Genetic diversity and morphological characteristics of native seashore paspalum in Indonesia

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Abstract. *Rahayu, Fatimah, Bae EJ, Mo YG, Choi JS. 2020. Genetic diversity and morphological characteristics of native seashore paspalum in Indonesia. Biodiversitas 21: 4981-4989.* Seashore paspalum (*Paspalum vaginatum*) is a warm-season turfgrass indigenous to tropical and coastal areas worldwide. The objectives of this study were to measure the genetic diversity and genetic variation of Indonesian seashore paspalum germplasm. Three turf quality, six morphological characters, and ten SSR (microsatellite) markers were used to assess genetic relationships and genetic variation among 22 germplasm resources from Indonesia and one commercial variety (Salam) from United States of America. The results showed significant variation for five morphological characters among 23 tested seashore paspalum accessions. The cluster analysis of morphological characters of 23 seashore paspalum accessions using 0,6 cut off divided into three morphological types: tall high-density, intermediate, and dwarf low-density ecotype. The genetic variation revealed 22 alleles with average number of alleles per locus was 2 and polymorphism information content (PIC) values average was 0.33. The microsatellite marker cluster analysis showed that 23 seashore paspalum accessions were grouped into two major groups, with a genetic similarity coefficient was 0,72. The low level of genetic diversity occurred among Indonesia natural grass germplasm and the genetic distance was relatively low between Indonesian germplasm and Salam variety. The genetic diversity and morphological characteristics will be useful for further study and utilization of Indonesian seashore paspalum germplasm.

Keywords: Genetic relationship, natural grass germplasm, *Paspalum vaginatum*, simple sequence repeat, SSR

INTRODUCTION

In Indonesia, genus P*aspalum* usually used as forage for ruminant such as *Paspalum dilatatum* or rumput Australi and *Paspalum notatum* or rumput bahia (Pebriansyah et al. 2014), *Paspalum conjugatum* or Jampang Pahit (Stuart 2015), and *Paspalum atratum* or rumput swallen (Marsetyo et al. 2019) or harvested as wild cereal such as *P. scrobiculatum* or kodo millet (Hariprasanna 2017) and used as local medicinal plants such as *Paspalum conjugatum* or Radupu in Tane Olen, East Kalimantan (Karmilasanti and Supartini 2011) and *Paspalum* sp. or Jiluang Gadis in Batin, ethnic group in Jambi Province (Jalius and Muswita 2013).

Seashore paspalum (*Paspalum vaginatum*) is a warm-season turfgrass originating in Asia or Africa, and can be used on golf courses, athletic fields, and commercial and residential lawns. Seashore paspalum has characteristics excellent tolerance to the high salt levels, takes less fertilizer and needs less irrigation than many other turfgrasses, also tolerates a wide range in soil pH levels, has minimal pesticide requirements, has good insect and disease tolerance, and is fairly shade tolerant (Acuna et al. 2019). In Indonesia, seashore paspalum has been widely used as ground cover in garden, ornamental parks, golf courses, and housing. Seashore paspalum of Salam variety introduced from America is widely used in Indonesia for grass golf courses, especially near the coast and saline areas. Seashore paspalum in Banda Aceh, Nanggroe Aceh Darussalam Province known as rumput lapang was reported as common forage for Kacang goat (Iskandar and Lubis 2010). Rahayu et al. (2014) reported that seashore paspalum could grow in media using volcano eruption sand at Merapi mountain area and clayey soil.

Morphological analysis is a useful tool to study the variation and characterization of germplasm. However, environmental factors and genetic composition can both influence variance in morphological analysis (Liu et al. 2019). Compared with morphological characterization analysis, the genetic variance at the molecular level is not influenced by environmental factors. Molecular marker techniques have been applied to assess the genetic diversity of seashore Paspalum such as RAPD (Liu et al. 1994), AFLP (Chen et al. 2009), EST-SSR (Li et al. 2016), SRAP (Liu et al. 2019), SSR (Shen et al. 2020) and high-density genetic map of seashore paspalum was also available (Qi et al. 2019). The fact that SSR markers are abundant, codominant, multi-allelic, highly polymorphic, and technically efficient makes SSR preferable and attractive as genetic markers (Shultz et al. 2013). Recent years, SSR markers have been developed and applied in *P. vaginatum* (Shultz et al. 2013; Jia et al. 2015; Li et al. 2016). SSR markers have been used in genetic diversity studies of *P. vaginatum* (Liu et al. 2019, Shen et al. 2020).

There is number of seashore paspalum germplasms distributed in Indonesia. However, there is limited information and research conducted in the characterization of these natural grass germplasm and there were no reports on genetic diversity especially seashore paspalum in Indonesia. Hence, this is the first study to investigate the genetic diversity and morphological characteristics of seashore paspalum in Indonesia. Therefore, the objectives of this study were to measure the genetic diversity and genetic variation of Indonesian seashore paspalum germplasm using turf quality, morphological characters and SSR (microsatellite) markers.

MATERIALS AND METHODS

Turfgrass collection

The survey and grass collection were conducted in mountains, highlands, karst areas, and coastal areas. Sampling site were determined based on experience in observing seashore paspalum grass in Java island surrounds the mountain areas of Merapi, Merbabu, Lawu, Sindoro, Dieng and Ungaran whereas the seaside areas were in Jepara, Bantul, Gunung Kidul, Pacitan, and Karimunjawa island while in Bali Island, the areas surveyed were Gilimanuk, Nusa Dua, Badung and Denpasar and also surveyed in Sumatra and Southeast Sulawesi. It was surveyed from March to April 2014 (Figure 1). The sampling was carried out using farming tools together with the local villagers.

The seashore paspalum was identified according to its morphological characteristics. Morphologically seashore paspalum is similar to bermudagrass (*Cynodon* spp.). Both species spread rapidly by stolons and rhizomes to form a fine-textured, dense turf with a deep root system. However, stolons and rhizomes of seashore paspalum are often much thicker and also darker green than those of hybrid bermudagrasses. Furthermore, seashore paspalum foliage regularly has a waxy (shiny) appearance compared to bermudagrass. This is especially pronounced on the underside (abaxial surface) of each leaf blade (Brosnan and Deputy 2008).

All of the identified seashore paspalum was recorded and photographed for the occurrence of evidence. Sampling locations were recorded as a basis for geographic distributions. The seashore paspalum samples (stolons or rhizomes) that have recorded then planted in greenhouse and maintained. The geographical position and characteristics of the ecological environment of each grass were presented in Table 1.

The vegetatively propagated seashore paspalum of twenty-two accessions collected during 2014 and cultivar "Salam" variety that widely use in Indonesia used as control were planted in April to June 2015 at experimental field of the Agriculture Faculty of University Sebelas Maret (UNS), Central Java, Indonesia ($7°56'07.09"$ S, $110°85'65.78"$ E). The research plots were arranged in a randomized complete block design with three replications. The plot size was 1.5 m x 1.5m with spaced 15 cm interval in the planting medium of clayey soil (Rahayu et al. 2014). It is maintained by mowing weekly or biweekly to prevent possible mixing among the accessions. Maintenance of grasses growth was applied with the irrigation of 100% evapotranspiration with 3 days interval and fertilizer NPK 4-1-2 (N 15 g m^{-2}) to ensure that the turf remained healthy and developed newly expanded leaf blades.
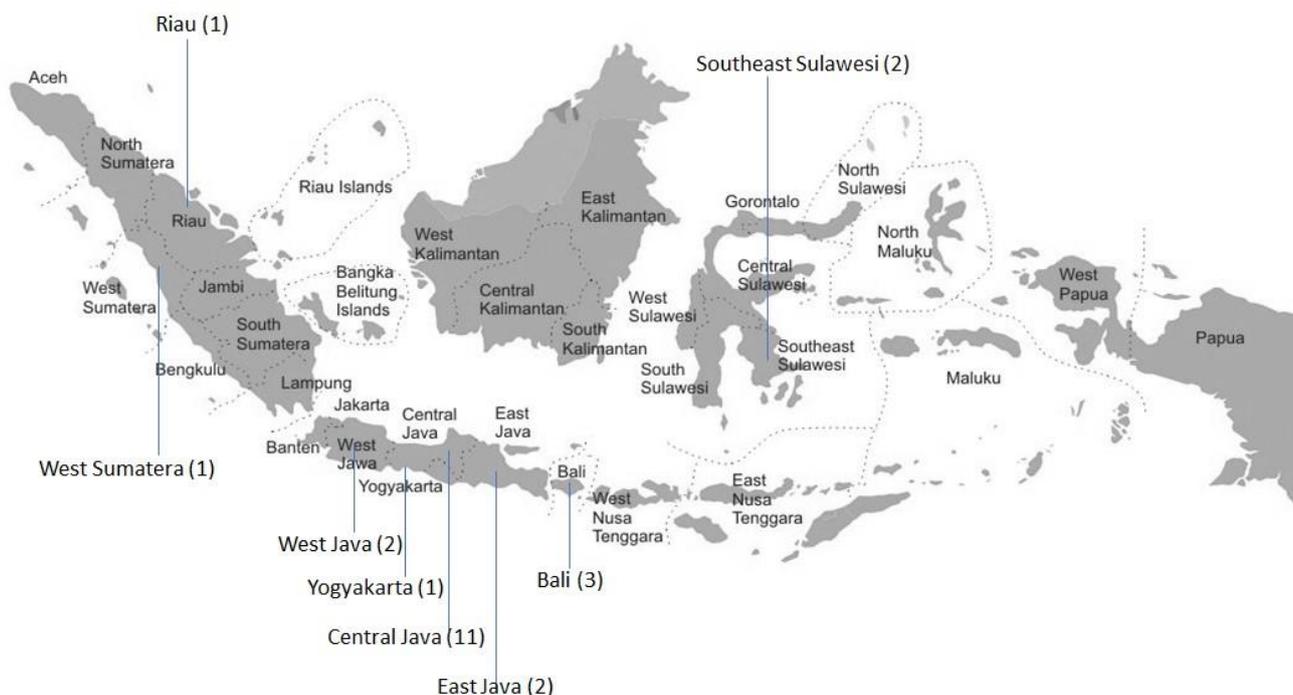

Figure 1. Map of Indonesia, showing the location of survey and sampling sites of seashore paspalum grass collected from 8 provinces. The lines indicating the province and the number of seashore paspalum sampled from each province.

Table 1. Data of collected seashore paspalum and sampling site used in this study

Population	Specific sample collection site					
	Location	Province	Site	Altitude (m)	Latitude	Longitude
PA	Jepara	Central Java	Seaside	3	6° 33' 55.09" S	110° 39' 35.87" E
PB	Kebumen	Central Java	Ground	20	7° 40' 09.47" S	109° 30' 05.05" E
PC	Tanah Lot	Bali	Beach	4	8° 37' 16.37" S	115° 05' 12.51" E
PD	Rumbai	West Sumatra	Roadside	5	0° 33' 8.23" S	101° 26' 2.78" E
PE	Siak	Riau	Roadside	11	0° 54' 25.84" S	102° 09' 06.89" E
PF	Bogor	West Java	Golf course	254	6° 35' 52.85" S	106° 50' 18.48" E
PG	Jepara 2	Central Java	Seaside	3	6° 33' 55.09" S	110° 39' 35.87" E
PH	Ponorogo	East Java	Lawn	98	7° 54' 20.13" S	111° 27' 27.57" E
PI	Magelang	Central Java	Golf course	390	7° 29' 36.67" S	110° 12' 48.08" E
PJ	Salam	Control variety	UNS field	155	7° 56' 07.09" S	110° 85' 65.78" E
PK	Temanggung	Central Java	Arable land	1150	7° 15' 01.52" S	110° 01' 42.12" E
PL	Klaten	Central Java	Lawn	350	7° 42' 20.99" S	110° 36' 23" E
PM	Kendari	Southeast Sulawesi	Roadside	18	4° 01' 06.60" S	122° 30' 52.63" E
PN	Wakatobi	Southeast Sulawesi	Seaside	5	5° 32' 49.56" S	123° 48' 06.41" E
PO	Jepara Kartini	Central Java	Seaside	4	6° 35' 24.24" S	110° 38' 52.46" E
PP	Jembrana	Bali	Seaside	3	8° 21' 00.41" S	114° 32' 52.03" E
PQ	Mojogedang	Central Java	Roadside	400	7° 32' 58.992" S	110° 59' 11.144" E
PR	Bogor 2	West Java	Golf Course	250	6° 35' 52.85" S	106° 50' 18.48" E
PS	Karanganyar	Central Java	Ground	350	7° 36' 25.463" S	110° 59' 12.098" E
PT	Tasikmadu	Central Java	Roadside	340	7° 34' 28.29" S	110° 55' 4.735" E
PU	Jembrana 2	Bali	Seaside	3	8° 21' 00.41" S	114° 32' 52.03" E
PV	Parangtritis	Yogyakarta	Roadside	10	7° 58' 33.94" S	110° 19' 00.30" E
PW	Ngawi	East Java	Lawn	74	7° 22' 15.28" S	111° 10' 22.49" E

Morphological characterization of seashore paspalum

The morphological characterization of seashore paspalum was measured using seven vegetative (turf quality and turf quantity character) on forty days after seedling (DAS) and two reproductive characters (spikelet length and grain per spikelet) on sixty DAS. The turf quality character of the grass covering the visual quality of texture, uniformity, shoot density, and growth rate, also level of surface coverage was observed within the plot. The turf quality and shoot density were evaluated following visual field assessment (VFA) of The National Turfgrass Evaluation Program (NTEP) method (NTEP 2008) with scale of 1-9 (1.0 = poorest possible quality and 9.0 = best possible quality) where the "6" score as the minimally acceptable value for the assessment. The turf quantity character such as plant height, leaf length, leaf width and internode length were measured with calipers ruler.

Molecular analysis

Genomic DNA was extracted from fresh leaves of each accession using DNA isolation method (Dellaporta 1983). Extraction buffer was added to crushed leaves tissues (sodium bisulfite was added shortly before use). Tube is shaken well and then heated in a water bath temperature of 65 °C for 15-30 minutes. A cold solution of chloroform: isoamyl alcohol (24:1, v/v) was added to buffer with a ratio (1:1, v/v) and shake for 10-15 minutes. Tubes centrifuged at a speed of 12,000 rpm for 15 minutes. Supernatant was transferred into a new tube. As much as 2/3 volume of cold isopropanol was added to the supernatant and then the tube is shaken gently. If necessary, 3M ammonium acetate can be added as much as 1/10 volume and the pellet resulted from centrifugation for 12 minutes was rinsed with cold

70% ethanol and then dried for 15-30 minutes. Stock DNA was dissolved in 50 μL of TE buffer (pH 8.0). The DNA was qualified in 0,8% agarose gel and quantified using nanodrop.

Molecular analysis was performed using 10 microsatellite primers in Table 2 developed from Paspalum genome (Shultz et al. 2013). PCR reactions containing 100 ng of genomic DNA, 10 pmol of each primer, 1.5 mM MgCl$_2$, 0.2 mM of each dNTP, and 0.2 units of Taq DNA polymerase (Dream Taq, Thermo Fisher Scientific US). The PCR condition was initial denaturation at 94 °C for 5 minutes followed by denaturation at 94 °C for 1 min, annealing at a temperature of 55 °C for 1 minute and 72 °C for 1 minute for 35 cycles followed and a final extension for 7 min at 72 °C. The PCR products were electroporated on 8% acrylamide gel or 2% agarose gel, followed by ethidium bromide staining. Then gel electrophoresis was visualized and documented using gel documentation (Bio-rad).

Data analysis

Data analysis of morphological characteristics was performed using Ms Excell and SPSS program to analyze the variation (ANOVA) of the morphological data. Duncan significant difference (DMRT) was determined at a five percent probability level to make the comparison between the mean values of each accession. The amplified bands of the PCR products were scored using PhotoCapMW. The molecular data were analyzed using Power marker to generate Polymorphism Information Content (PIC) and GeneAlex for AMOVA analysis. Cluster analysis was constructed using NTSYS ver.2.0 with SAHN and UPGMA trees.

Table 2. Primer list used in this study (Shultz et al. 2013)

Primer	Forward Sequence	Reverse Sequence	Repeat	Size (bp)	Chr
ctg02617	TATTTTGTGACATCGTCGGTGAGT	CTACTTGCTGTGACGGAAAGGATT	(CT)7	165-179	3
ctg02478	CAGGTTGCCTCACTCAGAAGAACT	TGTGTTTTCTTGGTCTTGGTCAGA	(TC)16	135-162	5
ctg01792	CTTTGGTAGCCATTGTGTTTACCC	TCCAGATGCCTTTAGTAGTTTGGC	(TTAT)4	170-185	-
ctg00695	TAGAATCCCAGAAACTCTGCAAGC	GTCGTCTCGCTAAACAATAAGCGT	(AGA)4	150-178	4
ctg05574	GATATCTGAAGCAGAATGCCCACT	AGAGGAGCTCTCGAAAAAGGTACG	(CGC)4	155-163	-
ctg13333	CTCTTCGCCAAGTACAAGATCCAG	TCTAGTATTCTGGATGGACGGACAA	(CTT)4	105-130	4
ctg598	CACAGACAAAGCACTGGATCTCAT	GATGCAGGGTGGAAATAAGAAGAA	(CTT)4	116-150	3
ctg02718	TGACAATGACTATGGTGGATACGG	AGACCAATACCATCCTCCTCCTTC	(GAG)3	77-180	4
ctg02534	GAGACGTTCGACAGGAACGC	ATGGCCAATTTGCTCATCCC	(GAT)4	140-206	6
ctg00721	AGGGGTATAAGAAGAAGCCTGCTG	CTCGTACTTTCCTCCTCCATAGCC	(GTG)4	125-130	1

RESULTS AND DISCUSSION

Morphological characterization of seashore paspalum

The morphological characteristics as standard parameters were used in this study to determine the performance of the seashore paspalum. The collected data can provide a preliminary evaluation of the accessions. The comparison of a number of Indonesian seashore paspalum accessions with commercial cultivar Salam variety provides a first screening of new plant material.

The coefficients variation (CV) observed of turfgrass quality, shoot density, and surface coverage were high (>50%) with the maximum CV value was surface coverage character (64,14%) while the CV of turfgrass quantity character leaf length, leaf width, internode length, spikelet length, grain per spikelet were less than 23% with the minimum was grain per spikelet (10,99%) (Table 3). The results indicated rich variation among 23 seashore paspalum accessions in turf quality but slightly different compare to turf morphological character (Figure 2).

The shoot density and turf quality rated with acceptable value (score > 6) observed in 17 accessions, within this the highest quality was "Salam" variety (score 8,5 and 9) while the low quality (score <6) observed in five accessions (PG, PM, PN, PP, and PU). High surface coverage rate (>90%) was found in 11 accessions (PB, PD, PM, PR, PS, PH, PI, PK, PL, PQ, and PT) while very low rate of surface coverage was found in two accessions PC and PO (5%) and Salam variety were also low (10%) (Table 3). In the turf quality characterization, PH from Ponorogo and PB from Kebumen was high in turf quality (score 8), shoot density (score 8), and surface coverage (90%) (Table 3).

The morphological characteristic of local seashore paspalum in vegetative phase was compared to Salam variety as the threshold. The plant height character separated into short/dwarf (< 10 cm) consisted of 4 accessions (PC, PF, PR, PU, and Salam variety) and tall (>10 cm) consisted of 18 accessions. The leaf width character separated into fine leaves (< 3 mm) consisted of 11 accessions (PA, PC, PD, PE, PF, PK, PM, PN, PS, PU, PV, and Salam) and wider leaves (> 4 mm) consisted of 11 accessions (PB, PG, PH, PI, PL, PO, PP, PQ, PR, PT, PW). The leaf length character separated into short leaves (< 3,5 cm) consisted of 5 accessions (PC, PF, PK, PU, PV, and Salam) and long leaves (> 3.5 cm) consisted of 17 accessions (PA, PB, PP, PE, PG, PH, PI, PL, PM, PN, PO, PP, PQ, PR, PS, PT, PW) (Table 3).

The morphological characteristic of local seashore paspalum in generative phase was compared to Salam variety as the threshold. The internode length character separated into short internode/dwarf (<2.17 cm) consisted of 8 accessions (PC, PD, PE, PK, PP, PT, PU, PV, and Salam) and long internode (> 2,1 cm) consisted of 14 accessions (PA, PB, PF, PG, PH, PI, PL, PM, PN, PO, PQ, PR, PS, PW). The spikelet length and grain per spikelet characters observed fewer seed and short spikelet in 13 accessions (PA, PC, PD, PF, PM, PN, PO, PP, PR, PS, PU, PW, PH, and Salam) and more seeds and longer spikelet in 9 accessions (PB, PE, PG, PI, PK, PL, PQ, PT, PV) (Table 3). Based on morphological characteristic in vegetative (plant height, leaf length, and leaf width) and generative phase (internode length, spikelet length, and grain per spikelet) revealed that PC (Tanah Lot, Bali) and PU (Jembrana, Bali) were similar to commercial "Salam" variety or dwarf low-density ecotype.

Figure 2. The natural grass of seashore paspalum from collected germplasm 40 days after seedling (DAS) that have formed dense colonies for the evaluation of quality, leaf color, and morphological characteristic

Table 3. Morphological characteristic of 23 seashore paspalum accessions analyzed in this study

Population	Shoot density (score)	Turf quality (score)	Surface coverage (%)	Turf height (cm)	Leaf length (cm)	Leaf width (cm)	Internode length (cm)	Spikelet length (cm)	Grain per spikelet
PA	7	6	30	20	8.7 ± 1.53[ef]	0.3 ± 0.03[a-c]	2.3 ± 0.46[c-h]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PB	8	7	90	24	4.8 ± 0.76[b-d]	0.5 ± 0.03[de]	2.3 ± 0.66[c-g]	2.7 ± 0.29[d]	14.0 ± 1.00[c]
PC	7	5	5	8	3.5 ± 0.50[a-c]	0.3 ± 0.00[a-c]	1.6 ± 0.49[a-d]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PD	7	7.5	90	17	5.8 ± 0.64[d]	0.3 ± 0.10[a-c]	1.7 ± 0.35[a-d]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PE	6.5	7	40	10	5.7 ± 1.16[cd]	0.3 ± 0.10[a-c]	1.9 ± 0.27[a-f]	0.9 ± 0.79[b]	8.3 ± 7.23[b]
PF	7	6.5	20	6	3.3 ± 0.76[ab]	0.3 ± 0.06[ab]	2.1 ± 0.79[c-g]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PG	4	4	50	21	5.0 ± 1.00[b-d]	0.4 ± 0.10[b-d]	2.1 ± 0.66[c-g]	1.7 ± 0.29[c]	14.7 ± 2.52[c]
PH	8	7.5	95	15	5.5 ± 0.50[b-d]	0.4 ± 0.10[b-d]	2.6 ± 0.17[e-h]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PI	6	6	95	22	6.2 ± 0.76[d]	0.5 ± 0.00[de]	2.8 ± 0.25[f-i]	2.7 ± 0.29[d]	15.7 ± 1.16[c]
PK	7.5	7.5	95	11	2.4 ± 0.51[a]	0.3 ± 0.00[a-c]	1.9 ± 0.21[b-f]	2.4 ± 0.36[d]	14.7 ± 1.53[c]
PL	6.5	6.5	98	22	5.5 ± 0.50[b-d]	0.4 ± 0.10[b-d]	2.7 ± 0.36[e-h]	2.3 ± 0.36[d]	13.3 ± 2.08[c]
PM	4.5	5	90	20	6.7 ± 0.58[de]	0.3 ± 0.06[ab]	3.3 ± 0.40[hi]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PN	4.5	4.5	70	33	8.5 ± 1.32[ef]	0.3 ± 0.06[ab]	3.6 ± 0.36[i]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PO	5.5	6	6.5	19	9.3 ± 3.22[f]	0.4 ± 0.06[b-d]	2.8 ± 0.75[f-i]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PP	5	5	25	16	4.8 ± 0.76[b-d]	0.4 ± 0.06[b-d]	1.9 ± 0.40[a-e]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PQ	5	6	100	18	5.0 ± 1.00[b-d]	0.5 ± 0.00[de]	2.8 ± 0.25[f-i]	1.7 ± 0.29[c]	12.3 ± 2.31[c]
PR	7.5	7.5	90	8	5.0 ± 1.00[b-d]	0.4 ± 0.06[b-d]	2.0 ± 0.20[c-f]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PS	7	7	90	13	5.0 ± 1.00[b-d]	0.3 ± 0.00[a-c]	2.9 ± 0.90[g-i]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PT	6	6	100	25	6.0 ± 1.00[d]	0.6 ± 0.15[e]	1.5 ± 0.25[a-c]	1.8 ± 0.29[c]	13.7 ± 2.31[c]
PU	5	5	10	4	2.5 ± 0.50[a]	0.2 ± 0.03[a]	1.1 ± 0.15[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PV	7	6.5	20	12	3.3 ± 0.29[ab]	0.3 ± 0.06[ab]	2.2 ± 0.29[c-g]	1.5 ± 0.00[c]	13.0 ± 0.00[c]
PW	7	7	10	22	6.7 ± 2.08[de]	0.4 ± 0.15[c-e]	2.4 ± 0.40[d-h]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
PJ (Salam)	8.5	9	10	2	1.7 ± 0.25[a]	0.2 ± 0.10[a]	1.1 ± 0.15[ab]	0.0 ± 0.00[a]	0.0 ± 0.00[a]
Mean	6.4	6.07	60.14	16.19	5.26	0.35	2.23	0.78	5.38
Minimum	4	4	5	2	1.73	0.18	1.1	0	0
Maximum	8.5	9	98	33	9.33	0.56	3.6	2.67	15.67
SD	3.26	3.11	38.57	8.7	0.94	0.06	0.53	0.1	0.59
CV (%)	50.88	51.28	64.14	53.75	17.86	17.34	23.8	13.17	10.99
Fprob	-	-	-	-	*	*	*	*	*

Note: Means followed by the same letter in a column are not significantly difference at $P \le 0.05$. SD= Standard deviation, CV (%) = Coefficient of variance and Fprob= F probability, * means significantly difference at $P \le 0.05$, "-" not counted.
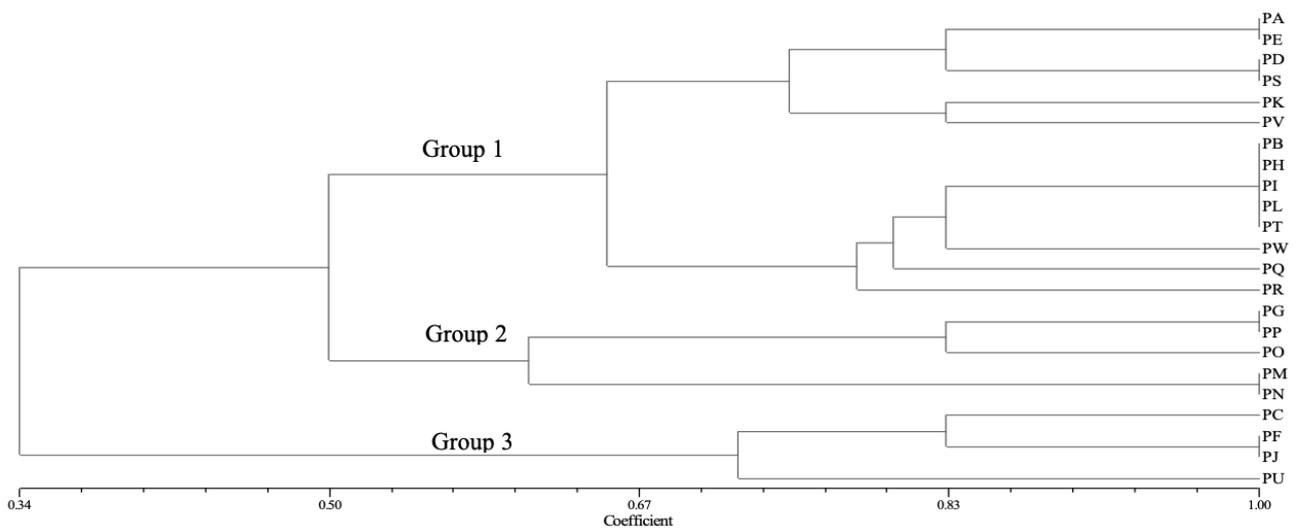

Figure 3. UPGMA dendrogram of seashore paspalum showing the genetic relationships between 23 seashore paspalum accessions based on turfgrass quality and morphological characterization revealed three major groups

A dendrogram was constructed from turfgrass quality and morphological characterization. When a cutoff value of 0.60 was used for genetic similarity among all accessions as the threshold for UPGMA clustering three major groups were observed. Group 1 consisted of 14 accessions (PA, PE, PD, PS, PK, PV, PB, PH, PI, PL, PT, PW, PQ, and PR). Group 2 consisted of 5 accessions (PG, PP, PO, PM, and PN). Group 3 consisted of 3 accessions (PC, PF, and PU) clustered with widely used seashore paspalum "Salam" variety (Figure 3).

Polymorphism analysis and genetic variation of seashore paspalum

Nine out of ten SSRs used for genotyping showed good polymorphism and were able to be measured in 19 Indonesian seashore paspalum germplasms (Figure 4). The genetic variation revealed 22 number of alleles with the average number of alleles per locus was 2 ranged from 2 (ctg598, ctg00721, ctg01792, ctg02478, ctg02534, ctg02617, ctg05574) to 4 (ctg02718). Gene diversity or expected heterozygosity (H_e) is the probability that two alleles are randomly selected from a population (Babu et al. 2017). Gene diversity value ranged from a low of 0,145 (ctg598) to a high of 0,66 (ctg00695 and ctg02718) with an average of 0,439 and heterozygosity (H_o) value ranged from a low of 0,00 (ctg02718) to a high of 0,78 (ctg02478) with the average of 0,442. PIC values provide information about the polymorphism of a marker. The PIC values average was 0.36 and ranged from a low of 0.13 (ctg598) to a high of 0.61 (ctg02718) (Table 4). The presence of 22 alleles in the 23 accessions indicates low genetic diversity and low in PIC value within the microsatellite markers.

An AMOVA study of Indonesian seashore paspalum was performed to analyze the distribution of genetic diversity between and within the populations. AMOVA analyses showed that 83% variance exists within individuals whereas 12% variance was present among populations (Table 5). The 23 of Indonesian seashore paspalum with 9 microsatellite primers yielded 59.26% in average of polymorphic bands. Central Java has the highest diversity with maximum P, Ne, and H values while Southeast Sulawesi has the smallest value for P, Ne, and He which infers a relatively low genetic variation (Table 6).

Cluster analysis was performed on similarity coefficient matrices calculated from molecular markers to generate a dendrogram. When a cutoff value of 0.70 was used for genetic similarity among all varieties as the threshold for UPGMA clustering two major groups were observed. Group 1 consisted of 5 accessions i.e: PA, PN, PM, PP, and PU clustered with widely used seashore paspalum "Salam" variety. This group consisted of seashore paspalum originated from Central Java (PA), South Sulawesi (PM and PN), and Bali (PP and PU). Group 2 consists of 13 accessions i.e: PB, PC, PE, PF, PD, PO, PG, PI, PT, PR, PS, PL, and PQ. In this group consisted of seashore

paspalum originated from Bali (PB), Riau, (PE), West Sumatra (PD, West Java (PF and PR), and Central Java (PB, PO, PG, PI, PT, PS, PL, and PQ) (Figure 5).

Table 4. Summary statistic of microsatellite primers used in this study

Primer	Allele number (Na)	Gene diversity (H_e)	Heterozygosity (H_o)	PIC
ctg598	2	0.145	0.158	0.135
ctg00695	3	0.666	0.684	0.592
ctg00721	2	0.488	0.316	0.369
ctg01792	2	0.453	0.538	0.350
ctg02478	2	0.494	0.789	0.372
ctg02534	2	0.450	0.684	0.349
ctg02718	4	0.660	0,000	0.610
ctg02617	2	0.161	0.176	0.148
ctg05574	2	0.432	0.632	0.339
Mean	2,333	0.439	0.442	0.363

Note: H_e: Expected heterozygosity, H_o: Observed heterozygosity, PIC: Polymorphism Information Content

Table 5. Summary of Analysis of Molecular Variance (AMOVA) of Indonesian seashore paspalum

Source	df	SS	MS	Est. Var.	%
Among populations	4	15.449	3.862	0.274	12%
Among individuals	14	29.867	2.133	0.106	5%
Within individuals	19	36.500	1.921	1.921	83%
Total	37	81.816		2.301	100%
Fst	0.119				
Nm	1.851				

Note: AMOVA: analysis of molecular variance; df: degrees of freedom; SS: sum of squares deviation; MS: mean squared deviation; Est. Var.: estimates of variance components; %: percentage of total variance contributed by each component

Table 6. Genetic diversity of Indonesian seashore paspalum populations analyzed by microsatellite data

Population	P (%)	Ne	I	Ho	He
Bali	77.78	1.550	0.471	0.333	0.309
Central Java	88.89	1.757	0.573	0.503	0.383
West Java	66.67	1.511	0.448	0.611	0.319
West Sumatra	44.44	1.444	0.308	0.444	0.222
Southeast Sulawesi	22.22	1.133	0.125	0.111	0.083
Riau	55.56	1.333	0.385	0.556	0.278
Mean	59.26	1.455	0.385	0.426	0.266

Note: P (%): number (percentage) of polymorphic loci; Ne: mean effective number of alleles; I: Shannon's information index, He: mean expected heterozygosity; Ho: mean observed heterozygosity
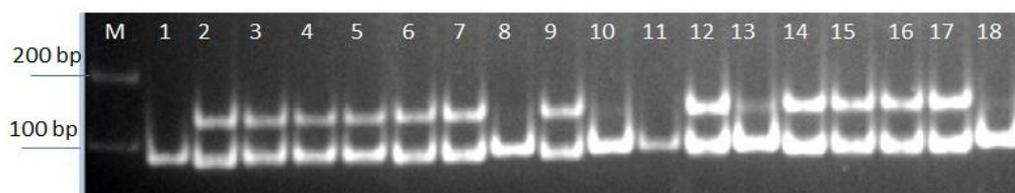
Figure 4. Electrophoregram of natural grass seashore paspalum germplasm using SSR marker ctg05574 in 2% agarose gel electrophoresis. Lane 1: 100 bp Ladder, Lane 2-19: seashore paspalum accessions. M= marker
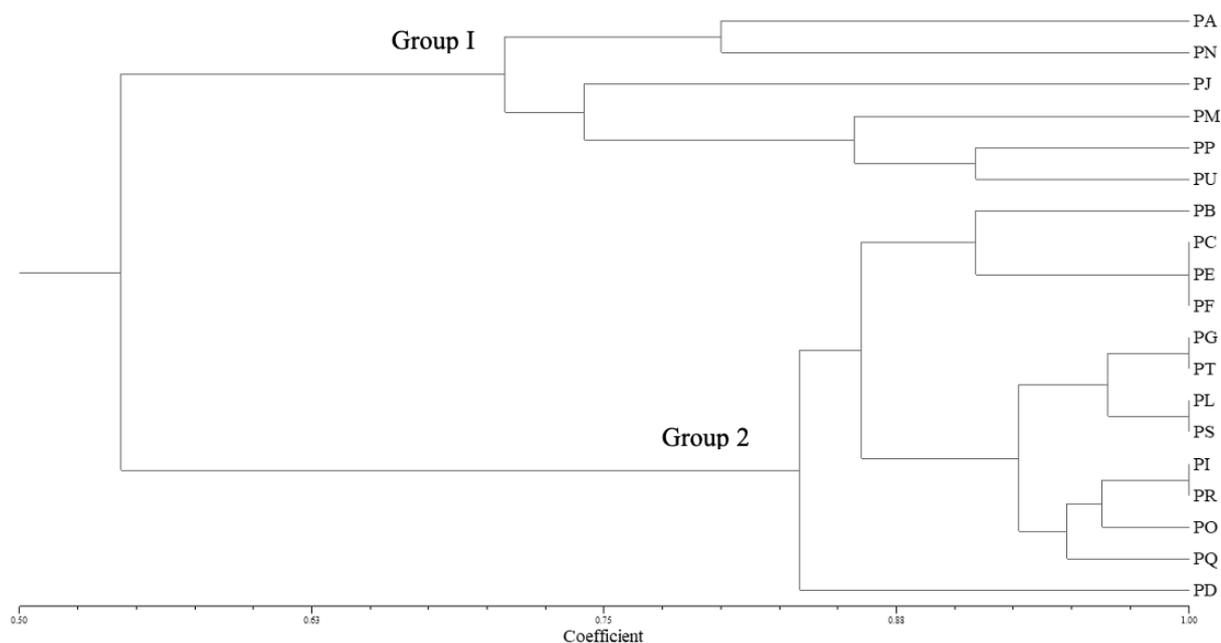

Figure 5. UPGMA dendrogram of seashore paspalum showing the genetic relationships between the 19 seashore paspalum accessions based on 9 microsatellite markers. Two major groups are labeled

Discussion

The genetic diversity analysis of Indonesian seashore paspalum collection showed that the PIC value in the present study (0.13-0.61; av=0.32) was similar to PIC value of Shultz et al. (2013) (0-0.78; av=0.31) that reported 17 seashore paspalum cultivars from America with 75 microsatellite markers but higher than Shen et al. (2020) (0.016-0.577; av=0.24) that reported 58 seashore paspalum accessions and four cultivars from China with 19 microsatellite markers. The microsatellite primer of ctg02718 and ctg00695 in this study, were giving higher PIC values than Shultz et al. (2013). It indicates the ability of these two markers to distinguish between and within individuals themselves in a population. The higher the PIC value of microsatellite marker indicates that the microsatellite markers have high potential in identifying genetic relationships. The gene diversity parameter in this study (0.14-0.66; av=0.44) was higher than Shultz et al. (2013) (0-0.80; av=0.34). In this study, the microsatellite loci revealed that more alleles number and high gene diversity have a high PIC value or positively correlated with the number of alleles and gene diversity. The gene diversity was high in Central Java which indicates the

presence of a large genetic diversity in Central Java collections as compared to those from other provinces. The within-population level genetic diversity of Indonesian seashore paspalum was obviously higher (83%) compared to the among population level (12%). It indicated that most variation in population genetics of Indonesian seashore paspalum occurs within populations.

Although the advancement of technology in genomics area, the Indonesian seashore paspalum collection remained uncharacterized at the molecular level such as in genetic diversity parameters. This has been one of limiting factors in their utilization for development of improved new turfgrass cultivars and conservation in Indonesia. The present study is the first major effort to characterize the Indonesian seashore paspalum collections at molecular level. This study shows that SSR technology is an effective and efficient method for genetic diversity analysis of Indonesian seashore paspalum.

During plantation, wide variation of natural grass accessions in turf density was clearly seen in experimental field (Figure 2). The highest variance was found in turfgrass quality, shoot density, and surface coverage. Thus, it can be used as parameter in further study of

Paspalum diversity and breeding. In this study, 17 accessions were observed high in shoot density and turf quality within this PH from Ponorogo and PB from Kebumen were the highest in turf quality, shoot density, and surface coverage.

The cluster analysis of turf quality and morphological characterization separated three major groups. Group 1 characteristics were high in turf density, turf quality and surface coverage compare to other groups. The plant height was tall, the leaves were long and wide and long internode, producing many seeds with large spikelets, this group named as tall high-density ecotype. Gaetani et al. (2017) reported that varieties with high shoot density having the potential to wear resistance and damage recovery that are superior to others. It could replace some commercial variety and recognized as reference standards for the turfgrass industry. Brosnan and Deputy (2009) reported that wear resistance in seashore paspalum may be associated with some physiological and morphological traits and shoot density seems consistently associated with traffic tolerance. Group 2 characteristics were low in turf density, turf quality, and surface coverage. However, the plant height was relatively tall, the leaves were long and wide and long internode while the spikelet was short and less seed per spikelet, this group named as intermediate ecotype between Group 1 and Group 3. Group 3, contains Salam variety, characteristics were low in the turf density, turf quality, and surface coverage, and the plant height was relatively low, the leaf length was short and leaf width was narrow while the internode length and spikelet length was short and less seed per spikelet, this group named as dwarf low-density ecotype (Figure 3). On the contrary, within this group, Salam variety was actually high in shoot density. The dwarf low-density ecotype could be used as the potential recipient for high shoot density ecotype in the breeding purpose for grass golf courses.

The cluster analysis based on microsatellite markers separated into two groups. Group 1 clustered with widely used seashore paspalum "Salam" variety with the characteristic of leaf width was narrow and Group 2 with the characteristic of leaf width was wider. This result was consistent with the findings of Shultz et al. (2013) which were clustered into two major groups. Group I consisted of seashore paspalum accessions with fine to medium-fine leaves and Group II consisted of seashore paspalum accessions with coarse leaves and predominant stems. Grouping based on leaf texture in seashore paspalum had been observed previously (Liu et al. 1994).

The cluster analysis of turf quality and morphological characteristics compare with cluster analysis of molecular marker resulted in slightly similarity in grouping. Group 1 (narrow leaves) in molecular marker cluster consisted of Group 2 (intermediate) and Group 3 (dwarf low-density ecotype) in morphological cluster while Group 2 (wide leaves) in molecular marker cluster similar to Group 3 in morphological cluster (tall-high density ecotype). However, both clustering does not discriminate against the natural grass seashore paspalum accessions by their geographical origin. It could be due to the limitations of the sampling strategies such as the different habitats and

environments were clustered together so that the material collected by the roadside might be a cultivar that has been widely used and accidentally collect similar material during sampling. García et al. (2007) and Liu et al. (2019) also reported that morphological markers do not discriminate individuals by their geographic origin. Certain accessions always have similar morphological characteristics when grown in the same environment; however, their genetic basis will not necessarily be the same. The accessions with a similar genetic basis clustered together based on the molecular markers; however, their morphological traits may be different because of the influence of the environment.

In conclusion, the genetic diversity of 23 Indonesian seashore paspalum assessed by microsatellite analysis detected narrow genetic diversity. The Contig02718 and Contig00695 markers have more alleles number, high gene diversity, and PIC value. The turf quality, shoot density, and surface coverage of PH (Ponorogo) and PB (Kebumen) accessions were high while PC (Tanah Lot) and PU (Jembrana) were similar to "Salam" variety in six morphological characters. The cluster analysis of morphological characterization separated three major groups. Group 1 was tall high-density ecotype. Group 2 was intermediate ecotype between Group I and Group 3. Group 3 was dwarf low-density ecotype. The cluster analysis of microsatellite markers divided into two groups. Group 1 clustered with "Salam" variety with narrow leaf width and Group 2 with wider leaf width and there was no geographical segregation of the natural grass seashore paspalum accessions. To enlarge the genetic base of seashore paspalum in Indonesia, the wide exploitation and identification of diverged genetic materials from the germplasm collection was required. It may contribute to plant breeders for the selection and develop new cultivars.

ACKNOWLEDGEMENTS

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