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Morphological and Genetic Variations in *Suaeda maritima* Based on Habitat

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We investigated the morphological and genetic variations found in *Suaeda maritima* growing on sand dunes at Jogeum-naru, Muan-gun, in Korea. Our objective was to determine how plant density, biomass, and morphological characteristics were affected by habitat and environmental factors. Differences in elevation dictated that habitats for this species be divided into three positions: low, middle, and high areas. The higher the elevation, the lower the amount of available water, total nitrogen, phosphate, and organic matter. Correlations between biomass and environment were statistically significant. For example, plant density and biomass declined as elevation increased. In low areas, the lengths of aerial stems and leaves were 17.98±0.46 and 7.83±0.12 mm, respectively; values measured at high areas were 0.70 and 0.83 times smaller, respectively. Likewise, lengths of main roots in low areas were 8.06± 0.21 mm; in higher areas, roots were 1.58 times longer. Leaf widths in low areas were 1.88±0.01 mm, and 1.16 times greater at higher elevations. Values for these parameters in middle areas were intermediate between those for the other two. Nevertheless, when we used an MVSP computer program to conduct a similarity coefficient analysis, we found that the morphological variations recorded at the three elevations were not matched by differences in DNA patterns, although genetic variations were observed within individual populations.

Keywords: DNA pattern, environmental factors, morphological characteristics, *Suaeda maritima*, variation

Five different halophytes *Suaeda asparagoides, Suaeda japonica, Suaeda maritima, Suaeda australis,* and *Suaeda malacosperma* grow mainly in low and middle areas, e.g., tidal flats and sand dunes, along the southwestern coast of Korea (Kim, 1971a, b; Lee, 1980; Kim and Min, 1983; Min, 1985; Ihm, 1987; Shim et al., 2001). These halophytes show morphological and physiological variations (Lee and Oh, 1989) along an environmental gradient that are created not by genetic differences but by the plasticity of environmental changes (Shea et al., 1975; Gottlieb, 1977; Valiela et al., 1978; Show and Vince, 1984; Seliskar, 1985b) or by fixed ecotypes (Turesson, 1922a, b; McNaughton, 1966; Pazourkova, 1973).

Halophytes species vary according to the salinity and soil water content of their habitat (Stalter and Batson, 1969; Brereton, 1971; Shea et al., 1975; Smart and Barko, 1980; Jefferies et al., 1981; Ungar, 1987; Jonathan et al., 2000). Seliskar (1985a) has described five different kinds of halophytes in various environments and has found that these variations are not genetically fixed; rather, they are caused by environmental differences as a result of cross-transplantation tests, which may be useful in identifying local soil water contents (Seliskar, 1985b). Shea et al. (1975) and Valiela et al. (1978) have also argued that, by electrophoretically comparing enzymes and total soluble proteins from the tall and short types of *Spartina alterniflora*, one can delineate them not as ecological types but as ecophenes. Recently, RFLP and RAPD approaches have been used to determine genetic similarities in populations of varying intra- and inter-specific taxa. In particular, the RAPD method, which reveals the difference in DNA fragment lengths created by PCR amplification, is also useful for identifying the degree of variation in inter-specific taxa (Roy et al., 1992). In Korea, research on halophytes has focused on environmental factors that influence the distribution of plants in coastal salt marsh areas. Those factors include salinity (Kim, 1971a, b, 1975; Kim et al., 1975; Ihm, 1987), soil water content (Ihm and Lee, 1985; Min, 1985), soil water potential and the ability of plants to adjust osmotically (Ihm, 1989), and the duration and frequency of seawater submergence (Lee, 1990). However, research data has been insufficient regarding morphological variations based on the habitats characteristic of those coastal salt marshes.

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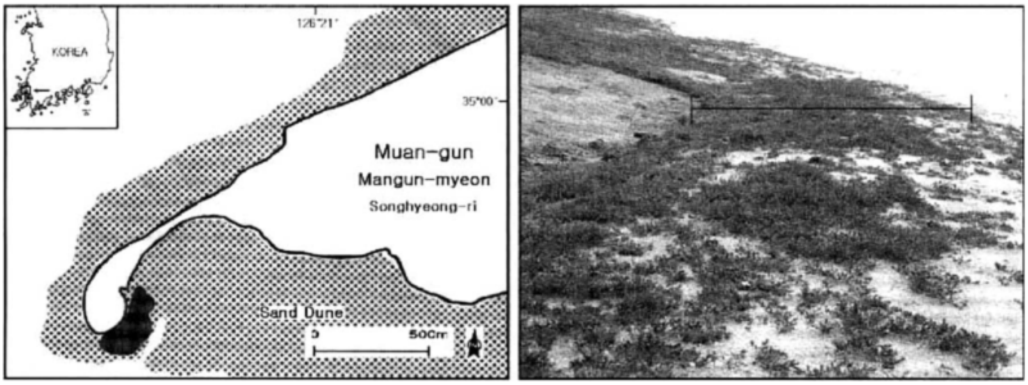


Figure 1. Maps of investigated area (dotted).

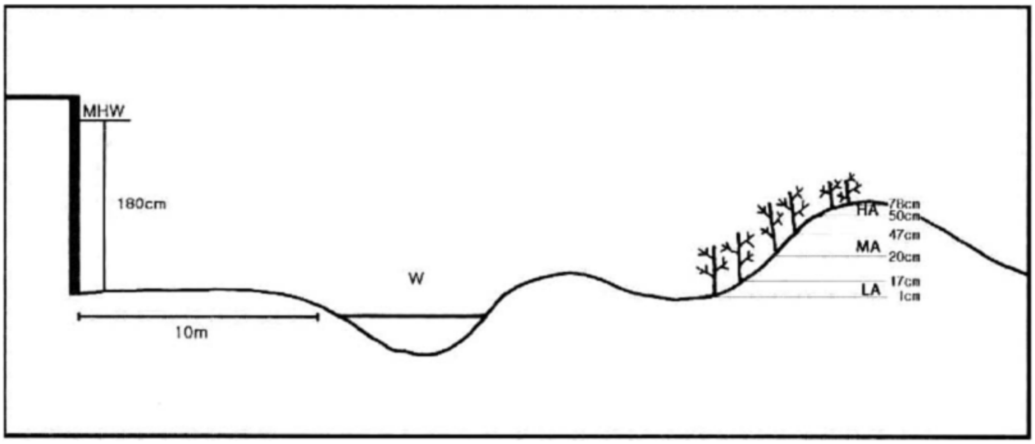


Figure 2. Profile of topography and vegetation in investigated areas. MHW indicates level of mean high water; W, saltwater; LA low areas; MA, middle areas and HA, high areas.

The objective of this study was to investigate the growth characteristics of halophytes such as *S. maritima* and to determine how their morphological and genetic variations are related to the elevation and slope of their habitats in the sand dunes of Muan-gun, Korea.

MATERIALS AND METHODS

Study Area

The study area was located on sand dunes at Jogeum-naru, Muan-gun, Korea (longitude 34°57N, latitude 126°17E), and was surrounded by a two-meter-high seawall spanning about 1 km (Fig. 1). Around the seawall was a dune measuring 400 m^2 and 45 m long, and shaped like a half-moon sandy plain. Areas in front of the seawall formed a mixed community of *Salsola komarovi*, *Aster tripolium*, *Atriplex gmelini*, *S. asparagoides*, *Chenopodium virgatum*, *Limonium tetragonum*, and *Zoysia sinica*. We divided the study area into three sections, according to elevation: the low areas, 1~18 cm, middle areas, 20~47 cm; and high areas, 50~78 cm from the bottom soil. The monthly submergence period for the middle areas averaged 2 h and 30 min. Inclination angle was approximately 37°, and a pure community of *S. maritima* covered 250-300 m^2 (Fig. 2)

Physico-Chemical Soil Analysis

Soil factors were analyzed by collecting four handsful of soil at each survey point. Soil water content was determined by placing 10 g of a fresh sample into a weighing bottle. After oven-drying for 48 h at 105°C, the sample was re-weighed, and water content was expressed as a percentage. The samples were then shadow-dried, crushed, and filtered through a 2 mm sieve. To measure its pH, electronic conductivity (E.C.), and salinity with an S.C.T. meter (YSI 63), we added 10 g of dried soil to distilled water in a 5 : 1

ratio (w/v), then shook the mixture for about 30 min, and filtered it through Whatman No. 44 paper. Organic matter content was determined by placing 10 g of shadow-dried soil into a jar and heating it for 4 h at 550°C in an electric furnace; the loss due to ignition was the calculated difference in weight. Available-phosphate (A-P) was measured according to the method of Bray (1948), and total nitrogen content was determined by the micro-Kjeldahl method (Jackson, 1967). Soil properties were defined with a particle size analyzer (SALD-301V, Shimadzu) by mixing and shaking 1 g of shadow-dried soil and 20 ml of distilled water for 4 h. Finally, UV-B and photosynthetic active radiation (PAR) were quantified with a quantum photo radiometer (Delta OHM) at each elevation.

Collection and Measurement of Plant Samples

S. maritima was evaluated according to its taxonomic literature, as compiled by Lee (1980, 1996) and Ohwi (1984). To distinguish morphological differences in plants as they related to specific elevations within the sandy plains of a particular dune, we installed 1 m x 1 m quadrates for random collection at each designated place in low, middle, and high areas (total of 30 units). Statistically significant parameters included: length, thickness, and width of leaves on stems branching from the middle of dwarf stalks, as well as the angle between the leaves and those stems. Leaf thicknesses and widths were measured with a micrometer after each leaf was cross-sectioned at the middle. Other parameters were: plant height above the ground; length of the main roots; number of twigs; number of seeds on short twigs; length, width and thickness of leaves. All surveyed characteristics were examined by ANOVA, and some individual samples were prepared for storage as specimens. To determine their biomass, the halophytes within each quadrate were collected, placed in polyethylene bags, and transported to the laboratory, where they were washed with tap water and oven-dried at 80°C to a constant weight. Population density in the quadrates was expressed in terms of the number of individuals per unit area.

RAPD Analysis

The plant leaves were collected from 3 quadrates (25 cm × 25 cm) on low, middle and high areas. The materials were rapidly delivered to the lab and the stored at −70°C before analysis. To extract genomic DNA, 100 mg of leaf tissue was crushed with two 50 μl grinding buffers plus 200 μl grinding buffer, 250 μl deionized distilled water (DDW) and 25 μl Proteinase K. After the reaction began, 40 μl of 10% CTAB and 100 μl of 5 M NaCl were added and the mixture was then centrifugally separated before approx. 500 μl of the upper liquid was used. This liquid was separated, using 24 : 1 chloroform : isoamylalcohol and PEG and then washed with 70% EtOH. Afterward, 200 μl DDW and 5 μl RNase A were added and the mixture was treated at 37°C for 30 min. Following the addition of 200 μl PCI and thorough mixing, the sample was centrifugally separated to remove the upper liquid before 400 μl 100% EtOH and 20 μl NaAc were added. After treatment at −70°C for 30 min, the mixture was centrifugally separated at 15,000 rpm for 10 min at 4°C and the sample was melted in 50 μl of TE buffer.

Basic PCR reaction conditions for DNA amplification were established by conducting tests on the density of the template DNA and determining the appropriate density for dNTP and Taq polymerase. The procedure involved mixing 30 ng of template DNA, 10 mM dNTP, 10 pM of random primer, 0.3 μl Taq polymerase (10 pM), 100 mM of 10× PCR buffer and 41.7 μl of DDW, bringing the total volume up to 50 μl. Two primers (OPA-02 and OPA-03) were selected from OPA and OPW kits (Operon, USA). PCR was performed with the GeneAmp PCR Systems 2400 (Applied Biosystems, USA). The DNA amplification comprised 45 cycles, each consisting of 5 min preheating at 94°C, 1 min at 94°C, 1 min annealing at 38°C and 1 min extension at 72°C, followed by 5 min of final extension at 72°C. Afterward, electrophoresis was conducted at 50 V for 45 min on a 1.2% agarose gel to which ethidium bromide had been added. The gel was viewed under a UV lamp.

RESULTS

Physico-Chemical Soil Characteristics

As the elevation decreased from high to low areas, soil water content, total nitrogen, available phosphate and organic matter content increased as a result of nutrient salts being deposited by incoming seawater (Table 1). This tidal action also increased the biomass and density of *S. maritima* plants. Although soil pH, electrical conductivity and salinity did not vary greatly by location, PAR and UV-B values

Table 1. Soil properties and environmental factors for *S. maritima* growing in different habitats in sand dunes of Jogeum-naru, Korea.

Habitat position	T-N (N/g)	A-P (/g)	PH	E.C. (us/ cm)	Salinity (ppt)	Organic matter (%)	Moisture content (%)	PAR (E/ S)	UV-B (W)	Elevation (cm)	Soil texture (%)		
											Clay	Silt	Sand
Low	323.3 ±13.3	46 ±2	7.04 ±0.02	861 ±2	0.5	3.27	9.58	0.269 ±0.005	0.314 ±0.002	18	0.25	0.35	99.4
Middle	133.3 ±16.7	31 ±3	7.22 ±0.01	886 ±3	0.5	1.05	6.18	0.281 ±0.007	0.321 ±0.001	47	0	0	100
High	58.3 ±8.7	28 ±1	7.31 ±0.01	819 ±2	0.4	0.56	3.29	0.345 ±0.005	0.333 ±0.002	78	0	0	100

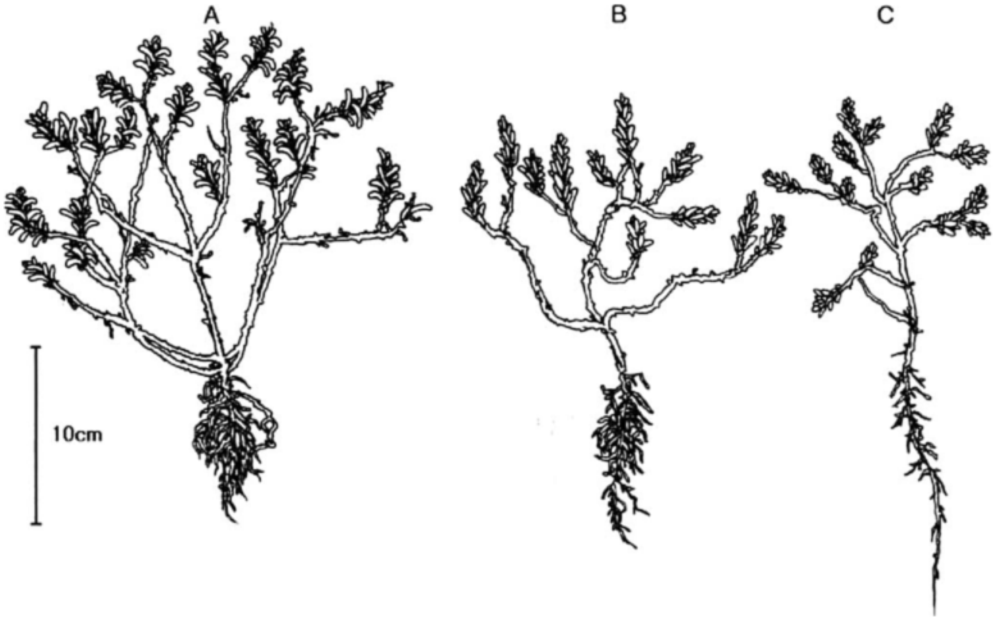


Figure 3. Population of *S. maritima* delineated in sand dune habitats: **A,** low areas; **B**, middle areas, and **C,** high areas.

increased with elevation. Sand content was 100% in the high and middle areas, while soils in the low area also contained 0.25% clay and 0.35% silt. As elevation increased, the proportion of coarse sand also rose by 50% of that recorded in the low area. We calculated that biomass was negatively correlated with UV-B and PAR but found positive correlations between plant biomass and environmental factors such as soil water content, organic matter, total nitrogen and available phosphate.

Morphological Variations

The ANOVA test showed statistically significant morphological differences among plants sampled in the 30 quadrate units. For example, as elevation increased, plant heights decreased by 0.78 to 0.70 times (cf. 17.98 ± 0.46 mm in low areas), while the main roots were lengthened by 1.15 to 1.58 times from a value of 8.06 ± 0.21 mm in low areas. In addi-

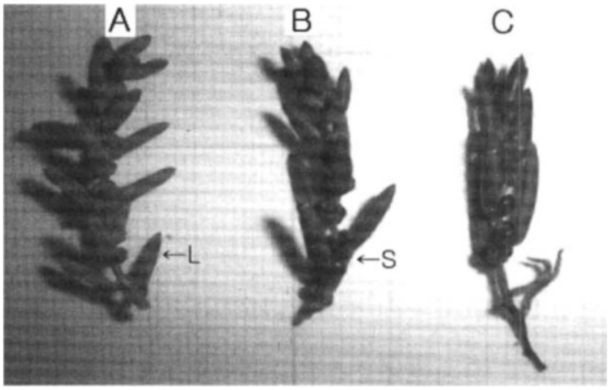


Figure 4. Leaves typicality of *S. maritima* in sand dune habitats. **A**, low areas; **B**, middle areas and **C**, high areas; One grid spans 1 mm; L, leaf and S, seed.

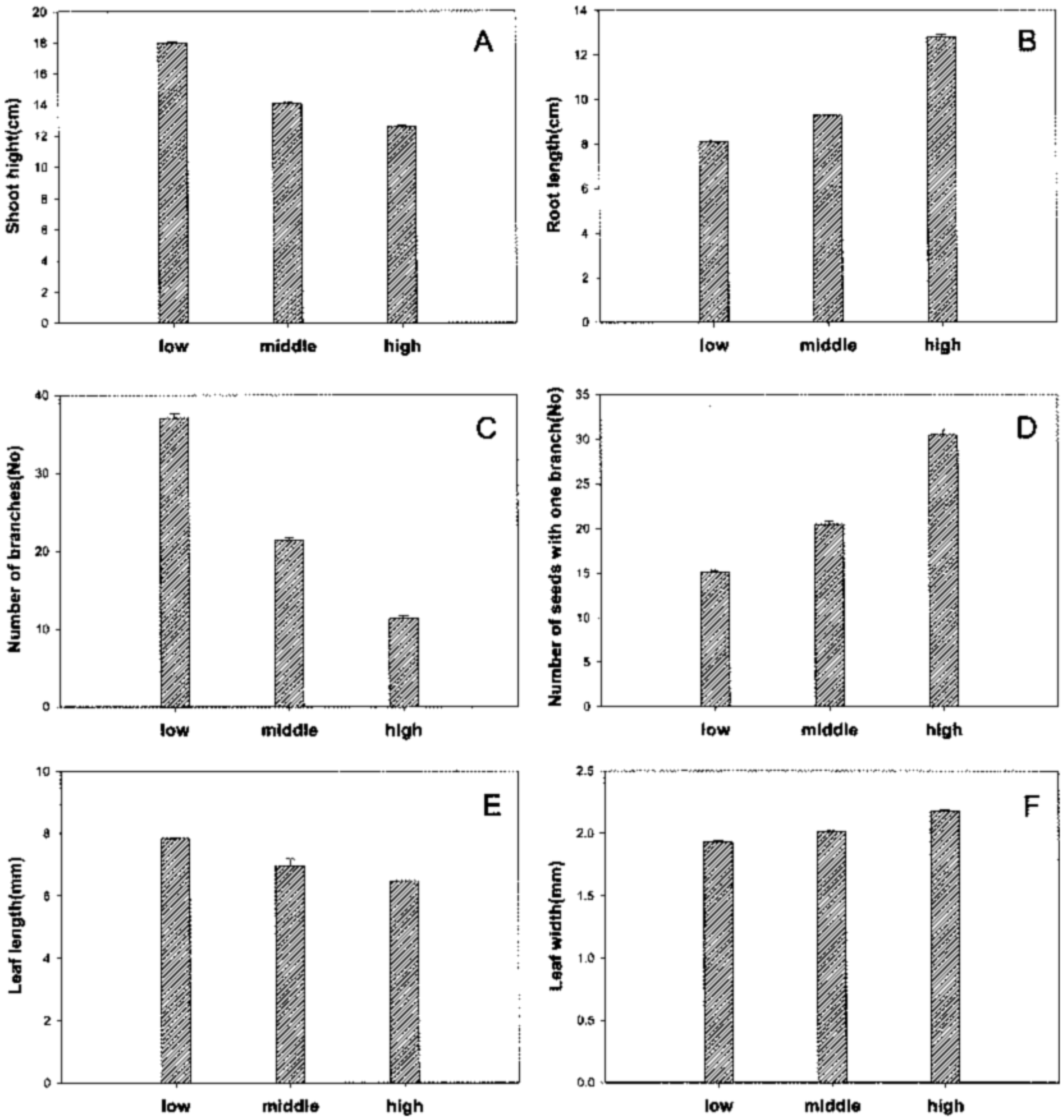
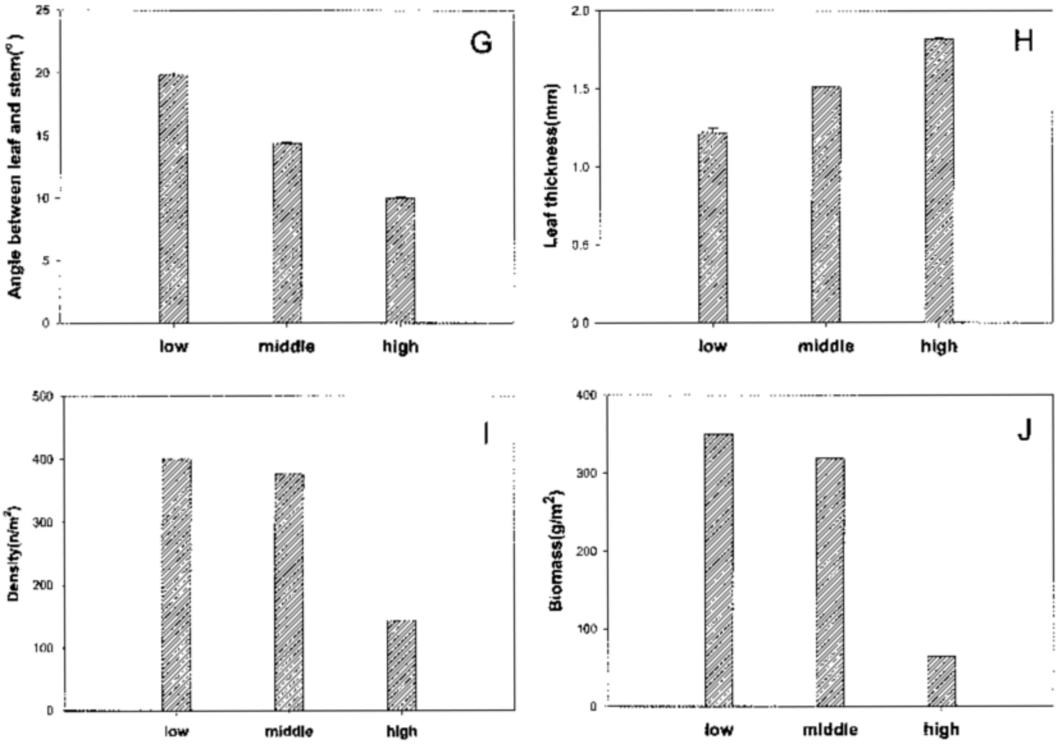


Figure 5. Comparison of *S. maritima* morphological characteristics among low, middle and high areas. **A**, shoot height; **B**, root length; **C**, number of branches; **D**, number of seeds with one branch; **E**, leaf length; **F**, leaf width; **G**, angle between leaf and stem; **H**, leaf thickness; **I**, density; **J**, biomass.

tion, the number of twigs on dwarf, third-order branches (37.1 in low areas) declined 0.58 to 0.31 times, while the number of seeds hanging on those short twigs (15 in low areas) increase 1.18 to 1.78 times (Fig. 3). Leaves that measured 7.83±0.12 mm long in low areas were 0.88 to 0.83 times shorter as elevation increased; their widths (1.88±0.01 mm in low areas) also increased 1.07 to 1.16 times; the tips of leaves with a filiform-linear shape on higher ground had more well-developed cortical spines; the angle between leaves and stems (19.8° in low areas) became 0.73 to 0.50 times smaller and leaf thickness (1.22 mm in low areas) increased 1.26 to 1.52 times (Fig. 4). Finally, plant density and biomass declined with increasing height above sea level (400 units/m^2, 376 units/m^2 and 144 units/m^2 in low, middle and high areas, respectively and 349.8 g/m^2, 319.0 g/m^2 and 63.6 g/m^2 corresponding biomass) (Fig. 5). As our determinant of elevational effects on plant morphology, we compared the ratio of leaf width to length as well as the ratio of leaf thickness to length. Each ratio declined significantly as elevation increased (Fig. 6).

Genetic Variations

We conducted RAPD analysis to survey the genetic



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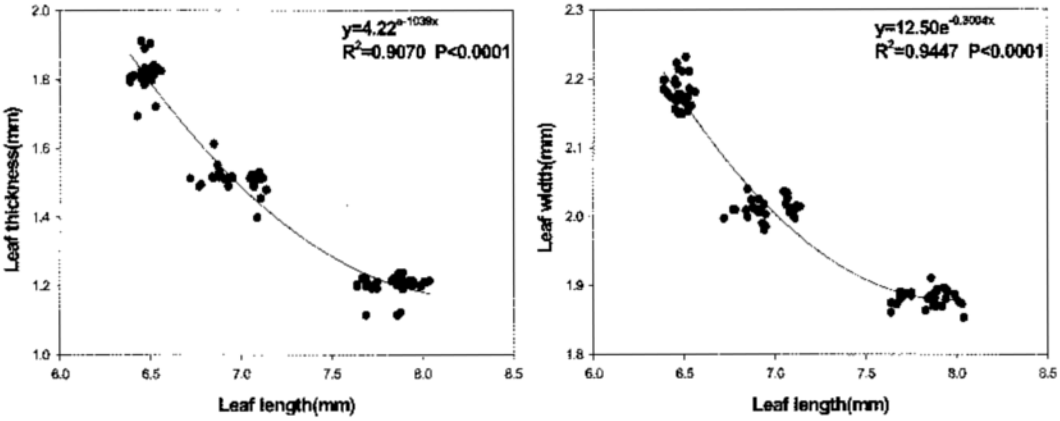

Figure 6. Relationships among leaf length, thickness and width.

variations between individual units of *S. maritima* as a function of elevation. Polymorphic bands were observed in the range of 340 to 1200 bp (Fig. 7). A comparison of their electrophoresis patterns confirmed four polymorphic and two monomorphic RAPD bands with the OPA-02 primer and five polymorphic and three monomorphic for OPA-03. The polymorphic bands were then subjected to similarity coefficient analysis using the MVSP computer program. This indicated that changes in elevation could not account for morphological variations; those results did not match the differences seen in DNA patterns (Fig. 8). Nevertheless, genetic variations were found within individual populations.

DISCUSSION

The genus *Suaeda* is widespread, occurring in two-phase communities dominated by annual species, *S.*

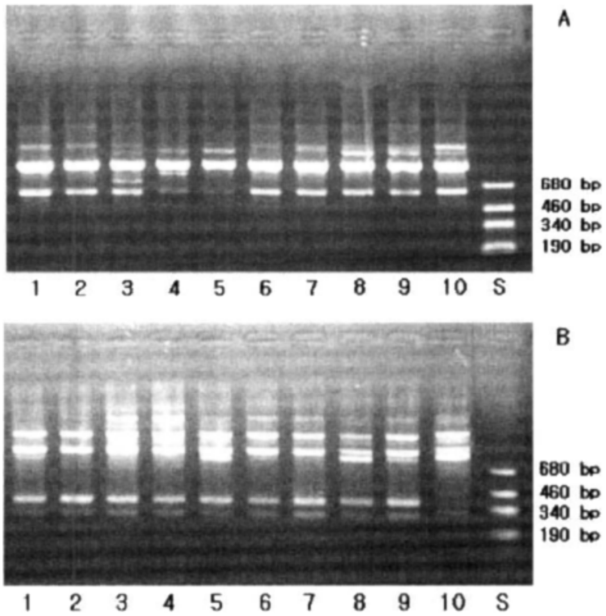

Figure 7. Random amplified polymorphic DNA patterns of *S. maritima* samples, using primers OPA-02 (**A**) and OPA-03 (**B**). Lane 1, high areas 1; Lane 2, high areas 2; Lane 3, high areas 3; Lane 4, middle areas 1; Lane 5, middle areas 2; Lane 6, middle areas 3; Lane 7, low areas 1; Lane 8, low areas 2; Lane 9, low areas 3; Lane 10, control. S, size marker.

maritima and *S. novae-zelandiae*, as well as communities dominated by perennial, shrubby species such as *S. fruticosa* and *S. californica* (Chapman, 1977). *S. maritima* is usually located on the coastal sand dunes of Korea, Australia, Malaysia, China, Taiwan, and Japan (Lee, 1996). Its growth pattern across the salt marsh zone (including low, middle and high areas) is mediated by differences in edaphic conditions, such as water content, elevation and PAR (Cooper 1982; Jonathan et al., 2000). In this study, we found that plant biomass was negatively correlated with PAR with regard to individual elevations, whereas water content, organic matter, total nitrogen and available phosphate were positively correlated with biomass. Those latter factors also caused morphologically significant differences according to elevation. In particular, water content, organic matter, total nitrogen and available phosphate declined at higher locations, while plant height and biomass declined, apparently due to the rise in UV-B values.

Because such dune habitats are associated with slopes, Hancock and Bringhurst (1978) have reported that when those slopes face northwest, leaves become broader and thinner in order to more effectively exploit the solar energy available in that shaded environment. Moreover, habitats especially in higher areas have coarse sand ratios as high as 50%, with a low capacity (i.e., 1.5 to 6.0%) for retaining soil moisture (Salisbury, 1942). Such conditions mean a greater incidence of water stress during the growing season. As a result, plants must produce longer main roots. In this study, we recorded that those roots were 1.58 times longer in the high areas compared with those in low areas. Likewise, above-ground plant portions must adapt to a more prostrate growth pattern to minimize losses from evaporation. Hsiao (1973) has also demonstrated that growth rates, stem and leaf elongation, and pore sizes are reduced under drought stress, a phenomenon we also recorded here with biomass in the low areas being 5.5 times greater than that of plants collected from the high areas.

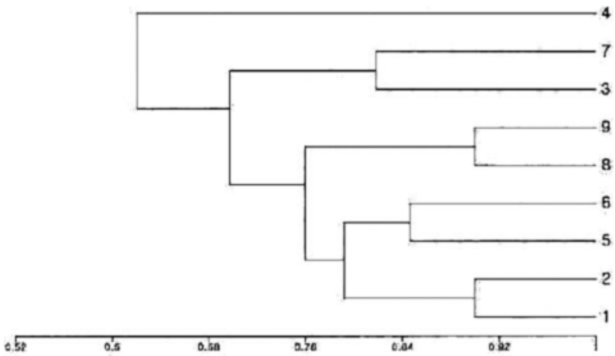

Figure 8. UPGMA dendrograms for samples from 9 *S. maritima* varieties, obtained by MVSP. **1**, high area 1; **2**, high area 2; **3**, high area 3; **4**, middle area 1; **5**, middle area 2; **6**, middle area 3; **7**, low area 1; **8**, low area 2; **9**, low area 3.

The angle measured between leaves and dwarf stems narrowed as elevation decreased, presumably due to the 37% slopes found with high areas. Because relative luminance was decreased in the low areas, the branching angle increased to accommodate any loss in photosynthetic capacity in individual units (Heley et al., 1992). These environmental conditions presumably affected plant height; size of the main roots; length, width, and thickness of leaves; and growth of other vegetative organs.

Although our study suggested that morphological variations did not match DNA patterns according to elevation, genetic variations did exist within populations. This may have been caused by frequent movement of seeds by the seawater and, subsequently, random establishment of seedlings. The influence of morphological variation on genetic variation is still unclear (Zhang et al., 2004). Therefore, we recommend further examination of the environmental gradients and cross-transplantation common to *Suaeda*

Table 2. Correlation coefficients among soil properties and environmental factors in *S. maritima* community.

	pH	Organic matter	Moisture content	E.C.	Salinity	A-P	T-N	Elevation	Biomass	PAR	UV-B
pH	1										
Organic matter	-0.987*	1									
Moisture content	-0.990**	0.953*	1								
E.C.	-0.461	0.311	0.583	1							
Salinity	-0.756	0.64	0.842	0.929	1						
A-P	-0.984*	1.000**	0.949	0.297	0.629	1					
T-N	-0.998*	0.994*	0.980*	0.411	0.719	0.993**	1				
Elevation	0.974*	-0.925	-0.996**	-0.649	-0.884	-0.919	-0.960*	1			
Biomass	-0.816	0.712	0.891	0.889	0.995**	0.702	0.783	-0.926	1		
PAR	0.844	-0.746	-0.912	-0.865	-0.989*	-0. 736	-0.813	0.943	-0.999**	1	
UV-B	0.942	-0.876	-0.981*	-0.731	-0.931	-0.869	-0.923	0.994**	-0.963*	0.975*	1

*<0.05, **<0.01.

maritima in order to better understand the relationship between morphological and genetic variability in that species.

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LITERATURE CITED

Bray RH (1948) Correlation of soil tests with crop responses to added fertilizer requirements: Diagnostic technique for soils and crops. The American Potash Institute, Washington DC, pp 53-86

Brereton AJ (1971) The structure of the species population in the initial stages of salt-marsh succession. J Ecol 59: 321-338

Chapman VJ (1977) Wet Coastal Ecosystems. Elsevier Scientific Publishing Company, New York, pp 1-29

Cooper AC (1982) The effects of standing water and drainage potential on the *Spartina alterniflora*-substrate complex in a North Carolina marsh. Estu Coast Mar Sci 11: 41-52

Gottlieb LD (1977) Genotypic similarity of large and small individuals in a natural population of the annual plant *Stephanomeria exigua ssp. coronaria* (Compositae). J Ecol 65: 127-134

Hancock JK, Jr, Bringhurst RS (1978) Inter-population differentiation and adaptation in the perennial diploid species *Fragaria vesca* L. Amer J Bot 65: 795-803

Heley WJ, Lindley ST, Levavasseur G, Osmond CB, Rasmus L (1992) Photosynthetic response of *Ulvarotundata* to light and temperature during emersion in an intertidal sand flat. Oecologia 89: 519-523

Hsiao TC (1973) Plant responses to water stress. Annu Rev Plant Physiol 24: 519-570

Ihm BS (1987) The distribution and growth of halophytes at the coastal marsh. Bull Inst Litt Biot 4: 71-79

Ihm BS (1989) Distribution of coastal plant communities in response to soil water potential and plant osmotic adjustment. Ph. D. thesis. Seoul National University, Seoul

Ihm BS, Lee JS (1985) Effects of salinity on growth of plants. Bull Inst Litt Biot 2: 33-40

Jackson ML (1967) Soil Chemical Analysis. Prentice-Hall, New Delhi, pp 227-271

Jefferies RL, Davy AJ, Rudmik T (1981) Population biology of the salt marsh annual *Salicornia europaea* agg. J Ecol 69: 17-31

Jonathan MH, Jacqueline AP, Rob HM (2000) Influence of environmental factors on the growth and interactions between salt marsh plants: Effects of salinity, sediment and waterlogging. J Ecol 88: 492-505

Kim CS (1971a) An ecological study on the process of plant community formation in tidal land. Kor J Bot 14: 163-169

Kim CS (1971b) An ecological study on the plant community formation in tidal land. Kor J Bot 18: 129-134

Kim CS (1975) A study on standing crops in *Phragmites communis* communities and their environmental factors. Kor J Bot 18: 129-134

Kim JH, Min BM (1983) Ecological studies on the halophyte communities at western and southern coasts in Korea (III). Kor J Bot 26: 53-59

Lee CB (1980) Illustrated Flora of Korea, p 318

Lee JS (1990) On the establishment of halophytes along tidal level gradients at salt marshes of the Mankyong and Dongjin River estuaries. Ph. D. thesis. Seoul

National University, Seoul

Lee KS, Oh GC (1989) Differences of *Suaeda japonica* populations from two different habitats in Sorae, Incheon, Korea. Kor J Ecol 12: 133-144

Lee WC (1996) Colored Standard of Korean Plants, p 624

Lee YN (1997) Flora of Korea, p 1237

McNaughton SJ (1966) Ecotype function in the *Typha* community type. Ecol Monogr 36: 297-325

Min BM (1985) Changes of soil and vegetation in reclaimed coastal lands, west coast of Korea. Ph. D. thesis. Seoul National University, Seoul

Ohwi J (1984) Flora of Japan, p 1066

Pazourkova Z (1973) Caryology of some forms of *Phragmites communis* Trin. *In* S Hejny, ed, Ecosystem study on wetland biomes in Czechoslovakia. Czechosl IBP/PT-PP Rep No 3. Trebon, pp 147-149

Roy A, Frascaria J, Mackay J, Bousquet J (1992) Segregation random amplified polymorphics (RAPDs) in *Betula alleghaniensis*. Theor Appl Genet 85: 173-180

Salisbury EJ (1942) The weed problem. Proc Royal Inst Gr Brit 31: 1-15

Seliskar DM (1985a) Morphometric variations of five tidal marsh halophytes along environmental gradients. Amer J Bot 72: 1340-1352

Seliskar DM (1985b) Effects of reciprocal transplanting between extremes of plant zones on the morphometric placity of five plant species in an Oregon salt marsh. Can J Bot 63: 2254-2262

Shea ML, Warren RC, Niering WA (1975) Biochemical and transplantation studies of the growth form of *Spartina alterniflora* in Connecticut salt marshes. Ecol 56: 461-466

Shim HB, Jung JY, Choi BH (2001) A taxonomic study on *Lilium* plants in Korea based on cluster analysis data. Kor J Plant Taxon 31: 381-386

Show AA, Vince SW (1984) Plant zonation in an Alaskan salt marsh. An experimental study of the role of edaphic conditions. J Ecol 72: 669-684

Smart RM, Barko JW (1980) Nitrogen nutrition and salinity tolerance of *Distichlis spicata* and *Spartina alterniflora*. Ecol 61: 630-638

Stalter R, Batson WT (1969) Transplantation of salt marsh vegetation, Georgetown, South Carolina. Ecol 50: 1037-1089

Turesson G (1922a) The species and variety as ecological units. Hereditas 3: 100-113

Turesson G (1922b) The genotypical responses of the plant. Hereditas 3: 211-350

Ungar IA (1987) Population characteristics, growth and survival of the halophyte *Salicornia europaea*. Ecol 68: 569-575

Valiela I, Teal JM, Deuser WG (1978) The nature of growth forms in the salt marsh grass *Spartina alterniflora*. Amer Nat 112: 461-470

Zhang S, Renquing W, Xinshan Q, Weihua G and Baimin S (2004) Morphological and RAPD variation of *Phragmites australis* along salinity gradient in the wetlands of the downstream of Yellow River, China. Kor J Ecol 27: 35-41