JJBS

Volume 14, Number 1, March 2021
ISSN 1995-6673
Pages 111 – 119
https://doi.org/10.54319/jjbs/140115

Jordan Journal of Biological Sciences

Genetic diversity of seagrass *Thalassia hemprichii* and *Enhalus acoroides* in coastal area of East Java

Hery Purnobasuki*, Sucipto Hariyanto and Putut Rakhmad Purnama

Biology Department, Faculty of Sciences and Technology, Airlangga UniversitySurabaya – INDONESIA

Received Mar 13, 2020; Revised July 19, 2020; Accepted July 25, 2020

Abstract

Seagrass degradation occurring worldwide in the last few decades led scientists and governments to take a role in saving this ecosystem. Seagrass transplantation is a direct action to increase seagrass density or recover damaged seagrass habitats. Regrettably, transplantation does not guarantee the success of coastal restoration. The transplants were not resistant against environmental stress at its new habitats. One factor that should be considered when sourcing plant donor materials is genetic diversity. In this paper, we assessed a donor site, Labuhan Beach, where the seagrass species of *Thalassia hemprichii* and *Enhalus acoroides* occur. RAPD (Random Amplified Polymorphism DNA) was performed to discover polymorphic fragments in order to interprete variation genetic of these species. Genetic diversity of the donor population was compared with the same species inhabiting an undisturbed conservation area (Baluran National Park). The data revealed that genetic diversity (allelic richness) of *T. hemprichii* of Labuhan population (0.19) was higher than the population in Baluran (0.15). Equally, for *E. acoroides* genetic diversity of the Labuhan population (0.21) was higher than at Baluran (0.16; t-test p=0.037). A phylogenetic analysis of the MatK region verified that the seagrass samples taken from Labuhan Beach were indeed *T. hemprichii* and *E. acoroides* based on identity with sequences in the GenBank database. This finding suggested that the seagrass population in Labuhan could be used as donor material; however, geographic distance and differing environmental conditions must also be considered.

Keywords: polymorphic, seagrass, transplantation, RAPD, MatK, Labuhan, Baluran.

1. Introduction

Seagrasses are seashore vegetation inhabiting in marine environment area on a wide-ranged substrate content of mud, sand, clay, gravel, or a mixture of them. In general, seagrass is known to have an essential role in aspects of coastal biodiversity, economic function, and the provision of environmental services for coastal ecosystems (Spalding *et al*., 2003). Ecosystem formed by seagrasses is as a habitat provider for coastal organisms, reducing waves of sea, stabilizing sediments, introducing oxygen into water bodies, providing sources of nitrogen, phosphorus in the food chain, also contributing to the supply of organic carbon in significant quantities (Beck *et al*., 2001; Duffy, 2006).

Unfortunately, seagrass habitat is one of the unfavorable habitats on this global ecosystem (Waycott *et al*., 2009; Short *et al*., 2011). The rate of decline in seagrass beds has been estimated at 7% per year since 1990, an alarming rate compared to coral reefs, mangroves, and tropical rain forests (Waycott *et al*., 2009). Environmental changes in Indonesia impact to wide degradation of seagrass, especially due to human activities disturbances such as building development, tourism activity, polluted runoff and mining (Riani *et al*., 2012). One of the efforts to conserve seagrass habitat is seagrass transplantation and restoration. This method has been tried in various types of seagrasses and planted at multiple depths (van Katwijk *et al*. 2009). Many environmental projects and various improvement schemes have been conducted as mitigation effort for seagrass losses. In Indonesia, restoration programs for seagrass have been developed (Riani *et al*., 2012). Seagrass transplantation has been attempted in species *Enhalus acoroides* at Ambon (Irawan, 2017) and Bintan (Harnianti *et al*., 2017) as well as *Thalassia hemprichii* at Jepara (Wulandari *et al*., 2013) and Bintan (Seprianti *et al*., 2017). To improve this condition, therefore, projects of transplantation have been conducted successfully just only for few seagrass species and, at some locations, have no success to outstandingly recover seagrass meadows (Paling *et al*., 2009). Stress associated with transplantation such as transplant shock and environmental changes induced a loss of shoot in the early stage of the transplantation period (Hughes and Stachowicz, 2004). Hence, seagrass transplantion must have the good opportunity and adaptation capability to face the changing conditions in the future (Kettenring *et al*., 2014).

* Corresponding author e-mail: hery-p@fst.unair.ac.id.

** **Abbreviation:** N: Number of amplification band, P: Number of polymorphic band, %: Percentage of polymorphic band, Na: Number of Observed Allele, Ne: Number of Effective Allele, Nm: Gene Flow, Gst: Genetic differentiation between Population, H: Heterozygosity / Ne'i Genetic diversity, Ht: Total population heterozygosity, Hs: Value of Heterozygosity in the Population, I: Shannon's Information index.

Genetic diversity is an essential aspect of ensuring the resilience of transplants in adapting to environmental changes. Genetic evaluation and genotypes identification of population variations have important role to genetic protection and plant breeding programs (Tahir *et al*, 2018). Procaccini and Piazzi (2001) explained that the increase in heterozygosity in donor plant populations was positively correlated with the success of *Posidonia oceanica* seagrass transplants. Furthermore, the similarity in the level of genetic diversity is also significantly associated with the growth and reproduction rate of seagrass *Posidonia australis* and *Zostera marina* (Williams, 2001; Sinclair *et al*., 2013).

T. hemprichii and *E. acoroides* seagrasses are described from Indonesia coast (Purnama *et al*., 2019). *T. hemprichii* is a dominant species for meadow-forming on sediments associated with coral reefs. This species is recognized by ribbon-like and curved leaves, up to 40 cm long often with tannin cells that prominent look red, purple or dark brown. The stem is short and erect, bearing 2-6 leaves. The rhizome is thick and covered with triangular-shaped leaf scars (Waycott *et al*., 2004; El Shaffai, 2011). While *E. acoroides* are considered to be the most extensive tropical seagrass species, leaf lamina is like a ribbon and has a length of up to 200 cm and a width of almost 2 cm. This species can be identified by the specific character such as sickle-shaped leaves, the tips of leaf are usually rounded and smooth. The leaves may appear colors spot due to tannin cells that appear red, purple or dark brown. The leaves of this species develop directly from the rhizome. The rhizome is covered in thick, usually, dark-colored bristles that are the persistent remains of the leaves. The rhizome is around 1.5 cm in diameter with numerous pale-colored roots (Waycott *et al*., 2004; El Shaffai, 2011). These seagrass species were also found in the coastal area of Labuhan Beach, Lamongan (Purnama *et al.,* 2018,). Otherwise, the high human activity on Labuhan Beach fishing activities, shellfish collection and the entry of domestic waste through rivers, is thought to have an anthropogenic impact on the seagrass population of seagrass population there. On the other hand, the seagrass population on Labuhan Beach looks quite dense even though it is outside the conservation area. This unique phenomenon is considered to be suspected as the genotypic diversity of seagrass populations, which depends on age, maturity of the meadows ecosystem, and the spatial structure (Reusch *et al*., 2000).

Random Amplified Polymorphic DNA (RAPD) is a popular molecular marker for detecting genetic diversity of plants at interspecific and intraspecific levels (Arif *et al*., 2010; Rawashdeh, 2011; Priya *et al*., 2005). A genetic marker was used also as tools of identification of varieties of plants (Zhao, et al., 2011). This RAPD marker has also succeeded in uncovering a decrease in genetic diversity of *Posidonia oceanica* due to anthropogenic disturbance in the Mediterranean Sea region (Micheli *et al*., 2005). The marker could show genetic and clonal diversity among the population (Reusch, 2001).

In this paper, we notice genetic diversity among the population of two species of seagrass T. hemprichii and E. acoroides in 2 different locations, Labuhan Beach (representing the area near the port) and the Bama Coast of Baluran National Park (represents a conservation area). This finding is comparison data of the genetic diversity of the same species in those different location. Another popular barcode, Matk region, also performed to dig up the presence of nucleotide polymorphism in two different habitats and to verify the identity of these two seagrass species. We also compare the environmental parameters such as sediment and seawater in both habitats. Finally, this finding can later be used to consider the feasibility of seagrass in Labuhan Beach as a donor site for a transplantation project.

2. Materials and Methods

A total of 20 individual seagrass samples from two localities were collected in May-June 2019. Sampling was carried out in Labuhan Coastal waters in Lamongan (6°52'40.8"S 112°12'50.5"E) and Bama Coastal Waters of Baluran National Park (7°50'39.8"S 114°27'39.8"E) province of East Java, Indonesia (Figure 1). At each location, five individuals of *T. hemprichii* and *E. acoroides* (taxonomy identification refer to den Hartog and Kuo, 2006) were taken from different patches within > 10 meters from each other to avoid samples from the same clone. All plants that have already collected then were rinsed carefully with fresh water in order to remove detritus and attached epiphytic algae. Each of primordial leaf from shoot was dried with silica gel and then preserved at the cold condition. The genetic diversity of seagrass samples were analyzed by RAPD-PCR analysis for the molecular data in the Laboratory of Molecular Genetics, Department of Biology Airlangga University. Whereas the sediment and seawater samples were analyzed and observed in the Laboratory of Environmental and Ecology, Department of Biology Airlangga University for environmental condition data.

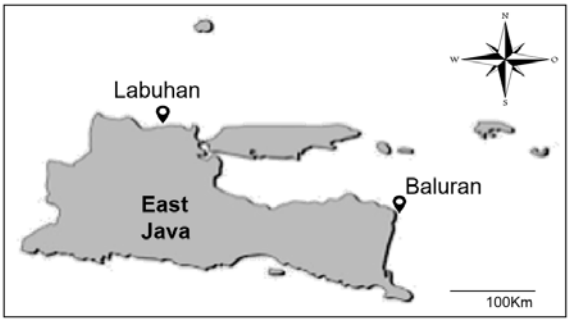

Figure 1. Map of sampling set location. Labuhan Beach and Baluran National Park.

The frozen leaves from the samples were crushed and mashed using a mortar and pestle. The genomic DNA from this leaves was extracted and analyzed using the Plant Genomic DNA Kit (New sera-Xtracta) refer to the protocols of DNA extraction.

The RAPD process was carried out using 25 ng DNA sample templates and using a two × GoTaq Green Mastermix (Promega) with a total reaction volume of 20 µl. A total of six RAPD primers were used in this study UBC127 (5'-ATCTGGCAGC-3'), OPA2(5'-TGCCGAGCTG-3 '), OPA4 (5'- AATCGGGCTG-3'), OPB12 (5'- CCTTGACGCA-3 '), OPD11 (5'-TGCCCGTCGT-3'), and OPH6 (5'- ACGCATCGCA- 3) 3 '). The PCR cycle was carried out by initiating denaturation at 95 ° C for 1 minute, followed by 38 cycles with 1 minute at 94 ° C, 1 minute at 37 ° C and 1.5

minutes at 72 ° C, and followed by a final extension of 10 minutes at 72 ° C.The PCR product from RAPD was visualized using 1.5% agarose gel in the 0.5x TBE buffer, while the MatK gene PCR product was visualized using 1% agarose gel in the 0.5x TBE buffer. A 100 bp DNA marker (Promega) was used on each of the electrophoretic gels.

MatK barcode amplification was performed using P646 5'-TAATTTACGATCAATTCATTC-3 'primer pairs and P647 5′-GTTCTAGCACAAGAAAGTCG-3 ′ (Lucas et al., 2012). The Cycle PCR used to amplify the Matk gene was 95 ° C for 5 minutes and followed by 35 cycles of 95 ° C for 1 minute, 54 ° C for 2 minutes, and 72 ° C for 2 minutes. The final extension was carried out at 72 ° C for 7 minutes. DNA sequences from the P646 primary strand Forward were carried out using Macrogen Inc. sequencing facilities (South Korea). The phylogenetic tree was generated by MEGA 7 using the Neighbor-Joining method.

Electropherograms were made manually by converting them into binary matrices that reflect the presence (1) and absence (0) of the allele. The matrix was tested using POPGENE 3.2 software.

Chemical and physical parameters of sediments are carried out to ammonium, phosphate, carbon, and size of sediment. Meanwhile, seawater samples are measured for mperature, salinity, turbidity, TDS, pH, hardness, heavy metals, and organic matters.

3. Results

As seen in Figure 2 that all primers can be well amplified on the *T. hemprichii* sample and generated a variety number of the band in both populations. Primer UBC 127 (Fig.2F) produced the most bands in *T. hemprichii,* which are 17 bands ranged from 300 bp up to 1500 bp. OPA2, OPA4, and OPB12 primers produce the same number of amplification bands by 12 bands (Fig2A-B-E). They were followed by OPD 11 by ten bands (Fig. 3D). Moreover, primer OPH 6 only produced five bands (Fig.2C).

Primer OPA2, OBP12 and UBC127 generated the same number of amplification band by 15 bands (Fig. 3A-E-F) Then, primer OPD11 and OPH6 produced 13 and 4 bands (Fig. 3D-C). The least produced primer was OPA 4, which produced only four bands (Fig. 3B). Moreover, consider to the least producing bands in this study, it shows that seagrass *E. acoroides* has a smaller number of locus then *T. hemprichii.*

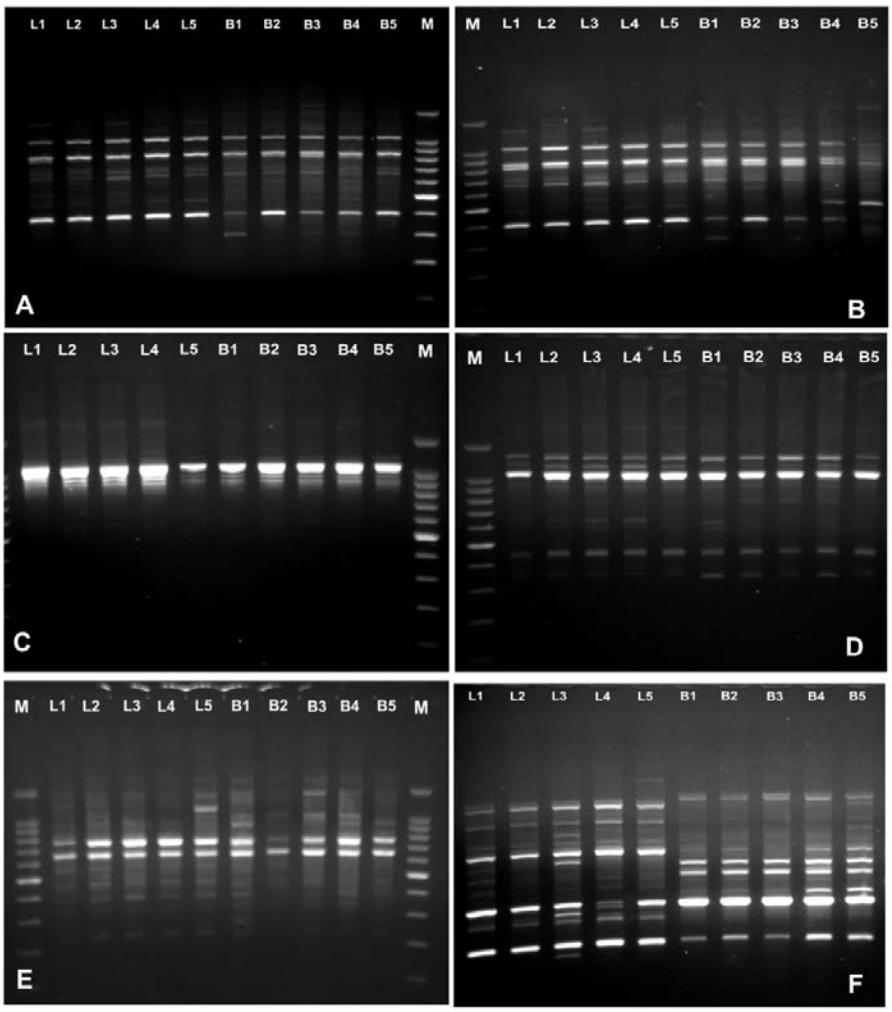

Figure 2. RAPD result from *T. hemprichii* using six primers. A. OPA2; B.OPA 4; C.OPH 6; D. OPD 11; E. OPB 12; F. UBC 127.

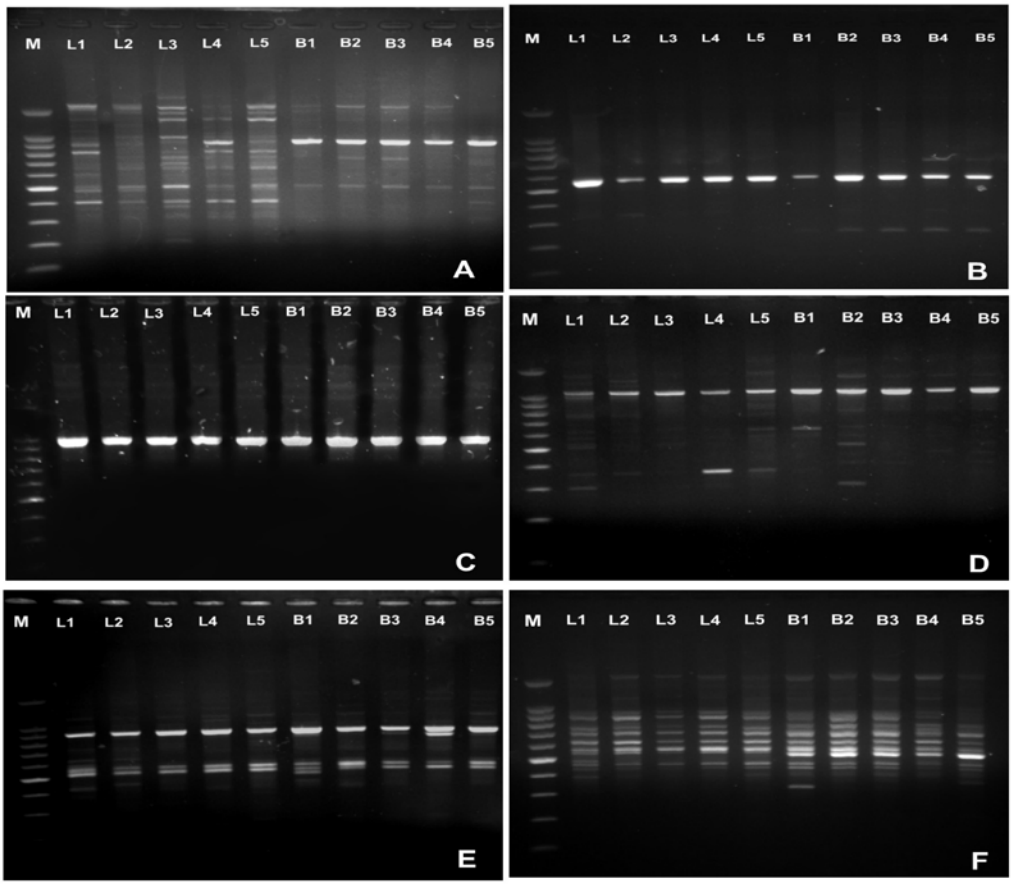

Figure 3. RAPD results from *E. Acoroides* using six primers. A. OPA2; B.OPA 4; C.OPH 6; D. OPD 11; E. OPB 12; F. UBC 127.

Table 1. Genetic diversity of *T. hemprichii* and *E. acoroides* population in Labuhan Beach and Baluran National Park.

Population	N	P	%	Na	Ne	H	I	Ht	Hs	Gst	Nm
Thalassia hemprichii											
Labuhan	68	35	51.47	1.5147	1.3266	0.1864	0.2772	0.1864	0.1864		
Baluran	68	29	42.65	1.4265	1.2683	0.1546	0.2302	0.1546	0.1546		
	68	**52**	**76.47**	**1.7647**	**1.4371**	**0.2585**	**0.3897**	**0.2585**	**0.1705**	**0.3405**	**0.9682**
Enhalus acoroides											
Labuhan	65	39	60.00	1.6000	1.3653	0.2116	0.3168	0.2116	0.2116		
Baluran	65	30	46.15	1.4615	1.2575	0.1550	0.2354	0.1550	0.1550		
	65	**50**	**76.92**	**1.7692**	**1.3882**	**0.2377**	**0.3657**	**0.2377**	**0.1833**	**0.2290**	**1.6831**

The seagrass population in Labuhan has a more extensive number of polymorphic bands and a percentage of the polymorphic band than the population in Baluran (Table 1). Other genetic parameters such as number of effective alleles, number of observed alleles, heterozygosity or Ne'i genetic diversity, total population heterozygosity, Shannon's information index, and value of heterozygosity in the population show the same condition in both seagrass species. Furthermore, genetic differentiation between the population of *T. hemprichii* is higher than the population of *E. acoroides*. In contrast, the population of *T. hemprichii* has fewer gene flow value than the population of *E. acoroides*.

Phylogenetic analysis from MatK barcode describes that species of *T. hemprichii* and *E. acoroides* taken from both locations. They are identically similar to the same species in the GenBank database (Fig.4). The barcoding analysis show that the identity of the seagrass sample from sampling locations are suitable. It also shows that the seagrass species from the different population was identic based on data in MatK region. Figure 4 also compares our sample to other seagrass species based on MatK sequence. *E. acoroides*, *T. hemprichii* together with *Halophila* spp. are clustered from single root Family Hydrocharitaceae. This data revealed that the samples of species are separated from other clusters from family Zosteraceae, Posidoniaceae, and Cymodoceae based on various seagrass accession numbers from GenBank.

The Nei genetic diversity (H) of the *T. hemprichii* population in Labuhan was slightly higher than the population in Baluran (t-test, $p = 0.173$). While the Ne'i genetic diversity of *E. acoroides* population in Labuhan was remarkably higher than the population in Baluran (t-test, $p = 0.036$); this finding indicated that the genetic differentiation in both populations was low rate.

The chemical-physical parameters of the environment are divided into sediment and seawater. The results of observing sediment parameters are in Table 2 below.

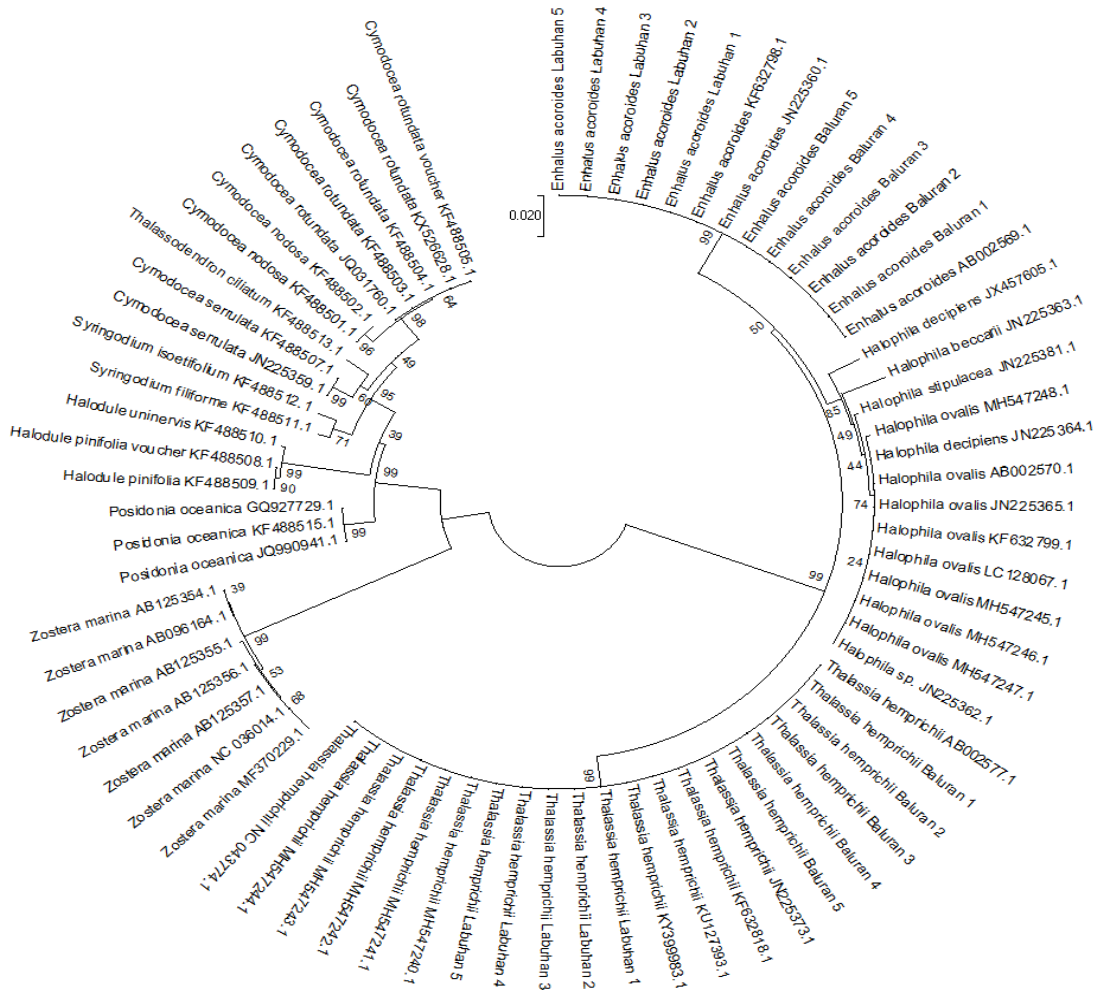

Figure 4. Phenogram of MatK region of the population of *T. hemprichii* and *E. acoroides* taken from two different sampling sites compared with various seagrass accession numbers from GenBank. The number in each branch indicated the percent of data coverage for internal nodes.

Table 2. The results of chemical-physical analysis from sediment of Labuhan and Baluran waters

		Locations	
Parameters	Units	Labuhan	Baluran
NH_3-N	%	0.057	0.087
P_2O_5	%	0.246	0.510
Carbon (C)	%	1.170	1.980
Gravel	%	0.14	13.70
Sand	%	60.94	84.74
Silt and Clay	%	38.92	1.56

Table 3. Result of chemical-physical analysis of seawater in Labuhan and Baluran waters

Parameters	Units	Locations	
		Labuhan	Baluran
Temperature	°C	28	26
Salinity	‰	34.5	35.1
Odour	-	No Odour	No Odour
TDS	mg/L	31078	31758
Turbidity	FTU	40.65	18.41
Colour	PtCo	0.38	0.06
pH	-	8.42	7.58
Hardness ($CaCO_3$)	mg/L	1870.36	2053.99
Iron (Fe)	mg/L	0.024	0.024
Fluoride (F)	mg/L	5.08	1.20
Cadmium (Cd)	mg/L	0.124	0.146
Chloride (Cl^-)	mg/L	17976.42	17140.68
Chromium (Cr_6^+)	mg/L	0.086	0.058
Manganese (Mn)	mg/L	0.133	0.132
Nitrate (NO_3^-)	mg/L	0.260	0.000
Nitrite (NO_2^-)	mg/L	0.000	0.000
Zinc (Zn)	mg/L	3.832	3.364
Cyanide (CN)	mg/L	0.000	0.000
Sulphate (SO4)	mg/L	2605.68	2736.66
Lead (Pb)	mg/L	1.537	0.159
Detergent (LAS)	mg/L	0.031	0.028
Potassium Permanganate (KMnO4)	mg/L	45.51	36.66

Based on Table 2, it can be seen that the nutrient content represented by the elements nitrogen, phosphate, and carbon shows that the sediments of Baluran waters are more abundant than Labuhan sediments. The sediment type of the two regions is dominated by sand substrates. However, the second-highest proportion of Labuhan sediments is in the form of silt and clay(38.92%), while in Baluran sediments is gravel (13.70%). The least proportion sediment of Labuhan is gravel (0.14%), while in Baluran it is silt and clay (1.56%).

The results of the analysis of seawater parameters in both regions was presented in Table 3. Labuhan waters temperature measured at the same time range shows higher results than Baluran waters, whereas salinity of seawater in Baluran has higher salt content. Seawater in Labuhan tends to be more turbid and has a higher pH, but the value is lower than Baluran waters. Some chemical materials such as fluoride, chloride, chromium, manganese, nitrate, zinc, detergent, and organic substances in the form of $KMnO_4$ in Labuhan waters are higher than Baluran, but not in cadmium, lead, and sulphate content. While the parameter data of iron, cyanide, and nitrite, both pools showed the same results.

4. Discussions

The genetic attribute of seagrass populations represented the relations of various progressions including a long-term changes related to species evolution (habitat fragmentation, population isolation, and shifts in distribution), gene flow, genetic drift, mutation, selection, and mating system (Slatkin, 1987; Schaal *et al*., 1998). In the present research RAPD survey of the two populations of tropical seagrass *E. acoroides* and *T. hemprichii* revealed that these seagrasses have a wide range of clonal diversity.

Four primers (OPA2, OPD 11, OPB 12, and UBC 127) are considered to be a potential marker to produce constant polymorphic bands pattern, clearness, amplification, and then used for further analysis for all samples. The other two primers (OPA4 and OPH6) did not well perform in the seagrass sample, especially in the DNA template extracted from *E. acoroides*, which provided a low number of PCR fragments. The DNA fragment was determined as polymorphic when some of them are available; however, it missing in different samples. Polymorphisms of RAPD markers are well-known and widely performed in population genetic research (Yang and Quiros, 1995).

Both *T. hemprichii* and *E. acoroides* of the Labuhan population have higher polymorphic fragments as well as the number of observed alleles than the Baluran population. It indicated that the seagrass population in Labuhan has high genetic variation. The Nei genetic diversity (H) of the *T. hemprichii* population in Labuhan was slightly higher than the population in Baluran (t-test, $p = 0.173$). While the Ne'i genetic diversity of *E. acoroides* population in Labuhan was remarkably higher than the population in Baluran (t-test, $p = 0.036$), this finding indicated that in both populations there is a low rate of genetic differentiation. The low rate of genetic differentiation in RAPD analysis has a higher tolerance of mutation due to RAPD amplification performed both in coding and non-coding region (Williams *et al*., 1990).

Genetic differentiation between populations (Gst) explains the proportion of genetic diversity in the entire population of each species. The Gst value of the *T. hemprichii* population was 0.3405, indicating 34% of total genetic variation was in the entire population, and 66% of total genetic variation was in each population, whereas the Gst value of *E. acoroides* was slightly fewer than *T. hemprichii* which is 0.229. So that 23% of total genetic variation represent in the entire population of two species. While 77% of total genetic variation was in each population of species.

Pharmawati *et al*. (2015) noted the number of observed heterozygosity of *E. acoroides* seagrass in Pramuka Island, Lembongan Island and Waigeo Island was 0.767; 0.436 and 0.582, respectively. That study employed five different primer pairs (Eaco1, Eaco9, Eaco19, Eaco51, Eaco55). Another study of *E. acoroides* in Aceh, Riau, Bangka Belitung, SeribuIsland, Banten, and Central Java, found the observed heterozygosity was 0.111-0.852 (Putra et al., 2018). Genetic diversity of *T. hemprichii* population in Indonesia also reported in a variety of Ne'i genetic diversity value range 0.184-0.534 (Hernawan, 2016).

Gene flow (Nm) of *E. acoroides* populations (1.6831**)** was higher than the population of *T. hemprichii* (0.9682). The genetic structure of marine organisms in Indonesia

coastal area is also influenced by oceanic condition such as ocean current of the Java Sea (Lind, 2009). Fruits of *E. acoroides* have capability to float for up to 10.2 days (Lacap *et al*., 2002) and have wide-distance dispersal (>1,000 km; Nakajima *et al*., 2017) while fruits of *T. hemprichii* stop floating after the fourth day (Wu *et al*., 2016). This finding suggested the increase in gene flow of *E. acoroides* between locations.

Seagrasses in favourable habitat inclined the genetic diversity (Larkin *et al*., 2006). In contrast, the present study revealed that both population seagrass of *E. acoroides* and *T. hemprichii* in the conservation area (Baluran National Park) has lower genetic diversity than the population in a disturbed area. This finding agrees with Putra *et al*. (2018) indicated that the population of E. acoroides with the lowest heterozygosity also found in the undisturbed area such as conservation area. A potential indicator of inbreeding through clonal reproduction can be shown by the excess of homozygosity. Small populations of organism can also multiply inbreeding and decrease genetic variation due to initiator impacts, deleterious mutations, and genetic drift which cause detrimental effects to genetic diversity (Procaccini *et al*. 2007. All those adverse effects reduce the fitness of the seagrass populations to ajust to environmental changes and lead them to extinction (Spielman *et al*., 2004; Leimu *et al*. 2006).

In this study, some immature fruit of *E. Acoroides* are found in the male and female flower of *T. hemprichii* during the sampling period (May-June) in Labuhan beach. Whereas, in the Baluran site, we only found male and female fruit of *T. hemprichii* and no reproductive organ of *E. acoroides* found there. This finding suggested that both generative and vegetative reproduction occur in Labuhan. Restricted flowering frequency and high-level inbreeding in the Baluran populations was one of the factors that influencing low clonal diversity. A complex combination of vegetative and generative reproduction support seagrass populations survives in fluctuate environmental conditions (Arriesgado *et al*., 2015).

Phylogenetic analysis of MatK barcode shows identical sequence among seagrass population in Labuhan, the population in Baluran, and sequence database obtained from GenBank. The sequence of MatK barcode of *E. acoroides* from both populations is identically similar to Enhalus acoroides AB002569.1; JN225360.1; KF632798.1. The MatK sequences of *T. hemprichii* from both populations is identically the same with *T. hemprichii* JN225373.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1; KF632818.1. Another seagrass from Family Hydrocharitaceae *Halophila* spp. is closer to *E. acoroides* then *T. hemprichii*. It can be seen in Figure 4, E. acoroides and Halophila spp. are clustered in one group. The same result was also reported by Tanaka *et al*. (1997) that put genus *Enhalus* and *Halophila* in one branch based on the MatK region. However, in different barcode region, RbcL, genus *Enhalus* is closer to *Thalassia* instead of Halophila. Furthermore, using a combined barcode with a matrix of 2388bp MatK and RbcL, genus of Enhalus and Thalassia were put in one branch (Tanaka *et al*. 1997). In the marine area, one of the largest families of aquatic angiosperms is Hydrocharitaceae, with a total of 135 known species of 16 known genera. The member of Hydrocharitaceae including both freshwater and marine aquatics (Christenhusz and Byng, 2016). Seagrasses themselves are regarded as an ecological group, not a taxonomical group. This condition indicates that many seagrass family members do not necessarily show a closely related relationship. Others seagrass families are Cymodoceae, Zosteraceae and Posidoniaceae (den Hartog and Kuo, 2006).

Seagrasses are a sensitive indicator of environmental changes especially in sediment loading, water quality, and other changes of ecosystem (Dennison *et al*. 1993). Water quality is potentially degraded due to some environmental quality degradation (Ruiz *et al*., 2001). The present study revealed different conditions in which sediments of undisturbed areas (Baluran) have a higher level of nutrient (ammonium, phosphate, and carbon). Run-off water from estuaries nearby suggested did not bring many nutrients to the coastal region. The high proportion of silt and clay of Labuhan sediment associated to the high level of turbidity in the water environment. While the different constituent elements of sediment of Labuhan and Baluran was contributed by fluctuate environmental condition. It seemed that both seagrass species were adaptable to a widerange of substrate types.

The most polymorphic donor material leads to increase in the genetic diversity of transplanted seagrass. It can be beneficial for transplantation achievement (Williams, 2001). Nevertheless, reducing the effects of restoration and improvement strategies on local gene pool is vital because of transplanting plants collected from far range of locations (Williams and Davis, 1996). Management and science-based protection approach supporting a consensus planning and legal authorities must be considered to minimize the effect of environmental stressors and put up wide range of effects on seagrass beds to protect these plants from fatal damage (Coles and Fortes, 2001; Kenworthy *et al*., 2006).

Another benefit of genotype diversity for ecosystems is supports stability of environmental and is also related to morphological plasticity of plants (Williams and Heck, 2001; Lewontin 1964). Increase of shoot density was beneficial for epiphytic invertebrates or other epifaunal organisms to refuge against a predator (Williams and Heck, 2001). Procaccini and Piazzi (2001) also reported that seagrass rhizome with higher elongation and ramification value was obtained from the population with higher genetic polymorphism. Thus, a high density of seagrass not only provides high productivity of the tidal zone but also serves as carbon sequestration, especially for *E. acoroides* and moreover, reduced impact of climate changes (Williams, 2001).

5. Conclusion

Seagrass *T. hemprichii* and *E. acoroides* were identified and verified, and they inhabited in Labuhan Beach, Lamongan Regency. Even though inhabiting anunfavorable area, the population of seagrass in Labuhan Beach provides high genetic diversity. The astonishing result indicates that genetic diversity of seagrass population in Labuhan is higher than in Baluran National Park, which is a conserved region. Therefore, the population of seagrasses in Labuhan beach is ideal to be a donor material for coastal restoration. Nonetheless, the

sourcing ecosystem should be kept maintained for the sustainability of seagrass ecosystem.

Acknowledgement

PUF Grant Research program from FST Airlangga University. Ir. Bambang Sukendro, M.M as Head of Baluran National Park and Setyo Hutomo,S.H., M.Si as Head of Region III KSDA Jember for permission to study in the conservation area and take the samples for this study.

Conflict of interest

The Authors declare that there is no conflict of interest with this work and the preparation of the manuscript.

References

Arif IA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan AA, Bahkali AH, Al Sadoon M and Shobrak M. 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet Mol Res.,* **9**: 2191-9198

Arriesgado DM, Kurokochi H, Nakajima Y, Matsuki Y, Uy W H, Fortes MD, Nadaoka L, Campos WL and Lian C. 2015. Genetic diversity and structure of the tropical seagrass *Cymodocea serrulata* spanning its central diversity hotspot and range edge. *Aqua Ecol.,* **49**(3): 357-372.

Beck MW, Heck KL, Ablem KW and Childers DL. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bios.,* **51**: 633−641

Christenhusz MJ and Byng JW. 2016. The number of known plant species in the world and its annual increase. *Phytotaxa*, **261**: 201-217.

Coles R and Fortes M. 2001. Protecting seagrass- approaches, and methods. In. Short, F. T., and Coles, R. G. (Eds). *Global Seagrass Research Methods* (Vol. 33). Elsevier.

den Hartog C and Kuo J. 2006. Taxonomy and Biogeography of Seagrasses. In Larkum, A. W., Orth, R. J., and Duarte, C. M. (Eds). *Seagrasses: Biology, Ecology, and Conservation* (p. 1-23). Dordrecht, The Netherlands: Springer.

Dennison WC, Orth RJ, Moore KA, Carter V, Kollar S, Bergstrom PW and Batiuk RA. 1993. Assessing water quality with submersed aquatic vegetation. *BioScience*, **43:** 86-95.

Duffy JE .2006. Biodiversity and the functioning of seagrass ecosystems. *Mar Ecol Prog Ser* **311**: 233−250

El Shaffai A. 2011. Field guide to seagrasses of the Red Sea. *IUCN, Gland, Switzerland and Total Foundation, Courbevoie,* France.

Harnianti N, Karlina I and Irawan H. 2017. Laju pertumbuhan jenis lamun *Enhalus acoroides* dengan teknik transplantasi polybag dan Sprig Anchor pada jumlah tunas yang berbeda dalam rimpang di perairan Bintan. *Intek Akua.,* **1**: 15-26.

Hernawan UE. 2016. Gene flow and genetic structure of the seagrass *Thalassia hemprichii* in the Indo-Australian Archipelago. Edith Cowan University, Australia (Ph.D. Thesis).

Hughes AR and Stachowicz, J. J. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences*, **101**: 8998-9002.

Irawan A. 2018. Transplantation of *Enhalus acoroides* on a sedimentary beach in Ambon Bay. In *IOP Conference Series: Earth and Environmental Science* **118**:. 012054). IOP Publishing.

Kenworthy WJ, Wyllie-Echeverria S, Coles RG, Pergent G and Pergent-Martini C. 2006. Seagrass conservation biology: an interdisciplinary science for protection of the seagrass biome. In *SEAGRASSES: BIOLOGY, ECOLOGY AND CONSERVATION* (pp. 595-623). Springer, Dordrecht.

Kettenring KM, Mercer KL, Reinhardt AC and Hines J. 2014. EDITOR'S CHOICE: Application of genetic diversity–ecosystem function research to ecological restoration. *J App Ecol.,* **51**: 339-348.

Lacap CDA, Vermaat JE, Rollon RN and Nacorda HM. 2002. Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. *Mar Ecol Prog Ser.,* **235**: 75-80.

Larkin P, Quevedo E, Salinas S, Parker J, Storey K and Hardegree B. 2006. Genetic structure of two *Thalassia testudinum* populations from the south Texas Gulf coast. *Aqua Bot.,* **85**: 198-202.

Leimu R, Mutikainen PIA, Koricheva J and Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *J Ecol.,* **94**: 942-952.

Lewontin RC. 1964. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics*, **49**: 49.

Lind CE. 2009. *Population genetics, phylogeography and the effects of aquaculture on genetic diversity of the silver-lipped pearl oyster, Pinctada maxima (Jameson)* (Doctoral dissertation, James Cook University).

Lucas C, Thangaradjou T and Papenbrock J. 2012. Development of a DNA barcoding system for seagrasses: successful but not simple. *PLoS One*, **7**: e29987.

Micheli C, Paganin P, Peirano A, Caye G, Meinesz A and Bianchi CN. 2005. Genetic variability of *Posidonia oceanica* (L.) Delile in relation to local factors and biogeographic patterns. *Aqua Bot*., **82**: 210-221.

Nakajima Y, Matsuki Y, Arriesgado DM, Campos WL, Nadaoka K and Lian C. 2017) Population genetics information for the regional conservation of a tropical seagrass, *Enhalus acoroides*, around the Guimaras Strait, Philippines. *Cons Gen.,* **18**: 789-798.

Paling EI, Fonseca M, van Katwijk MM and van Keulen M. 2009. Seagrass restoration. In: Perillo GME, Wolanski E, Cahoon DR, Brinson MM (eds) *Coastal Wetlands*: an integrated ecosystems approach. Elsevier, Amsterdam, p 687−713

Pharmawati M, Putra I, Syamsuni YF and Mahardika IGNK. 2015. Genetic diversity of *Enhalus acoroides* (L.) Royle from coastal waters of Pramuka Island, Lembongan Island, and Waigeo Island, Indonesia, based on microsatellite DNA. *Adv Sci Lett.,* **21**: 199-202.

Priya TA, Manimekalai V and Ravichandran P. 2015. Intra specific genetic diversity studies on *Calotropis gigantean (*L) R. Br. - using RAPD markers. *European J Biotech Biosci.,* **3**: 7-9

Procaccini G and Piazzi L. 2001. Genetic polymorphism and transplantation success in the Mediterranean seagrass *Posidonia Ocean Restor Ecol.,* **9**: 332−338

Procaccini G, Olsen JL and Reusch TB. 2007. Contribution of genetics and genomics to seagrass biology and conservation. *J of Exp Mar Biol Ecol.,* **350**: 234-259.

Purnama PR, Purnama ER, Manuhara YS, Hariyanto S and Purnobasuki H. 2018. Effect of high-temperature stress on changes in morphology, anatomy and chlorophyll content in tropical seagrass *Thalassia hemprichii*. *AACC Bioflux,* **11**: 1825-1833.

Purnama PR, Hariyanto S, Manuhara YSW and Purnobasuki H. 2019. Gene expression of antioxidant enzymes and heat shock proteins intropical seagrass *Thalassia hemprichii* under heat stress. *Taiwania*, **64**: 117-123.

Putra ING, Syamsuni YF, Subhan B, Pharmawati M, and Madduppa H. 2018. Strong genetic differentiation in tropical seagrass *Enhalus acoroides* (Hydrocharitaceae) at the Indo-Malay archipelago revealed by microsatellite DNA. *Peer J.,* **6**: e4315.

Rawashdeh IM. 2011. Genetic diversity analysis of *Achillea fragrantissima* (Forskal) Schultz Bip Populations Collected From Different Regions of Jordan Using RAPD Markers. *Jor J Biol Sci.*, **4**: 21-28.

Reusch TB. 2001. New markers old questions: population genetics of seagrasses. *Mar Ecol Prog Ser.*, **211**: 261-274.

Reusch TBH, Stam WT and Olsen JL. 2000. A microsatellite based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Molr Ecol*., **9**: 127-140.

Riani E, Djuwita I, Budiharsono S, Purbayanto A and Asmus H. 2012. Challenging for seagrass management in Indonesia. *J Coast Dev.*, **15**: 234-242.

Ruiz JM, Perez M and Romero J. 2001. Effects of fish farm loadings on seagrass (Posidoniaoceanica) distribution, growth that and photosynthesis. *Mar Pollut Bull.,* **42**:749–760.

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT and Smith WA. 1998. Phylogeographic studies in plants: problems and prospects. *Mol Ecol.,* **7**: 465-474.

Seprianti R, Karlina I and Irawan H. 2017. Laju pertumbuhan jenis lamun *Thalassia hemprichii* dengan teknik transplantasi sprig anchor dan polybag pada jumlah tegakan yang berbeda dalam rimpang di Perairan Kabupaten Bintan. *Intek Akua.*, **1**: 56-70.

Short FT, Polidoro B, Livingstone SR and Carpenter KE .2011. Extinction risk assessment of the world's seagrass species. *Biol Conserv.*, **144**: 1961−1971

Sinclair EA, Verduin J, Krauss SL, Hardinge J, Anthony J and Kendrick GA .2013. A genetic assessment of a successful seagrass meadow (*Posidonia australis*) restoration trial. *Ecol Manage Restor*., **14**: 68−71

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. *Science*, **236**: 787-792.

Spalding M, Taylor M, Ravilious C, Short F and Green E. 2003. The distribution and status of seagrasses. World atlas of seagrasses. University of California Press, Berkeley, California, 5-26.

Spielman D, Brook BW and Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proc Nat Acad Sci.,* **101**: 15261-15264.

Tahir NAR, Lateef DD, Omer DA, Kareem SHS, Ahmad DA and Khal LH. 2018. Genetic diversity and structure analysis of pea grown in Iraq using microsatellite markers. *Jor J Biol Sci.,* **12**: 201-207.

Tanaka N, Setoguchi H and Murata J. 1997. Phylogeny of the family hydrocharitaceae inferred from rbcL and matK gene sequence data. *J Plant Res.,* **110**: 329-337.

Van Katwijk MM, Bos AR, De Jonge VN, Hanssen LSAM, Hermus DCR and De Jong DJ. 2009. Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Mar Poll Bull*., **58**: 179-188.

Waycott M, Duarte CM, Carruthers TJB and Orth RJ. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc Natl Acad Sci.,* USA **106**: 12377−12381

Waycott M, McMahon K, Mellors J, Calladine A and Kleine D. 2004. A guide to tropical seagrasses of the Indo-West Pacific.

Williams SL. 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. *Ecol Appl.*, **11**: 1472−1488

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuc Acids Res.*, **18**: 6531–6535

Williams SL and Davis CA. 1996. Population genetic analyses of transplanted eelgrass (*Zostera marina*) beds reveal reduced genetic diversity in southern California. *Rest Ecol.*, **4**: 163-180.

Williams SL and Heck KL. 2001 Seagrass community ecology. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Sunderland, MA, p 317–337.

Wu K, Chen CNN and Soong K. 2016. Long distance dispersal potential of two seagrasses *Thalassia hemprichii* and *Halophila ovalis*. *PloS one*, **11**: e0156585.

Wulandari D, Riniatsih I and Yudiati E. 2013. Transplantasi lamun *Thalassia hemprichii* dengan metode jangkar di Perairan Teluk Awur dan Bandengan, Jepara. *J Mar Res.,* **2**: 30-38.

Yang X and Quiros CF. 1995. Characterizing the celery genome with DNA-based genetic markers. *J Amer Soc Hort Sci.*, **120**: 747-751.

Zhang J, Zhou F, Chen C, Sun X, Shi Y, Zhao H and Chen F. 2018. Spatial distribution and correlation characteristics of heavy metals in the seawater, suspended particulate matter and sediments in Zhanjiang Bay, China. *Plos One,* **13**: e0201414.

Zhao MZ, Zhang YP, Wu WM, Wang C, Qian YM, Yang G and Fang JG. 2011. A new strategy for complete identification of 69 grapevine cultivars using random amplified polymorphic DNA (RAPD) markers. *African J Plant Sci*., **5**: 273–280.