestingly, PCR products using the URP 02 and 05 primers showed various DNA polymorphisms, even within the population of Geojedo. The populations of Gaedo and Jedo exhibited almost the same RAPD patterns, but the URP 05 and 11 primers might play an important role in differentiating between the Gaedo and Jaedo populations. Table 2 showed the total, inter-common, intra-species- specific, and polymorphic fragments generated by five primers. Five primers produced a total of 58, 46, and 65 DNA frag-

Table 2. The number of observed bands generated by RAPD-PCR using 5 random primers in the populations of eelgrass (Zostera marina) from Geojedo, Gaedo, and Jedo

Primer	No. of average band per lane			No. of common bands			No. of specific bands			No. of polymorphic bands		
	Ge	Ga	Je	Ge	Ga	Je	Ge	Ga	Je	Ge	Ga	Je
URP 02	2.8 (14)	2.2 (11)	2.3 (14)	5	10	12	8	1	2	5	2	4
URP 04	1.8 (9)	1.4 (7)	1.3 (8)	7	7	6	1	0	1	0	0	0
URP 05	3.6 (18)	2.0 (10)	2.0 (12)	5	5	6	8	5	6	11	2	0
URP 09	2.0 (10)	2.0 (10)	2.0 (12)	10	10	12	0	0	0	0	2	1
URP 11	1.4 (7)	1.6 (8)	3.1 (19)	5	6	7	2	2	12	2	5	5
Total no.	11.6 (58)	9.2 (46)	10.7 (65)	32	38	43	19	9	21	18	11	10
Average no. per primer	15.8	9.2	13	6.4	7.6	8.6	3.8	1.8	4.2	3.6	2.2	1.6

The total number of bands generated by a primer in eelgrass collected from Geojedo(Ge), Gaedo (Ga), and Jedo (Je) is shown in parentheses.

ments, with an average of 15.8, 9.2, and 13 per primer from the populations of Geojedo, Gaedo, and Jedo, respectively. In addition, 43 inter-common fragments, with an average of 8.6 per primer, were obtained from the population of Jedo, which were the greatest observed bands. Furthermore, 9 intra-species-specific fragments, with an average of 1.8 per primer, were obtained from the population of Gaedo, which were the smallest observed bands. In the population of Geojedo, the number of specific and polymorphic fragments had the greatest observed bands of 19, and 18, respectively. The population of Geojedo had a moderate similarity of 48-77% with Gaedo, which had a similarity of 48-70% with Jaedo (Table 3). However, similarity within the population of Geojedo had higher value of over 70%, which was a somewhat lower value than that of Gaedo and Jedo. This indicates that Z. *marina* occurring on the southern coastal waters shows inter and intra-species DNA polymorphisms. The phylogenetic tree was constructed from the similarity matrix of the populations from Geojedo, Gaedo, and Jaedo using MVSP program ver. 3.1 (Fig. 3). Individuals from Geojedo, Gaedo, and Jedo formed a stronger monophyletic group. Jedo was more closely related to Gaedo than to Geojedo. The distance obtained between Jaedo and Gaedo populations was 0.08, whereas that obtained between Jedo and Gaedo and Geojedo was 0.16.

Discussion

It is known that variability in the populations may be associated with undergoing the process of differential press

Table 3. Similarity matrix of DNA polymorphic fragments generated by RAPD-PCR using 5 random URP primers in the populations of eelgrass (Zostera marina) from Geojedo, Gaedo, and Jedo based on MVSP program (ver. 3.1)

	Ge 1	Ge 2	Ge 3	Ge 4	Ge 5	Ga 1	Ga 2	Ga 3	Ga 4	Ga 5	Je 1	Je 2	Je 3	Je 4	Je 5	Je 6
Gel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ge 2	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ge 3	80	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ge 4	72	69	88	0	0	0	0	0	0	0	0	0	0	0	0	0
Ge 5	84	70	75	85	0	0	0	0	0	0	0	0	0	0	0	0
Gal	70	57	56	63	73	0	0	0	0	0	0	0	0	0	0	0
Ga 2	63	60	50	57	66	84	0	0	0	0	0	0	0	0	0	0
Ga 3	60	57	48	54	63	80	84	0	0	0	0	0	0	0	0	0
Ga 4	66	63	52	60	70	88	94	88	0	0	0	0	0	0	0	0
Ga 5	73	60	58	66	77	94	88	84	94	0	0	0	0	0	0	0
Je 1	66	63	52	60	70	77	82	77	87	82	0	0	0	0	0	0
Je 2	63	60	50	57	66	84	77	73	82	77	94	0	0	0	0	0
Je 3	60	57	48	54	63	80	73	80	77	73	88	94	0	0	0	0
Je 4	60	50	50	56	63	78	63	78	66	72	76	81	86	0	0	0
Je 5	54	52	44	50	57	72	66	81	70	66	80	85	90	96	0	0
Je 6	63	52	51	58	66	81	66	72	70	76	80	85	90	96	91	0

Ge, Geojedo; Ga, Gaedo; Je, Jedo.

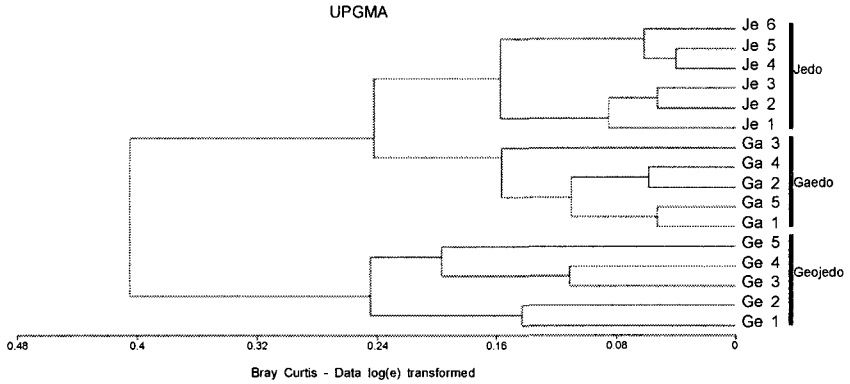

Fig. 3. Dendogram of the populations of *Zostera marina* from Geojedo, Gaedo, and Jedo based on random amplification of polymorphic DNA fragments.

of gene flow, genetic drift, and natural selection caused by environmental differences [17] within micro-geographic distance and among geographical sites. Based on geographical characteristics, sampling sites (Geojedo, Gaedo, and Jedo) were located in the southern region. In the southern region, the coastlines of the South Sea have a complex coastal topography, with many islands, estuaries, and bays. These sites do not form a semi-closed bay, but have various types of ecosystems because they are affected by both inshore and offshore water currents. Theoretically, it is possible to form a genetically homogeneous population regardless of geographical separation because of gene transfer by planktonic larvae at small and large scales. Most of this current genetic population structure has been obtained with the direct analysis of fishes and invertebrates [7,14,54].

In contrast to fishes and invertebrates, hydrophilic pollination and reproductive shoots carrying seeds play an important role in enhancing gene flow in *Z. marina*. Variability in pollination of *Z. marina* is less than 48 h and maximum distance of pollen and seeds estimated at 15 m [1,2,8]. Furthermore, reproductive shoots are strongly influenced by water currents [15,38]. Ruckelshaus [47] reported that the ability for seed dispersal in *Z. marina* was higher than in other terrestrial angiosperms. It is not known how far other *Zostera* species can disperse in the ocean. Ewanchuk and Williams [12] reported that it was difficult for drifting reproductive shoots to re-establish in nature. Possibly, it was difficult for the species of *Z. marina* to become accustomed to the effective gene flow and genetically homogeneous populations between distant regions. Likewise, this study found evidence of genetic differences between geographically separated populations of *Z. marina*. At a local scale, the populations from Gaedo and Jedo, where geographic distance is below 10 km, have a strong genetic relationship. At a large geographic scale, the populations from Geojedo and Gaedo/Jedo, where distance is over 100 km, have a long genetic distance and weak genetic relationship. Although the three populations had homogenous environmental characteristics and a relatively small number of available samples, a genetically distinct population was found in this study. This is because limited effective gene flow is caused by seed dispersal according to geographical scale. A close genetic relationship between Gaedo and Jedo populations is the result of an easier gene transfer than that of Geojedo population and is caused by their geographical frontier. The correlation of genetic and geographic distances have been reported [38,39,40,44-46, 57,58] which achieved similar results with our present study.

Olsen et al. [40] revealed high levels of genetic diversity in the populations of *Z. marina* from the Wadden Sea, although the meadow has been declining. Some researchers suggested that loss of seagrass habitat was associated with decreasing genetic diversity of seagrass populations [4,48, 58,59]. There is not available on the percentage in terms of the reduction of seagrass coverage in Geojedo, Gaedo, and Jedo estimates; however, our present study shows that possibly populations are composed of many heterogeneous individuals and high genetic diversity. These findings are similar to the results achieved by Olsen et al. [40]. Our current genetic diversity of Geojedo, Gaedo, and Jedo populations may be not the result of deterioration in seagrass coverage, but may be a process shaped by factors intrinsic to the species (i.e., high out-crossing). Consequently, no bottleneck has been found in the populations from Geojedo, Gaedo, and Jedo and their allele frequencies and gene pools are changing through successive generations. At present, they form an effective population size against internal and external factors.

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초록 : RAPD 방법을 이용한 거제도, 개도, 제도해역에서 채집한 말잘피 개체분석

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본 연구는 말잘피 군집의 유전적 구조 및 다양성 분석은 잘피 관리를 위해서 시행했다. 거제도, 개도, 제도연안에 서식하는 잘피를 대상으로 RAPD 분석에 따른 유전적 다양성이 높은 것으로 나타났다. 거제도는 개도 및 제도와는 100 km 이상 떨어진 지역에 서식하는 잘피의 유전적 거리는 0.16으로 나타난 반면에, 개도와 제도의 유전적 거리는 0.08 정도로 보이고 있다. 거제도에 서식하는 잘피의 개체내 유전적 유사도는 70% 이상으로 나타났으나, 개도와 제도에 비하면 낮은 유사도를 보이고 있다. 따라서 남해안에 서식하고 있는 잘피의 개체는 지역적 거리에 따라 유전적으로 상이한 집단을 형성하고 있다. 이러한 원인은 시스트 확산 및 유전자 이동를 제한 때문인 것으로 추정된다.