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Genetic diversity analysis among 123 accessions of castor (*Ricinus communis* L.) using SSR marker and its association to agronomic traits

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Abstract. *Anggraeni TDA, Waluyo B, Sugiharto AN, Kuswanto. 2022. Genetic diversity analysis among 123 accessions of castor (*Ricinus communis *L.) using SSR marker and its association to agronomic traits. Biodiversitas 23: 1211-1221.* Castor (*Ricinus communis* L.) breeding program targets to create dwarf-type plants suitable for mechanical harvesting. Characterization of plants using morphological and molecular markers can enhance the successful breeding program. This study aimed to investigate the diversity of castor accessions using Simple Sequence Repeat (SSR) markers and find the SSR markers that are significantly associated with the agronomic traits. The castor germplasm collection consisting of 123 accessions from the islands throughout Indonesia, breeding lines, and introduced accessions displayed a moderate genetic diversity. The germplasm collection showed high variation in plant height, node length average, number of nodes, 100 seed weight, and oil content. The association analysis using GLM (General Linear Model) and MLM (Mixed Linear Model) could detect six associations between SSR marker alleles and agronomic traits. Among the marker alleles, two marker alleles had an overlap association. RcSSR-12_175 had an association with plant height and flowering day, RcSSR-23_113 had an association with node length average and branch number, and RcSSR-4_125 had an association with seed weight/raceme. This finding could provide information on Indonesia's castor accession status. The linked-trait SSR markers are useful in marker-assisted selection for breeding high yield dwarf type-castor.

Keywords: Association, castor, diversity, dwarf, simple sequence repeat markers

INTRODUCTION

Castor (*Ricinus communis* L.) produces a non-edible oilseed. Its seed oil contains a high ricinoleic acid (90%) and has unique properties. The oil and its derivates are beneficial as raw materials for industries, such as coatings, polymers, lubricants, biodiesel, cosmetics, medicinal uses (Mubofu 2016), and larvicide (Wibowo et al. 2010). Therefore, it acquires a lot of attention in the world market oil. Castor oil world consumption increased by more than 50%, from 400,000 tons in 1985 to 610,000 tons in 2010 (Severino et al. 2012), or on average increases by 3-5 % per year (Anjani 2012). However, the castor oil industry experienced a decline due to the lack of availability of castor seed as a raw material for castor oil. The castor seed total world production in 2011 was 2.2 million tons, decreased in 2015 to 1.5 million tons, and continued to decrease to 1.2 million tons in 2020 (FAOSTAT 2022). One of the reasons is that 96% of the world's castor seed production is still carried out by small farmers, with minimal mechanization in particular for harvesting (Severino and Auld 2013). The average castor seed production in Indonesia from 2012 until 2014 was 1.42 thousand tons per year (Statistics Indonesia 2022).

The natural growth type of castor as a perennial plant hinders mechanical harvesting. Plants in good growing conditions can reach a height of 3-5 m and continue to produce new branches and panicles (Oswalt et al. 2014; Santoso et al. 2014). These characteristics lead to reducing yields and difficulty in harvesting. Therefore, it needs a dwarf-type plant (less than 1.5 m) that exhibits an erect stem, a few branches, a long primary raceme (20-40 cm), and a harvesting age of fewer than 150 days (Lavanya et al. 2018). The major producing countries, such as India, China, and Brazil have created dwarf varieties with high seed yields. However, specific production environments and cropping systems will require cultivars that adapt well to regionally environmental conditions (Severino et al. 2012). Therefore, it is still needed to create dwarf-type castor that has specific adaptation.

Development of dwarf-type castor through breeding program requires well-characterized germplasm. The selection of plants with desirable traits demands genetic material with high genetic diversity (Yulianah et al. 2020; Sadiyah et al. 2021). Castor germplasm accessions showed a variation in morphological and agronomic characters (Rukhsar et al. 2018; Wahibah et al. 2020). Besides variation on phenotypic traits, some genetic diversity analysis using molecular markers have been reported, such as Simple Sequence Repeat (SSR) (Senthilvel et al. 2016), Sequence Related Amplified Polymorphism (SRAP) (Agyenim-boateng et al. 2019), Inter Simple Sequence

Repeat (ISSR), and Random Amplified Polymorphic DNA (RAPD) (Kim et al. 2021). SSR markers have advantages compared to other molecular markers. The marker is highly informative, codominant, multi-allele, reproducible, and transferable among related species (Vieira et al. 2016). Some studies on genetic diversity and agronomic trait analysis have been conducted on castor, such as Wang et al. (2017), Wahibah et al. (2020), Dharajiya et al. (2020), and Anggraeni and Purwati (2022).

Genetic diversity analysis using SSR markers and its association to agronomic traits in castor accessions originated from the islands throughout Indonesia have not been reported. Therefore, this study aimed to investigate the genetic diversity of castor accessions from different islands in Indonesia using SSR markers, observe the variation of agronomic traits, and find the SSR significantly associated with the agronomic traits.

MATERIALS AND METHODS

Study area

The experiment was carried out during the 2020 planting season (February to November) in Karangploso Experimental Field, Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI), Malang, Indonesia. The field site was located at an altitude of 515 m asl. The soil type was inceptisol and the average rainfall was 1500 mm/year. According to Schmidt-Fergusson classification, the climate type is D, average temperature and humidity are 22 to 25°C and 64 to 84%, respectively (Statistics of Malang District 2020).

Plant materials

Plant materials were 123 accessions of castor germplasm from the ISFCRI and the Department of Agronomy, Faculty of Agriculture, Brawijaya University, Malang, Indonesia. The germplasm collection consisted of 41 breeding lines (selection for dwarf-type), 73 Indonesian castor germplasm, introduced accessions from three Asian countries (China, Japan, and Thailand), and two Indonesian commercial varieties (Table 1).

SSR marker analysis

DNA extraction

Genomic DNA was extracted from leaf tissue using GeneALL exgene Plant SV mini kit (Geneall Biotechnology Co. Ltd. South Korea), following the instruction provided. SSR markers were developed from forty sequences of plant hormones and zinc finger gene family in the *Ricinus communis* genome available in NCBI (https://www.ncbi.nlm.nih.gov/gene) and TIGR (http://www.plantgdb.org/RcGDB) database (Feng et al. 2019). SSR motif detection and primer design were used Batch3 primer design software (You et al. 2008). The criteria of the repeat number were $\geq$10 for di-, $\geq$5 for tri, and $\geq$4 more tetra-, penta-, and hexanucleotides. The parameter set up for primer designing were PCR primer length of 15-25 and 20 bp as optimum with a product size of 150-400 bp, annealing temperature of 55-61°C with 60°C as optimum temperature, and minimum GC content of 40-60% with 50% being the optimum. All primers were synthesized by Integrated DNA Technology (Coralville, IA, USA). SSR primers used in the study are shown in Table 2.

Polymerase Chain Reaction (PCR) and electrophoresis

PCR amplifications were performed in a total volume of 50 µL, containing 2 µL genomic DNA, 25 µL Dreamtaq green PCR master mix (2x), each 1 µL of forward and reverse primer, and 21 µl nuclease-free water. PCR reactions were conducted in T100 Thermal Cycler (Applied Biosystem, USA). Initial denaturation was for 3 minutes at 95°C, then followed by 35 cycles of denaturation for 30 seconds at 95°C, annealing at 56.5-62°C for 30 seconds, and extension at 72°C for 1 minute. The final extension was for 5 minutes at 72°C. The PCR products were separated by electrophoresis on 1% standard agarose and 2% metaphor agarose (Lonza) followed the modification method of Murianingrum et al. (2018) in 70 mL 1x TBE buffer and GelRed Nucleic Acid Stain Biotium using Bio-Rad Horizontal Electrophoresis System at 60 V for 180 minutes. The PCR products were visualized under a UV transilluminator and documented using Geldoc Wealtec KETA (Wealtec Corp).

Agronomic trait measurement

The accessions were arranged in an augmented design block with one breeding line and two Indonesian commercial varieties as the control plants. The block was 3.3x0.5 m, consisted of 30 random accessions and 3 control plants. Each accession was planted in a row consisting of ten plants with plant spacing 50x100 cm. Each block was replicated four times.

The phenotypic measurements were recorded on three plant samples for each accession. The parameters observed were thirteen agronomic traits, including seven plant architecture and six yield component traits. Plant height was measured on the primary stem, starting from the base plant on the ground and ending at the base of the raceme. Node length and the number of nodes were calculated from the primary stem. The flowering day was recorded as an average of 10 plants per accession. Raceme length, capsules per raceme, and seed weight per raceme were measured as an average of raceme in the first to the third branch. Total seed weight per plant was determined by weighing the seed from primary, secondary, and tertiary branches after air drying. Oil content was measured based on w/w (Merkouropoulos et al. 2016).

Data analysis

Amplicons of molecular data were scored according to their product size for every accession and primer combination. Marker polymorphism was defined by allele size, allele number, genotype number, observed heterozygosity (Ho), major allele frequency, and Polymorphism Information Content (PIC) using PowerMarkers version 3.25 (Liu and Muse 2005). Genetic diversity was determined by the number of alleles, the effective number of alleles, observed heterozygosity, expected heterozygosity, and percentage of

polymorphic loci using GenAlEx v. 6.5 (Peakall and Smouse 2012).

A phylogenetic tree revealed genetic relatedness among accessions was constructed using Nei's genetic distance calculated with PowerMarker 3.25 and drawn using Mega 6.0 (Tamura et al. 2013) with 1000 bootstrap replications. STRUCTURE version 2.3.4 was used to identify the genetic structure and assign individuals to populations (Pritchard et al. 2000a). The estimated number of populations (K) was set from 1 to 9, with a 100,000 step burn-in and 100,000 MCMC iterations. The genotype with a score >0.80 was considered pure and <0.80 as admixture (Anandan et al. 2016).

Variation of the agronomic traits was analyzed using Analysis of Variance for RCBD augmented design using augmented RCBD packages (Aravind et al. 2021). Correlation among the traits was measured using Pearson correlation with a significant value of 0.05 by Hmisc packages (Harrel 2021). The statistical analysis was done in R software version 4.1.0 (R Core Team 2020).

The association between phenotype and molecular data was analyzed using the General Linear Model (GLM) and Mixed Linear Model (MLM) considering Q-matrix from STRUCTURE software and Kinship data to reduce the spurious association caused by population structure. The analysis was using TASSEL version 3.0 (Bradbury et al. 2007). The significant association between SSR markers and agronomic traits was identified on the R2 and p-value <0.05.

Table 1. List of castor germplasm collections studied

Pop. code	Acc. code	Source	Island	Type	Pop. code	Acc. code	Source	Island	Type
P1	A1-A41	Breeding line	-	Dwarf	P4	Rc-M5	Lebek	Madura	Normal
P2	Rc-S1	Medan, North Sumatra	Sumatra	Normal	P5	Rc-Sing1	Singaraja	Bali	Normal
P2	Rc-S2	Medan, North Sumatra	Sumatra	Normal	P5	Rc-Sing2	Singaraja	Bali	Normal
P2	Rc-S3	Tapanuli, North Sumatra	Sumatra	Normal	P5	Rc-Sing3	Singaraja	Bali	Normal
P2	Rc-S4	Tapanuli, North Sumatra	Sumatra	Normal	P5	Rc-Sing4	Singaraja	Bali	Normal
P2	Rc-S5	Jambi	Sumatra	Normal	P6	Rc-NTB1	Lombok	Lombok	Normal
P2	Rc-S6	Jambi	Sumatra	Normal	P6	Rc-NTB2	Lombok	Lombok	Normal
P2	Rc-S7	Jambi	Sumatra	Normal	P6	Rc-NTB3	Beraringan	Lombok	Normal
P2	Rc-S8	Lampung	Sumatra	Normal	P6	Rc-NTB4	Lokok Rangan	Lombok	Normal
P3	A-grt	Garut, West Java	Java	Normal	P6	Rc-NTB5	North Lombok	Lombok	Normal
P3	Rc-KF1	Central Java	Java	Normal	P6	Rc-NTB6	Lokok Rangan	Lombok	Normal
P3	Rc-KF2	Central Java	Java	Normal	P6	Rc-NTB7	Tampes	Lombok	Normal
P3	Rc-KF4	Central Java	Java	Normal	P6	Rc-NTB8	Sumbawa	Sumbawa	Normal
P3	Rc-KF5	Central Java	Java	Normal	P7	Rc-NTT1	Maumere	Flores	Normal
P3	Rc-smrg3	Semarang, Central Java	Java	Normal	P7	Rc-NTT2	Kupang	Timor	Normal
P3	Rc-grbg2	Grobogan, Central Java	Java	Normal	P7	Rc-NTT3	Soe	Timor	Normal
P3	A-smg	Semarang, Central Java	Java	Normal	P7	Rc-NTT4	Bobonaro	Timor	Normal
P3	A-suko1	Sukoharjo, Central Java	Java	Normal	P7	Rc-NTT5	Soe	Timor	Normal
P3	A-suko2	Sukoharjo, Central Java	Java	Normal	P7	Rc-NTT6	Atambua	Timor	Normal
P3	Rc-39	Asembagus, East Java	Java	Normal	P7	Rc-NTT7	Atapupu	Timor	Normal
P3	Rc-40	Asembagus, East Java	Java	Normal	P7	Rc-NTT8	East Sumba	Sumba	Normal
P3	Rc-43	Banyuwangi, East Java	Java	Normal	P7	Rc-NTT9	East Sumba	Sumba	Normal
P3	Rc-45	Purwodadi, East Java	Java	Normal	P8	Rc-77	East Kalimantan	Kalimantan	Normal
P3	Rc-54	Ponorogo, East Java	Java	Normal	P8	Rc-96	East Halmahera	Halmahera	Normal
P3	Rc-59	Malang, East Java	Java	Normal	P8	Rc-97	East Halmahera	Halmahera	Normal
P3	A-Jys	Malang East Java	Java	Normal	P8	Rc-98	Tidore	Tidore	Normal
P3	Rc-101	Tuban East Java	Java	Normal	P8	Rc-99	East Tidore	Tidore	Normal
P3	Rc-105	Asembagus, East Java	Java	Normal	P8	Rc-100	West Halmahera	Halmahera	Normal
P3	Rc-106	Asembagus, East Java	Java	Normal	P8	Rc-102	Bula	Seram	Normal
P3	Rc-107	Asembagus, East Java	Java	Normal	P8	Rc-104	Bula	Seram	Normal
P3	Rc-108	Asembagus, East Java	Java	Normal	P9	Rc-I1	Introduced	China	Dwarf
P3	A-tbn1	Tuban, East Java	Java	Normal	P9	Rc-I2	Introduced	China	Dwarf
P3	A-tbn2	Tuban, East Java	Java	Normal	P9	Rc-I3	Introduced	China	Dwarf
P3	A-tbn3	Tuban, East Java	Java	Normal	P9	Rc-I4	Introduced	China	Dwarf
P3	A-tbn4	Tuban, East Java	Java	Normal	P9	Rc-I5	Introduced	China	Dwarf
P3	A-lmg1	Lamongan, East Java	Java	Normal	P9	Rc-I6	Introduced	China	Dwarf
P3	A-lmg2	Lamongan, East Java	Java	Normal	P9	Rc-I7	Introduced	Thailand	Dwarf
P3	A-lmg3	Lamongan, East Java	Java	Normal	P9	Rc-I8	Introduced	Thailand	Dwarf
P4	Rc-M1	Bata-Bata	Madura	Normal	P9	Rc-I9	Introduced	Japan	Dwarf
P4	Rc-M2	Kangean	Madura	Normal	P10	ASB 81	Commercial varieties		Normal
P4	Rc-M3	Palengaan	Madura	Normal	P10	Agribun ASB 119	Commercial varieties		Normal
P4	Rc-M4	Kangean	Madura	Normal					

Note: Pop: population; Acc: accession

Table 2. SSR markers were used in the present study

Primer ID	Repeat	Primer sequence	Expected amplicon size	Annealing temp.	Predicted gene	Gene ID
RcSSR-4	(AGA)5	F: CCTGTTGAGAGAGGCTATGG R: CCACAAGATCGACTGTTTCC	136	56.5°C	Indole-3-acetic acid GH3.17	LOC8285710
RcSSR-5	(GTG)8	F: TGAGGCTGAGCTAGAGTTGG R: GCTGAGATACAACGGAAGGA	149	56.5°C	Auxin responsive protein IAA13	LOC8265723
RcSSR-12	(AAAAG)5	F: CACATCAAGAAAGGGAGCAAG R: AGGGTGGTTTTCATGACTGC	163	58.5°C	Auxin response Factor 3	LOC8281869
RcSSR-23	(TCCACC)4	F: TCCCCAGCTCCTACTGAAGA R: GAGAAGGAGATGCCGATTTG	127	62.0°C	Zinc finger protein (ZAT 10)	LOC8272263
RcSSR-40	(AGA)10	F: CCCCTGCTTCCTGAACTAGA R: CCAAGCATAGTCCTCAAATGAA	156	58.5°C	Gibberelin-2-beta-dioxygenase2	LOC8265362

RESULTS AND DISCUSSION

Genetic diversity using SSR markers

The summary statistics of five SSR markers among 123 accessions of castor germplasm are shown in Table 3. Five polymorphic SSR markers produced 26 alleles with an average of 5.2 alleles per locus. The allele size ranged from 113 bp to 184 bp. The average number of genotypes was 7.6. RcSSR-5 had the highest number of genotypes with 13 genotypes. RcSSR-12 and RcSSR-23 only produced a homozygous band, as indicated by the zero value of Ho. The other three markers yielded homozygous and heterozygous bands. PIC value determined the relative informativeness of each marker. MAF indicates the frequency of allele that is most common in a population. The MAF had an average of 0.460 and ranged from 0.340 to 0.545. The PIC value of the SSR loci across 123 castor accessions averaged 0.6 and ranged from 0.5 for RcSSR-12 to 0.68 for RcSSR-5, indicating a moderate to high marker's ability to differentiate the accessions. The polymorphism generated by RcSSR-40 primer as representative SSR primers and accessions are shown in Figure 1.

The genetic diversity parameters of castor populations are shown in Table 4. The allele number ranged from 1.6 for commercial varieties to 4.4 for breeding lines. The adequate number of alleles showed the number of equally frequent alleles that would take the expected heterozygosity. The highest Ne was achieved by the breeding lines with the highest He value. The observed heterozygosity of all populations had an average of 0.032. Population from Madura, Bali, Kalimantan, Halmahera-Tidore-Seram, and commercial varieties did not have heterozygous genotypes in their SSR locus in this study (zero value of Ho). The expected heterozygosity (He) is an indicator of genetic diversity. He value ranged from 0.3 to 0.683. Breeding line, Sumatra, Java and introduced accessions had high genetic diversity. Overall, the gene diversity of castor collection from Indonesia was 0.486, indicating a moderate level of genetic diversity.

The genetic relationship among accessions is shown by the Neighbor-Joining (NJ) tree based on Nei's genetic distance (Figure 2). The castor germplasm collection is separated into three groups (Figure 2A). There were mixed accessions in each group. Breeding lines, accessions from Sumatra, Java, Lombok-Sumbawa, and introduced were separated into three clusters. Accessions from Madura were incorporated in groups I and III. Accessions from Bali, Flores, Timor, and Sumba were placed in groups I and II. Both commercial varieties were placed in group III. Generally, there was little geographical origin pattern of the grouping of the accession based on genetic distance using SSR markers in this study.

Table 3. Summary statistics of five polymorphic SSR markers

Marker name	Allele size	Allele number	Genotype number	Ho	MAF	PIC
RcSSR-4	113-150	6	6	0.000	0.447	0.671
RcSSR-5	149-172	6	13	0.124	0.340	0.682
RcSSR-12	143-175	4	4	0.000	0.545	0.505
RcSSR-23	113-142	4	4	0.000	0.529	0.514
RcSSR-40	138-184	6	11	0.098	0.419	0.656
Mean		5.2	7.6	0.044	0.460	0.606
SD		0.980	3.720	0.061	0.077	0.080

Note: Ho: observed heterozygozity; MAF: major allele frequency; PIC: polymorphic information content

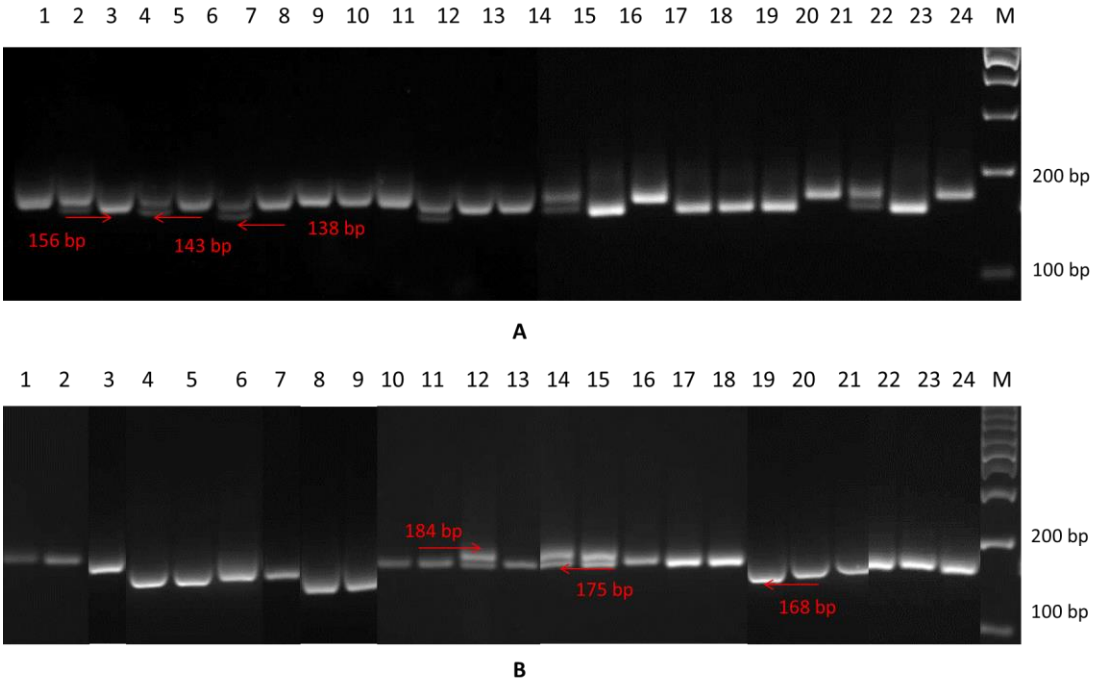

Figure 1. Banding patterns generated by RcSSR-40 primer. A. breeding lines (1-24 = A1-A24), B. some accessions originated from Sumatra (1-3), Java (4-6), Madura (7), Bali (8 and 9), Lombok-Sumbawa (10-13), Flores-Timor-Sumba (14-18), Halmahera-Tidore (19-21), and Introduced accessions from China (22-24)

Table 4. Genetic diversity parameters of 10 castor populations

Population	Accession number	Na	Ne	Ho	He	PPL (%)
P1: Breeding line	41	4.400	3.288	0.049	0.683	100
P2: Sumatra	8	3.200	2.615	0.025	0.602	100
P3: Java	29	4.000	2.442	0.058	0.566	100
P4: Madura	5	2.000	1.742	0.000	0.416	100
P5: Bali	4	2.400	2.027	0.000	0.475	100
P6: Lombok-Sumbawa	8	2.800	1.982	0.075	0.402	80
P7: Flores-Timor-Sumba	9	2.800	1.974	0.667	0.443	100
P8: Kalimantan-Halmahera-Tidore-Seram	8	2.800	1.853	0.000	0.438	100
P9: Introduction	9	3.000	2.159	0.044	0.533	100
P10: Commercial varieties	2	1.600	1.600	0.000	0.300	60
Total	123					
Mean		2.900	2.168	0.032	0.486	94
SE		0.167	0.102	0.010	0.025	4.27

Note: Na=number of allele; Ne=effective number of allele; Ho=observed heterozygosity; He=expected heterozygosity; PPL=percentage of polymorphic loci.

Population structure using STRUCTURE grouped the collection into three subpopulations (Q) as inferred by delta k=3 (Figure 2B). Similar to the previous clustering method, the accessions from different islands were grouped in the same subpopulations. In Q1, the most members were breeding lines and Java accessions. The accessions from Sumatra mainly joined the Q1. The majority of accessions from Lombok-Sumbawa and Flores-Timor-Sumba grouped in Q2. These islands are located in the Southern East of the archipelago and are known as Nusa Tenggara islands. Seven accessions from Kalimantan-Maluku islands (Halmahera, Tidore, and Seram) were grouped in Q3, and only one accession joined the Q2.

The barplot of the subpopulation showed the genotype with mixed and pure alleles (inferred by the color in every line) (Figure 2C). The barplot showed the proportion of genotype with pure allele was bigger than the mixed one. The assigning accession to the subpopulation is also used in association analysis to reduce the spurious association due to population structure in the germplasm collection.

Agronomic trait variation

The 123 accessions of castor showed high variation in the agronomic trait, including plant architecture and yield traits. Plant height, number of nodes, and node length average showed a significant effect of total entries, check, accessions, and interaction between check and accessions. Whereas stem diameter, branch number, and capsules number per raceme were not different among the accessions (Table 5). Raceme length showed a significant difference of check. For yield traits, 100-seed weight and oil content showed a significant effect of total entries, check, accessions, and interaction between check and accessions. Flowering day is only difference between the check. Seed weight per raceme and total seed weight per plant of the accessions were significantly different from the check plants (Table 6).

The mean, minimum and maximum values of the agronomic traits among 123 accessions are shown in Table 7. The germplasm collection had a wide range of plant height, the number of nodes, and node length averages. Accession A-11 (breeding line) had the lowest plant height (24.50 cm), whereas A-Suko1 (Java) had the highest one (329.33 cm). The number of nodes of the main stem ranged from 7 to 33.50. Node length average had a mean value of 6.89 cm with the minimum and maximum values of 1.66 and 16.42 cm, respectively. Raceme length is varied between 9.33 to 112 cm.

Accession A-3 had the earliest flowering day (48 days) and accession Rc-105 (Java) had the latest one (143 days). Accession Rc-S5 (Sumatra) had the highest value of total seed weight per plant and 100 seed weight. Rc-S5, Rc-S7, and Rc-S8 had bigger seeds than other accessions. Their 100 seeds weight was more than 80 g. This characteristic was only found in accession from Sumatra, indicating that this accession type is specific to Sumatra. The oil content had a mean value of 36.47% and ranged from 20.47 to 51.15%.

Pearson's correlation coefficient (r) measures the strength of linear association between two variables (Table 8). Among the plant architecture traits, the highest correlation corresponded to the plant height and nodes length average (r = 0.82) and plant height with the number of nodes (r = 0.78). For the yield component traits, a high correlation was found between capsules per raceme with seed weight per raceme and total seed weight per plant. Plant height and the number of nodes had a significant positive correlation with the flowering day. Branch number and oil content did not significantly correlate with the other traits.

Association between agronomic traits and SSR markers

The presence of genetic variation in phenotype and genotype of the germplasm population can be used to detect the association between molecular markers and agronomic traits. This association can be utilized in marker-assisted selection for increasing the selection process efficiently. The result of association analysis using GLM (P+G+Q) and MLM (P+G+Q+K) are shown in Table 9. The significant association is indicated by p-value <0.05 and <0.01. The association level is inferred by the R^2 value. Among the SSR markers, all markers had an association with castor plant architecture and yield traits, except RcSSR-5 as detected by GLM. While using MLM, RcSSR-4, RcSSR-12, and RcSSR-23 were significantly associated with plant architecture and yield traits of castor.

Plant height had significant association with RcSSR-12_175 and RcSSR-23_142 (GLM). Using MLM association with RcSSR-12 could also be detected between RcSSR-12_175 and plant height. For the number of nodes, the association was only detected by the GLM model. For node length average, there were associations with five marker alleles that could be detected by GLM. However, using MLM there was only left one association between RcSSR-23_113 and the trait. RcSSR-4_125 was the only marker allele that was significantly associated with yield component traits (seed weight/raceme) using the MLM model. In overall, the strongest association between traits and SSR markers in this study was flowering day and RcSSR-12_175 as indicated by probability values of 0.0008. The result of association analysis in this study also revealed the overlap correlation between the marker alleles and the traits. For instance, RcSSR-12_175 had an association with plant height, node length average, raceme length, flowering day, and 100-seed weight. In addition, RcSSR-23 _113 had an association with node length average, branch number, raceme length, and flowering day.

Table 5. Mean squares from ANOVA for augmented randomized complete block design for plant architecture traits measured

Source	Df	Plant height	Number of nodes	Node length average	Canopy width	Stem diameter	Branch number	Raceme length
Block	3	655^{ns}	29.30^{ns}	1.14^{ns}	313^{ns}	0.05^{ns}	3.329^{ns}	182.2^{ns}
Total entries	122	4708*	20.17*	9*	740^{ns}	0.449^{ns}	207.92^{ns}	357.0^{ns}
Check	2	6387*	88.22**	7.71^{ns}	51^{ns}	0.254^{ns}	1.591^{ns}	1930.4*
Accessions	119	4540*	18.83*	8.83*	711^{ns}	0.445^{ns}	1.691^{ns}	326.8^{ns}
Check vs accessions	1	20415**	43.94*	31.78*	5563*	1.347^{ns}	3.521^{ns}	803.4^{ns}
Error	6	840	3.72	2.13	421	0.367	1.408	346.7

Note: *: Significant at the 0.05 probability level; **: Significant at the 0.01 probability level; ^{ns}: Non significant at the 0.05 probability level

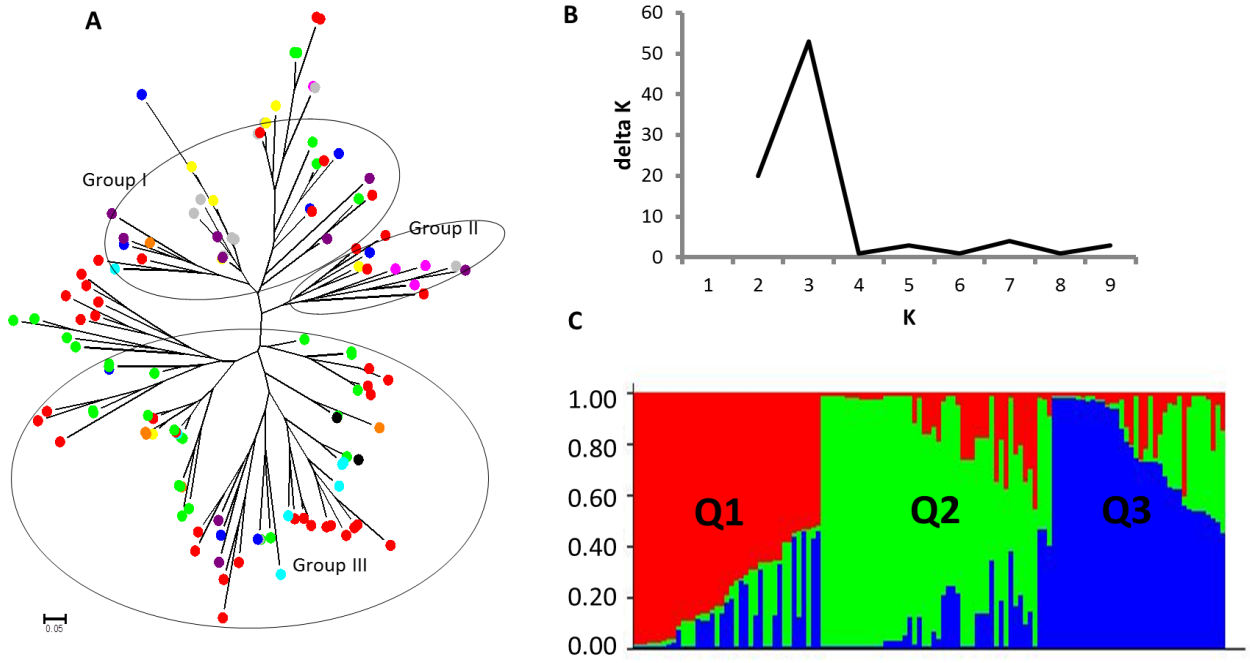

Figure 2. The genetic relationship and population structure of 123 castor accessions. A. Neighbor-joining (NJ) tree based on Nei's genetic distance. The color circle represents the population P1 (red), P2 (blue), P3 (green), P4 (orange), P5 (pink), P6 (yellow), P7 (grey), P8 (light blue), P9 (purple), and P10 (black). B. Delta K showed the true K = 3. C. Barplot showed three subpopulations (Q) of castor collections

Table 6. Mean squares from ANOVA for augmented randomized complete block design for yield component traits measured

Source	df	Flowering day	Capsules per raceme	Seed weight per raceme	100 seed weight	Total seed weight per plant	Oil content (%)
Block	3	60.3^{ns}	1223.0^{ns}	1253.0^{ns}	8.98^{ns}	14081^{ns}	23.53^{ns}
Total Entries	122	236.7^{ns}	441.2^{ns}	440^{ns}	172.38**	2883^{ns}	62.23*
Check	2	481.1*	221.1^{ns}	678.5^{ns}	81.27*	4943^{ns}	16.35^{ns}
Accessions	119	233.9^{ns}	441.2^{ns}	414.5^{ns}	174.38**	2725^{ns}	62.69*
Check vs accessions	1	77.9^{ns}	885.3^{ns}	2995.6*	116.76*	22425*	99.50*
Error	6	65	316.7	344.8	12.70	3130	14.29

Note: *: Significant at the 0.05 probability level; **: Significant at the 0.01 probability level; ^{ns}: Non significant at the 0.05 probability level

Table 7. Mean, minimum and maximum values of agronomic traits

Variables	Mean	SD	Min.	Accession	Max.	Accession
Plant height/PH (cm)	119.74	74.14	24.50	A11	329.33	A-Suko1
Number of nodes/NN	15.83	4.32	7.00	A-lmg3	33.50	Rc-S5
Node length average/NL	6.89	2.97	1.66	A-37	16.42	Rc-58
Canopy width/CW (cm)	144.32	29.06	84.33	A-10	213.00	Rc-18
Stem diameter/SD (cm)	2.74	0.70	1.37	A-8	4.34	A-Jys
Branch number/BN	2.83	1.64	0.67	A-21, Rc-NTB8, Rc-S7	6.67	Rc-I8
Raceme length/RL (cm)	57.23	18.25	9.33	Rc-I3	112.00	A-6
Flowering day/FD	69.07	16.25	48.00	A-3	143.00	Rc-105
Capsules per raceme/CR	45.61	21.29	6.00	A-28	128.33	A-36
Seed weight per raceme/SW (g)	33.65	24.15	1.79	A-28	115.00	Rc-102
Total seed weight per plant/TSW (g)	94.83	63.68	3.53	A-28	317	Rc-S5
100 seed weight/100SW (g)	29.94	13.20	8.00	Rc-98	98.00	Rc-S5
Oil content/OIL (%)	36.47	8.25	20.47	Rc-KF1	51.15	Rc-NTT5

Table 8. Pearson's correlation coefficient between 13 agronomic traits

	PH	NN	NL	CW	SD	BN	RL	FD	CR	SW	TSW	100SW	OIL
PH	1												
NN	0.78**	1											
NL	0.82**	0.44**	1										
CW	0.59**	0.43**	0.57**	1									
SD	0.72**	0.62**	0.57**	0.68**	1								
BN	-0.02	0.10	-0.06	0.33**	0.21**	1							
RL	-0.04	-0.07	-0.03	0.21*	-0.03	-0.01	1						
FD	0.77**	0.71**	0.57**	0.38**	0.57**	-0.05	-0.15	1					
CR	0.22*	0.10	0.25**	0.38**	0.28**	0.10	0.37**	0.05	1				
SW	0.36**	0.32**	0.33**	0.41**	0.36**	0.00	-0.09	0.25**	0.75**	1			
TSW	0.30**	0.23**	0.32**	0.47**	0.16**	0.09	0.35	0.16**	0.74**	0.87**	1		
100SW	0.23**	0.30**	0.16	0.05	0.32**	-0.23	-0.16	0.44**	0.25**	0.24**	0.22*	1	
OIL	-0.06	-0.01	-0.06	-0.15	-0.10	-0.02	-0.08	-0.06	-0.04	-0.06	-0.02	-0.02	1

Note: PH: plant height; NN: number of nodes; NL: node length average; CW: canopy width; SD: stem diameter; BN: branch number; RL: raceme length; FD: flowering day; CR: capsules per raceme; SW: seed weight per raceme; TSW: total seed weight per plant; OIL: oil content

Table 9. Association of SSR markers with agronomic traits of plant architecture and yield in castor collections using GLM (P+G+Q) and MLM (P+G+Q+K) model

Trait	Marker allele	GLM (P+G+Q)			MLM (P+G+Q+K)		
		F ratio	p-value	R^2	F ratio	p-value	R^2
Plant height	RcSSR-12_175	5.57	0.02	0.04	5.11	0.02	0.04
	RcSSR-23_142	4.15	0.04	0.03	3.94	Ns	0.03
Number of nodes	RcSSR-23_127	4.82	0.03	0.04	0.28	Ns	0.002
Node length average	RcSSR-12_143	4.14	0.04	0.03	3.13	Ns	0.03
	RcSSR-12_175	4.64	0.03	0.03	2.86	Ns	0.02
	RcSSR-23_113	9.55	0.002	0.07	6.60	0.01	0.05
	RcSSR-23_142	5.98	0.016	0.04	2.52	Ns	0.02
	RcSSR-40_168	9.03	0.003	0.06	0.04	Ns	0.003
Canopy width	RcSSR-12_143	4.57	0.03	0.03	4.72	0.03	0.03
	RcSSR-40_143	5.12	0.03	0.04	2.86	Ns	0.02
Branch number	RcSSR-12_143	6.23	0.01	0.05	3.81	Ns	0.03
	RcSSR-23_113	6.19	0.01	0.05	5.42	0.02	0.04
Raceme length	RcSSR-12_175	4.52	0.04	0.04	1.65	Ns	0.02
	RcSSR-23_113	4.27	0.04	0.03	1.26	Ns	0.01
Flowering day	RcSSR-4_150	4.70	0.03	0.04	3.30	Ns	0.03
	RcSSR-12_175	11.78	0.0008	0.08	5.59	0.02	0.05
	RcSSR-23_113	10.37	0.002	0.08	3.88	Ns	0.03
	RcSSR-40_156	4.68	0.03	0.04	2.08	Ns	0.02
Seed weight/raceme	RcSSR-4_125	6.89	0.009	0.05	4.78	0.03	0.04
	RcSSR-23_142	4.30	0.04	0.04	3.94	Ns	0.03
	RcSSR-40_184	4.46	0.04	0.04	0.39	Ns	0.003
100-seed weight	RcSSR-4_125	6.20	0.01	0.05	2.10	Ns	0.02
	RcSSR-12_175	4.67	0.03	0.04	4.00	Ns	0.03
	RcSSR-23_127	5.27	0.02	0.04	1.15	Ns	0.009
	RcSSR-23_138	5.11	0.03	0.04	1.52	Ns	0.013
Total seed weight/plant	RcSSR-4_125	8.94	0.003	0.07	3.35	Ns	0.03
	RcSSR-4_136	5.73	0.018	0.05	2.23	Ns	0.02
	RcSSR-23_142	6.31	0.01	0.04	3.07	Ns	0.03
	RcSSR-40_175	5.39	0.02	0.04	2.66	Ns	0.02
Oil content	RCSSR-12_163	4.18	0.04	0.03	2.59	Ns	0.02

Discussion

The level of genetic diversity in a germplasm collection is crucial in breeding effort. This study evaluated a collection of castor germplasm which included 41 breeding lines for dwarf-type castor selection and 73 accessions from the islands throughout Indonesia. Many researchers have documented the genetic diversity of castor germplasm. Vasconcelos et al. (2016) assessed the genetic diversity of castor genotypes in Brazil using AFLP and ISSR markers. Muraguri et al. (2020) analyzed the genetic diversity of castor collection from Africa, China, and India using variation in castor chloroplast genomes. Kim et al.

(2021) used ISSR and RAPD markers to examine the genetic diversity of global castor germplasm. The research used either a world germplasm collection or a collection from a single country. The recent study was the first to look at the genetic diversity of castor accessions from several Indonesian islands. The result demonstrated that castor collections originating from a variety of Indonesian islands have moderate genetic diversity (as indicated by the He/Gene diversity score of 0.5). Senthilvel et al. (2016) and Wang et al. (2017) reported a modest degree of genetic diversity in castor collections. On the other hand, Seo et al. (2011) found a low He value (0.30) among Korean castor accessions. The discrepancies in the diversity estimates (He value) in many researches could be due to variances in sample size and the representativeness of the germplasm collections (Senthilvel et al. 2016; Wang et al. 2017). In addition, given the differences in geographical characterization between the archipelago and continental countries, the diversity of the Korean castor collection used by Seo et al. (2011) could be smaller than in our study.

The genetic relationship among castor accessions could result in three groups. The accessions were not separated based on their origin. There were admixtures among the various populations. There were accessions from nearby islands and long-distance accessions in the group. Several researchers have documented the lack of spatial organization of castor germplasm. According to Pecina-Quintero et al. (2013), there was a tendency for little clustering by geographical origin of castor accessions from Chiapas, Mexico. Castor accessions from different locations on two Indian islands did not split based on their geographic origin (Kanti et al. 2015). Castor collections have a limited geographic structure due to diverse sources of germplasm accessions and the prevalence of genetic material exchange via human migration (Foster et al. 2010). The population structure analysis could assign individual accession based on their estimated population (Q) and lower the likelihood of false-positive in association analysis (Pritchard et al. 2000b).

Plant architecture (plant height, node length, branch number, and raceme length) and seed weight per plant are target traits in castor breeding programs for a dwarf type with a high yield (Merkoroupoulos et al. 2016). The 41 breeding lines employed in this study had heights less than 1.5 m, whereas the other genotypes had plant heights of more than 1.5 m. Some accessions may potentially reach a height of 3 meters. A few accessions had plant height less than 1.5 m (34%). The average plant height of all introduced accessions was 92.75 cm. This finding implied that the germplasm collection had enough variation for parents' hybridization candidates or the selection.

The dwarf-type castor prefers erect plants with a long primary raceme. The castor collections' average raceme length was 57.23±18.25 cm. Castor dwarf hybrid has a primary raceme 55-63 cm in length (Merkouropoulos et al. 2016). The finding implies that the accessions in this present study had the potential as genetic material for the dwarf-type castor with long primary raceme. There were no significant differences in seed weight per raceme and total seed weight per plant across accessions for yield attributes, indicating that the dwarf plant has the potential to produce a yield comparable to castor plants with a high stalk. Hu et al. (2016) reported that underplanting density smaller than 12,000 seedling/hm^2, the dwarf plant had a lower yield, but above that, the dwarf plant had a higher seed yield than the high-stalk type.

The association analysis utilized GLM (fixed effect) and MLM (a fixed and random effect). MLM can incorporate information about relationships among individuals. The average relationship between the accessions is estimated using kinship (K) determined from the markers data. An approach that combines input from both Q and K improves statistical power compared to Q alone (Bradbury et al. 2007). Using the GLM, there were fourteen and sixteen associations between the marker alleles and plant architecture and yield component traits. The MLM model reduced the number of significant associations. There were only six associations for both plant architecture and yield traits.

There was an overlap association between the marker and the agronomic traits. RcSSR-12_175 had a significant association with plant height, node length average, and flowering day. Plant height and node length average had a highly significant correlation as indicated by Pearson's Correlation coefficient. Plant height is determined most by stem elongation. The length or number of internodes in the stem is connected to stem elongation (Feng et al. 2019). Castor plant height was reduced by stem internode removal (Alemaw and Merera 2015). Nugraha et al. (2016) observed a substantial relationship between two iron toxicity tolerance traits in rice and the same SNP marker. Terryana et al. (2017) reported similar findings in soybean marker-trait association. Furthermore, plant height positively connected with the flowering day and significantly linked to the same marker, RcSSR-12_175. However, the correlation between the agronomic characters and the SSR marker alleles in this study was not high (indicated by the low value of R^2). As a result, before being employed as marker-assisted selection, these linked-trait markers should be validated with another breeding population to ensure consistency.

SSR markers were created in this work using the sequences from gene families with varied expressions in dwarf and high-stalk castor and *Jatropha* plants (Hu et al. 2017; Shi et al. 2018; Feng et al. 2019). *Jatropha curcas* and *Ricinus communis* share high sequence similarities and gene families (Hu et al. 2017; Lu et al. 2021). The SSR markers from the coding region can produce marker-trait association efficiently (Izzah et al. 2016). RcSSR-12 and RcSSR-23 were the SSR markers that MLM found to have significant relationships. RcSSR-12 is a part of the plant hormone gene, Auxin Response Factor 3 (ARF3). Auxin response factor plays a role in affecting the auxin response and transcribing the signal of a gene (Roosjen et al. 2018). According to Feng et al. (2019), one member of the RcARF gene family in the dwarf castor was up-regulated compared with the high-stalk castor. RcSSR-23 is a marker from repeat sequences in the Zinc finger protein (ZAT 10) gene. This protein can attach to DNA, RNA, and proteins. They have many biological processes, including plant growth

and stress resistance (Han et al. 2020). According to Shi et al. (2018), dwarf plants emerge from ectopic expression of the *J. curcas* zinc finger gene in transgenic tobacco. The discovery of the trait-linked marker in this study paves the way for further research into the function and regulation of genes in castor dwarf-type. Furthermore, the overlap association between SSR markers and the agronomic traits is noteworthy for the gene pyramiding strategy via marker-assisted selection (Dormatey et al. 2020). Gene pyramiding is the act of combining desired traits by stacking numerous genes into a single genotype (Lombardo et al. 2016).

To conclude, the 123 accessions used in the study had a moderate genetic diversity. The genetic relationship analysis revealed three groups with little geographic structuring. Plant height, node length average, number of nodes, 100-seed weight, and oil content varied significantly across the Indonesian accessions. Furthermore, the association analysis indicated six significant correlations between the SSR markers and plant height, node length average, canopy width, branch number, flowering day, and seed weight per raceme.

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