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Effects of the Loess Coating on Seed Germination and Seedling Growths of the Eelgrass, *Zostera marina*

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Seagrass bed is an important component in coastal and estuarine ecosystems, providing food and habitats to a wide variety of marine organisms. Recently, seagrass coverage has declined significantly due to anthropogenic impacts such as cultural eutrophication and reclamation, and thus efforts are under way to prevent further losses and restore disturbed seagrass habitats worldwide. Seagrass transplantation techniques for habitat restoration include vegetative and seed-based methods. Seagrass seeds can be collected easily, and sowing seeds is an economically effective method for large-scale restoration. However, large numbers of seed can be lost by seed predation and physical disturbance in the planting areas. In the present study, *Zostera marina* seeds were coated with loess to reduce seed loss by predation and sweeping away by the water currents, and germination rates of coated seeds and seedling growth were examined to assess the feasibility of the seed-coating method for large-scale restoration. Germination rate of the coated seeds with loess was significantly higher than that of the uncoated seeds. Additionally, seedling growths were not significantly different between the coated and the uncoated seeds. These results suggest that coating of eelgrass seeds with loess enhances success of seed germination with no harmful effects on seedling growth. Therefore, the seed coating method using loess may be an effective and applicable seed-based transplanting technique for large-scale restoration.

Key Words: seed-coating, eelgrass, restoration, loess, transplanting method, *Zostera marina*

INTRODUCTION

Seagrasses are an important component of estuarine and coastal ecosystems, and are among the most productive plant communities (McRoy and McMillan 1977), providing habitat and food for a wide variety of marine organisms (Heck and Westone 1977; Orth *et al.* 1984; Summerson and Peterson 1984; Huh and Kitting 1985). However, significant losses of seagrass coverage due to natural and anthropogenic impacts have been reported from many regions of the world (Pulich and White 1991; Gorden *et al.* 1996; Short and Wyllie-Echeverria 1996). Because of the essential roles of seagrass habitat in estuarine and coastal ecosystems, numerous projects for seagrass habitat restoration have been attempted worldwide (Fonseca *et al.* 1994, 1998; Short *et al.* 2002; Seddon 2004).

Seagrass transplanting techniques include vegetative transplanting methods and seed-based methods (Calumpong and Fonseca 2001; Seddon 2004; Pickerell *et al.* 2005). The most frequently used seagrass transplanting method to date is planting adult shoots in disturbed areas (Davis and Short 1997; Fonseca *et al.* 1998; Fishman *et al.* 2004). However, this technique has been proven to be labor intensive and expensive (Fonseca *et al.* 1994; Davis and Short 1997). Additionally, collecting adult plants may have significant harmful effects on the donor bed (Bird *et al.* 1994; Orth *et al.* 2006). Consequently, there has been growing interesting the use of seagrass seeds as an alternative method of seagrass habitat restoration. The major advantage of seed-based methods is that once suitable numbers of seeds have been collected, they can be sown quickly and easily over large areas. However, because currents and bioturbation can transport seeds to unsuitable sites, there is no guarantee that they will germinate where they are sown; thus, it is difficult to create a seagrass bed in a specific location (Moore *et al.* 1993; Davis and Short 1997). In addition, a large proportion of seagrass seeds may be lost to predation and disease (Wigand and Churchill 1988; Fishman and Orth 1996; Inglis 2000; Nakaoka 2002), and only a small portion of the seeds in sediments usually develops successfully into adult shoots. Bacteria and fungi are likely prime suspects in causing the death of some seeds (Kremer 1993), but

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*Corresponding author (klee@pusan.ac.kr) few studies have addressed this problem on seagrasses. Some fungi can damage seeds by the production of enzymes that destroy specific compounds in seeds and toxins which can cause the inhibition of germination and breakdown of membranes (Baskin and Baskin 2001).

Because of its antibacterial and detoxification property, and strong absorption ability, loess has been widely used in livestock feed, medicine, natural dying, soil conditioner, and removal of harmful algal blooms (Cho *et al.* 2000; Kim and Kang 2000; Kim 2003; Jung *et al.* 2004). Therefore, we expected that seed coating using loess can reduce seed mortality through decreasing fungal infection and physical protection from predation, and consequently lead to increasing success of seed germination. Additionally, coated seeds will be less drifted by the water currents because they have more weight. In the present study, we compared success of germination and seedling growth between coated and uncoated eelgrass seeds with loess to assess the feasibility of the seed-coating method for large scale eelgrass habitat restoration.

MATERIALS AND METHODS

Seed collection and coating with loess

Seed-bearing shoots of eelgrass were collected from Jindong Bay (35°06’N, 128°32’E; Fig. 1) and Koje Bay (34°45’N, 128°30’E; Fig. 1) during July 2005 and were stored in flowing seawater aquariums for two months. The seeds were manually collected from the debris in the aquariums, and then were kept in seawater of 4°C in a dark chamber (Granger *et al.* 2002).

The loess, which was collected at Kijang, Busan in September 2003, was dried at room temperature for months and grounded using mortars and pestles. Eelgrass seeds were coated using mixture of loess powder ($\leq 250\ \mu$m) and distilled water (1:9 w / w) just before sowing the seeds in the planting site (Lee and Park 2006).

Seed planting and assessment of seedling growth

Seed planting site was located in a bare area in Koje Bay (34°45’N, 128°38’E) on the southern coast of Korea (Fig. 1). This area originally had large seagrass meadows, but significant seagrass declines have occurred due to human activities such as seawall and port construction. The mean water depth at the planting site was about 3 m relative to the mean sea level (MSL). Sediments at the site consisted of 84.9% sand, 14.9% silt and 0.2% clay (Park *et al.* 2005). No naturally dispersed eelgrass seeds were found in the sediment of the planting site during the experiment.

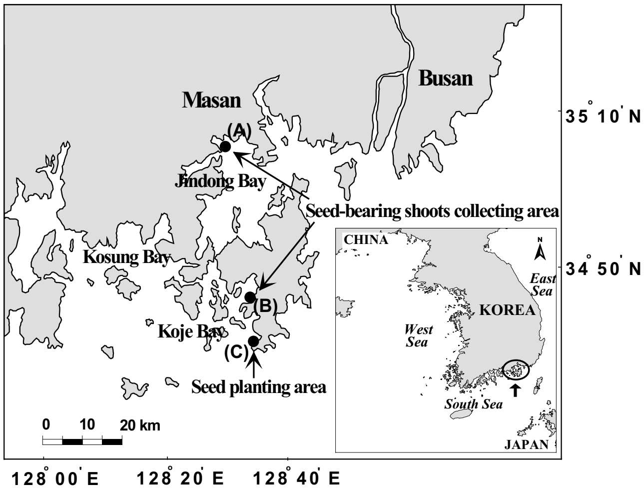

Fig. 1. Study sites in Koje Bay and Jindong Bay on the southern coast of Korea. Seed-bearing shoots of eelgrass were collected at Jindong Bay (A) and Koje Bay (B) in July 2005, and collected seeds were planted at Koje Bay (C) in November 2005.

In each planting plot (1 m × 1 m), 200 coated or uncoated seeds were sown by hand using SCUBA in November 2005. Each of coating and un-coating (control) treatments had six replicated planting plots. Shoot density, shoot height, leaf numbers, and leaf width of the eelgrass seedlings in each plot were monitored from December 2005 to March 2006 monthly.

Statistics

All values are reported as means ± 1SE. Statistical analyses were performed on a microcomputer using a general linear model procedure (SAS). Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics. Differences in seedling density, shoot height, leaf number, and leaf width between coated and uncoated seed planting plots and among sampling time were tested for significance using a two-way ANOVA. When a significant difference between variables was observed, the means were analyzed using a Tukey multiple comparison test to determine where the significant differences occurred between variables.

RESULTS

Coated eelgrass seeds with loess

Coated eelgrass seeds with loess became larger in size and much heavier than uncoated eelgrass seeds (Fig. 2). The average diameter and length of the coated eelgrass

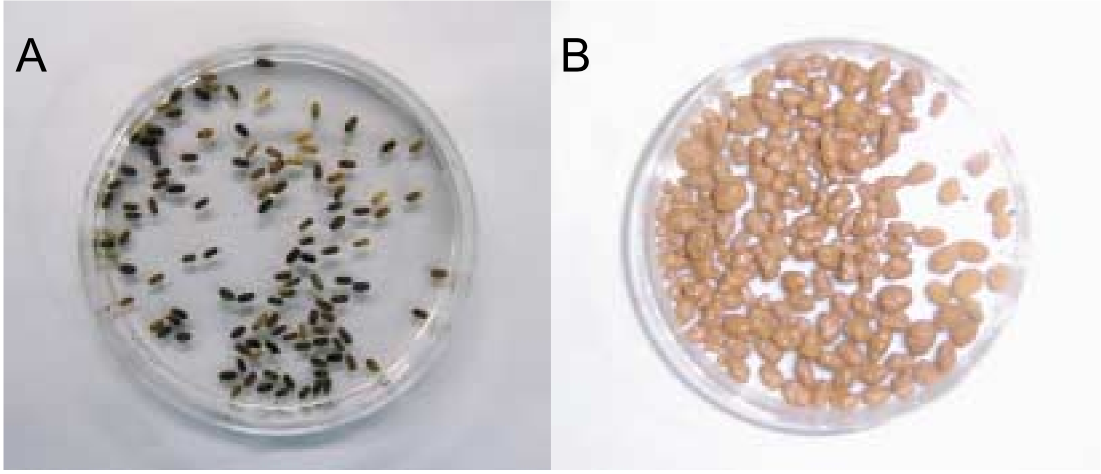

Fig. 2. Uncoated eelgrass seeds (A) and coated eelgrass seeds with the loess (B).

Table 1. Summary of ANOVA results for shoot density, shoot height, leaf width and leaf numbers of the *Zostera marina* seedlings at the coated and uncoated seed plots during December 2005 to March 2006

Variable	Source	SS	df	MS	F-ratio	P value
Shoot density	Seed treatment	776.0208	1	776.0208	30.07	< 0.0001
	Time	278.0625	3	92.6875	3.59	0.0217
	Treatment*Time	66.0625	3	22.0208	0.85	0.4731
Shoot height	Seed treatment	13.3723	1	13.3723	1.78	0.1849
	Time	1866.0123	3	622.0041	82.56	< 0.0001
	Treatment*Time	10.1991	3	3.3997	0.45	0.7168
Leaf width	Seed treatment	0.0066	1	0.0066	0.03	0.8614
	Time	1.7198	3	0.5733	2.66	0.0504
	Treatment*Time	0.5578	3	0.1859	0.86	0.4618
Leaf numbers	Seed treatment	0.1256	1	0.1256	0.48	0.4875
	Time	11.4454	3	3.8151	14.72	< 0.0001
	Treatment*Time	0.2031	3	0.0677	0.26	0.8533

seeds were about 4 mm and 5 mm, respectively, whereas, those of the uncoated seeds were 1-1.5 mm and 3-4 mm, respectively. Weight of the coated seeds was about 80 mg per seed, while uncoated seeds weighed about 10 mg per seed.

Seedling density and growth

Seedling density during the study period was significantly higher ($P < 0.001$) in the plots sown with coated-seeds than plots with uncoated seeds (Fig. 3, Table 1). Seedling density at the coated-seed plots (18.7 ± 3.6 shoots m^{-2}) was about twice that of uncoated-seed plots (9.2 ± 1.4 shoots m^{-2}) in March 2006. However, shoot height, leaf width, and leaf numbers of the seedlings were not significantly different during the study period ($P = 0.18$, $P = 0.86$, and $P = 0.49$) between coated and uncoated seed plots (Fig. 4, Table 1). Mean shoot height, leaf width, and leaf numbers of the seedlings in both

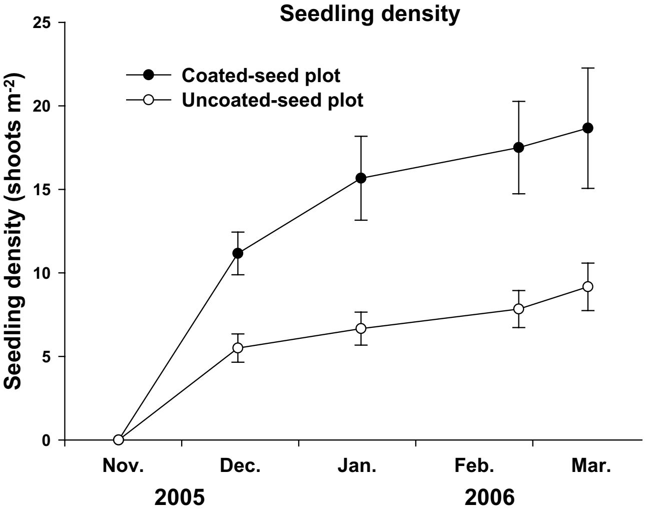

Fig. 3. Seedling density of *Zostera marina* at the planting plots with coated and uncoated seeds in Koje Bay from November 2005 to March 2006.

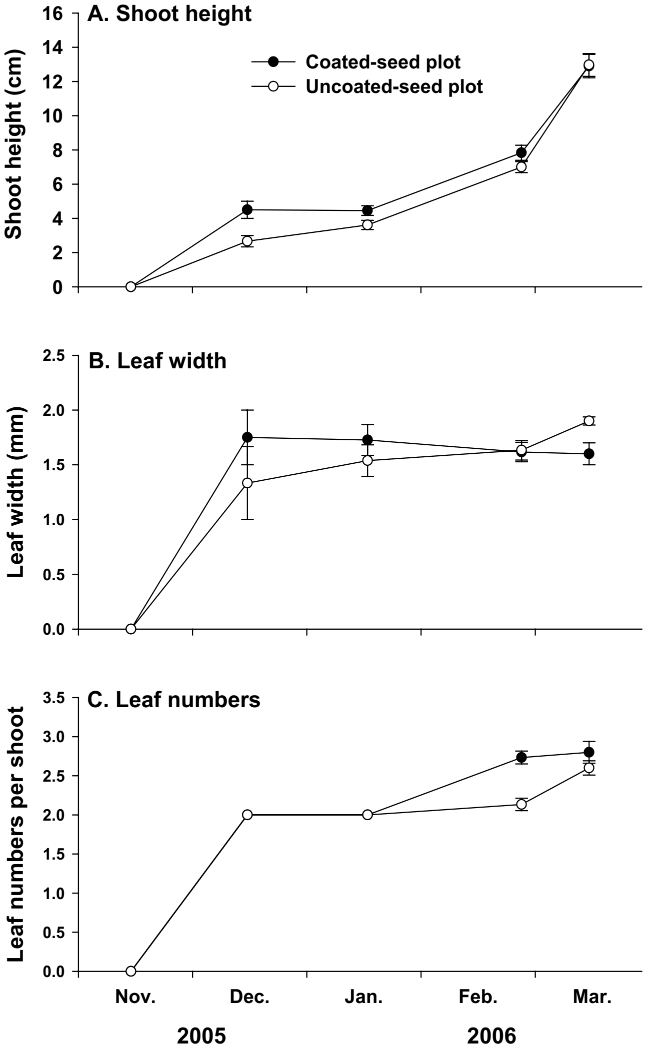

Fig. 4. Temporal changes in shoot height (A), leaf width (B), and leaf numbers per shoot (C) of the seedlings of *Zostera marina* at the planting plots from November 2005 to March 2006.

coated and uncoated-seed plots were about 13 cm, 1.5-2.0 mm, and 2.5-3.0 leaves shoot^{-1}, respectively, in March 2006 (Fig. 4).

DISCUSSION

More eelgrass seedlings established successfully through germination of coated seeds with loess than uncoated seeds. About 9% of coated seeds sown in the planting plots successfully germinated, whereas about 5% of uncoated seeds germinated in the planting plots. Seed predation is a significant factor causing seed loss in the eelgrass beds (Fishman and Orth 1996; Harwell and Orth 1999; Nakaoka 2002; Orth *et al.* 2003). Seeds are rich in nutritional component such as starch, protein, fat, carbohydrate and crude fiber, and thus they are more digestible than other seagrass parts such as leaves, rhizomes and roots (Montaňo *et al.* 1999; Nakaoka 2002). Some predators consumed up to 65% of the eelgrass seeds in sediments (Fishman and Orth 1996); thus seed predation has a considerable negative impact on seed abundance in sediments of eelgrass beds (Wigand and Churchill 1988; Fishman and Orth 1996; Kaldy and Dunton 1999; Holbrook *et al.* 2000; Nakaoka 2002). Abundant distribution of the decapod crustaceans, which are eelgrass seed predators, has been reported in eelgrass beds on the coasts of Korea (Han *et al.* 1995; Fishman and Orth 1996; Huh and An 1997; Nakaoka 2002; Kim *et al.* 2005). In the present study, therefore, seed coating with loess probably prevented, at least partially, seed predation through physical protection. Lower seed predation in the plots planted with coated seeds than uncoated seeds was reflected in higher seedling density in the planting plots with coated seeds.

Transport of seeds from the parental beds to unsuitable sites for germination may be one of primary factors to limit seedling recruitment (Orth *et al.* 1994; Seddon 2004). Recent work found that *Z. marina* seeds could be transported dozens of kilometers from the parental beds by the water current (Reusch 2002; Harwell and Orth 2002). A large proportion of eelgrass seeds sown in planting plots may be lost to transport to unsuitable areas. A coated seed with loess was about 8 times heavier than that of uncoated seed. The heavier coated seeds will be less drifted by the water currents, and consequently more seeds will remain and germinate in the planting plots with coated seeds than uncoated seeds.

Additionally, anaerobic conditions can stimulate germination of eelgrass seeds (Moore *et al.* 1993; Brenchley and Probert 1998; Bradley and Stolt 2006). The planting plots in the present study were characterized by a high sand content in the sediment, in which the sediment is more oxygenated than in muddy sediment sites. Seed coating using loess may allow the seeds to locate in more anaerobic conditions, and this reducing condition may enhance germination of coated seeds compared to the uncoated seeds. Antibacterial and detoxification property of loess may reduce seed mortality, which can cause by bacterial or fungal infection, and consequently also led to increasing seedling recruitment rates in planting plots with coated seeds.

In the present study, seedling establishment rates were relatively low compared to the previous seed-based transplanting experiments of Orth *et al.* (1983, 1994, 2003), Harwell and Orth (1999), Granger *et al.* (2002), and Pickerell *et al.* (2005). Low seedling establishment in this study may be caused by the sediment characteristics of the planting plots. The planting plots of this study mainly consisted of sandy sediment, in which the rates of seed germination and seedling establishment are usually lower than in muddy sediment (Handley and Davy 2002; Bradley and Stolt 2006). Muddy sediments tend to have higher levels of anoxia to stimulate germination of seeds, and allow rhizome and roots expansion into the sediments, while sandy sediments tend to diffuse oxygen more quickly, and obstruct rhizome expansion and root penetration into sediments (Short 1987; Moore *et al.* 1993; Fonseca *et al.* 1998; van Katwijk and Wijgergangs 2004; Park *et al.* 2005).

Growth of the seedlings recruited through germination of coated seeds was not significantly different with that of uncoated seeds. This result implies that seed coating with loess has no negative impacts on eelgrass seedling growths after germination. In conclusion, coating of eelgrass seeds with loess enhances success of seed germination with no harmful effects on seedling growth. Therefore, the seed coating method using loess may be an effective and applicable seed-based transplanting technique for large-scale restoration.

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