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Genetic Diversity of Castor Bean (*Ricinus communis* L.) Revealed by ISSR and RAPD Markers

HyokChol Kim [1], Pei Lei [1], Aizhi Wang [1], Shuo Liu [1], Yong Zhao [2,3], Fenglan Huang [2,3,*], Zhenliang Yu [4], Guoli Zhu [5], Zhibiao He [5], Deyun Tan [6], Hongwei Wang [7] and Fanjuan Meng [1,*]

1 College of Life Science, Northeast Forestry University, Harbin 150040, China; 15104601275@163.com (H.K.); 18800465845@163.com (P.L.); mfj19751@163.com (A.W.); ls977573@163.com (S.L.)
2 College of Life Science, Inner Mongolia University for the Nationalities, Tongliao 028043, China; 15334946958@163.com
3 Inner Mongolia Key Laboratory of Castor Breeding, Tongliao 028043, China
4 Heilongjiang Provincial Hydraulic Research Institute, Harbin 150080, China; yzl56123@163.com
5 Tongliao Academy of Agricultural Science, Tongliao 028000, China; 18800465846@163.com (G.Z.); hezhibiao@139.com (Z.H.)
6 Academy of Agricultural Sciences, Zibo 255000, China; tandeyun@126.com
7 Shanxi Academy of Agricultural Sciences, Fenyan 032200, China; jzswhw@163.com
* Correspondence: huangfenglan@imun.edu.cn (F.H.); m13313626779@163.com (F.M.)

Abstract: Castor (*Ricinus communis* L.), known as castor oil plant or castor bean, is a non-edible oilseed crop. In the present study, the genetic diversity among 54 samples (3 wild and 51 cultivated) collected worldwide was evaluated using inter-simple sequence repeats (ISSRs) and random amplified polymorphic DNA (RAPD) markers. A total of 9 ISSR primers produced 83 high-resolution bands with 61 (74.53%) as polymorphic. The percentage of polymorphic bands per primer and the genetic similarity coefficient ranged from 54.55% (UBC-836) to 100% (UBC-808) and from 0.74 to 0.96, respectively. A total of 11 out of 20 RAPD primers amplified unique polymorphic products with an average percentage of polymorphic bands of 60.98% (56 polymorphic bands out of a total of 90 bands obtained). The percentage of polymorphic bands per primer ranged from 25% (OPA-02 and B7) to 90.91% (B21) with the genetic similarity coefficient ranging from 0.73 to 0.98. The unweighted pair group method with arithmetic averages (UPGMA) dendrogram using two molecular markers divided 54 castor genotypes into three groups. Furthermore, based on morphological data, all 54 castor varieties were grouped into three main clusters. The genetic diversity analysis based on two molecular makers showed that most varieties from China were closely related to each other with three varieties (GUANGDONGwild, ZHEJIANGWild, and HANNANWild) belonging to a wild group separated from most of the cultivated castor samples from China, India, France, and Jordan. These results suggested that the cultivated castor contains a narrow genetic base. Accordingly, we recommend that wild castor genetic resources be introduced for breeding novel castor varieties. Furthermore, the Vietnam, Malaysia, Indonesia, and Nigeria accessions were clustered into the same group. The results of principal coordinate analysis (PCoA) and UPGMA cluster analysis were consistent with each other. The findings of this study are important for future breeding studies of castor.

Keywords: *Ricinus communis* L.; genetic diversity; inter simple sequence repeat (ISSR); random amplified polymorphic DNA (RAPD)

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1. Introduction

Castor (*Ricinus communis* L.) is an annual or perennial plant in Euphorbiaceae and is indigenous to the southeastern Mediterranean basin, East Africa, and India [1]. The castor is known to be highly tolerant in drought [2,3] and its leaves can be used as the food of silkworm. In addition, castor bean with an oil content of 45–55% in the seeds is considered

as one of the top 10 oil crops worldwide [4,5]. Castor oil is the non-drying oil with high viscosity and is not easily clogged even under a low temperature of −56 °C [6]. Therefore, castor oil can be used for the production of biofuel in the chemical industry [7,8]. The demand for castor products such as castor oil and its derivatives has increased worldwide since 1991 [9–11]. After 2014, the import rate of castor raw materials in China has increased over 90% [10,12]. To date, many methods or techniques have already been used to improve breeding and productivity of castor [13–16].

Analyses of genetic diversity are very important for the development of genetic resources and new varieties of castor. In the past, genetic diversity studies based on morphological and biochemical characteristics have been conducted in many animal and plant varieties [17,18]. In particular, morphological traits are important phenotypic indicators in the development of sustainable crop breeding [19]. However, the study of genetic diversity based only on the morphological and geographical characteristics is ineffective, due to the strong influence of environmental complexity and genetic responses of plants. To date, many stable and efficient molecular markers have been developed. For example, studies of genetic diversity using molecular markers, such as simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and single nucleotide polymorphisms (SNPs) have been widely applied in castor [20,21]. However, these methods have shown some disadvantages such as the high cost of AFLP and radioactive labeling of RFLP [22,23]. In recent years, developing SSR markers through mining SSR motifs in the whole genome has reduced the cost greatly, but has shown somewhat lower congruence with dominant marker data [24,25]. Both random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) have been used to study the genetic diversity and evaluate the genetic mutations of many plants [26,27]. ISSR technologies were found to be reproducible and effective to uncover polymorphism and obtain more composite marker patterns [28,29]. In contrast, RAPD has low rate of replicability, but is very efficient in analyzing genetic diversity because it does not need sequence data to design molecular primers [30,31].

Generally, both ISSR and RAPD markers are used in combination to improve their resolving power for genetic analysis of many plants [32]. In the past decade, combined morphological and molecular analyses have been widely used to investigate genetic diversity and phylogenetic relationships. For example, morphological characters and RAPD were demonstrated useful for reconstructing a *Citrus* phylogeny [33] and morphological traits and both RAPD and ISSR markers were evaluated to discriminate 15 potato cultivars [34] and to establish unique identification profiles in 26 sorghum varieties [35].

To obtain data of high quality, the study of genetic diversity based on both morphological characters and molecular markers (i.e., RAPD and ISSRs) is very important for castor breeding and production. To date, there are only a few studies available on analyzing the genetic variation by using combined RAPD and ISSR markers and morphometric characters. In this study, we aim to resolve the relationship between wild and cultivated varieties of caster from different accessions collected worldwide. These results provide the basic information for cultivating new varieties of caster and contribute to the selection and verification of novel varieties and the preservation of castor germplasms.

2. Materials and Methods

2.1. Plant Materials

The seeds of 54 castor varieties from different regions of the world were provided by the Inner Mongolia Key Laboratory of Castor Breeding, China. During the experiment, four quantitative traits (seed length (mm), width (mm), thickness (mm), and weight (g)) were measured on ten random samples. Detailed information on these 54 accessions is showed in Table 1 and Figure 1. Seed coat colors were scored by comparison with standards by multiple observers.

Table 1. List of the castor beans (*R. communis* L.) included in the work.

No.	Variety	Location	Length (mm)	Width (mm)	L/W Ratio	Thickness (mm)	Weight (g)
1	GUANGDONGWild	Guangdong (China)	10.24 ± 0.52 ^{f}	6.60 ± 0.19 ^{de}	1.55 ± 0.04 ^{bc}	4.96 ± 0.18 ^{de}	0.19 ± 0.02 ^{de}
2	ZHEJIANGWild	Zhejiang (China)	10.44 ± 0.33 ^{f}	6.46 ± 0.17 ^{e}	1.62 ± 0.03 ^{b}	4.97 ± 0.19 ^{de}	0.16 ± 0.01 ^{de}
3	HAINANWild	Hainan (China)	9.85 ± 0.15 ^{g}	5.97 ± 0.03 ^{e}	1.65 ± 0.03 ^{ab}	4.26 ± 0.09 ^{e}	0.13 ± 0.01 ^{e}
4	Lubri	India	13.12 ± 0.78 ^{cd}	8.32 ± 0.53 ^{c}	1.58 ± 0.11 ^{bc}	6.08 ± 0.29 ^{bc}	0.36 ± 0.05 ^{cd}
5	SANGRAM9	India	13.15 ± 0.51 ^{cd}	8.25 ± 0.26 ^{c}	1.59 ± 0.55 ^{bc}	6.13 ± 0.26 ^{bc}	0.35 ± 0.03 ^{cd}
6	HYCASTOR	India	12.56 ± 0.28 ^{cd}	8.76 ± 0.08 ^{bc}	1.43 ± 0.02 ^{d}	6.34 ± 0.15 ^{bc}	0.37 ± 0.03 ^{cd}
7	CSR.181	France	14.10 ± 0.21 ^{c}	8.17 ± 0.32 ^{c}	1.73 ± 0.03 ^{a}	6.38 ± 0.31 ^{bc}	0.35 ± 0.02 ^{cd}
8	CSR.63	France	12.32 ± 0.28 ^{d}	8.29 ± 0.17 ^{c}	1.49 ± 0.03 ^{cd}	5.99 ± 0.16 ^{c}	0.32 ± 0.03 ^{d}
9	Middle East Castor	Jordan	14.53 ± 0.57 ^{bc}	8.83 ± 0.26 ^{bc}	1.65 ± 0.03 ^{ab}	6.53 ± 0.11 ^{b}	0.39 ± 0.02 ^{cd}
10	A063	CAOF	12.13 ± 0.28 ^{d}	8.09 ± 0.15 ^{c}	1.50 ± 0.02 ^{c}	5.83 ± 0.09 ^{c}	0.25 ± 0.04 ^{d}
11	YUNbima5	Yunnan (China)	14.21 ± 0.58 ^{c}	9.32 ± 0.69 ^{bc}	1.52 ± 0.15 ^{c}	6.49 ± 0.30 ^{bc}	0.45 ± 0.02 ^{c}
12	YUNbima4	Yunnan (China)	14.99 ± 0.72 ^{bc}	10.49 ± 0.31 ^{b}	1.43 ± 0.10 ^{d}	6.99 ± 0.28 ^{b}	0.55 ± 0.02 ^{b}
13	SHANXIbima	Shanxi (China)	14.31 ± 0.34 ^{c}	9.08 ± 0.27 ^{bc}	1.58 ± 0.01 ^{bc}	6.65 ± 0.10 ^{b}	0.42 ± 0.07 ^{c}
14	zhebima3	Tongliao (China)	12.20 ± 0.46 ^{d}	7.96 ± 0.39 ^{cd}	1.53 ± 0.05 ^{c}	5.91 ± 0.30 ^{c}	0.30 ± 0.04 ^{d}
15	zhebima4	Tongliao (China)	12.42 ± 0.51 ^{d}	8.20 ± 0.09 ^{c}	1.51 ± 0.05 ^{c}	6.78 ± 0.28 ^{b}	0.31 ± 0.03 ^{d}
16	Tongbima5	Tongliao (China)	13.13 ± 0.76 ^{cd}	8.12 ± 0.91 ^{c}	1.62 ± 0.26 ^{b}	6.16 ± 0.52 ^{bc}	0.32 ± 0.04 ^{d}
17	Tongbima5	Tongliao (China)	11.60 ± 0.42 ^{e}	7.88 ± 0.31 ^{cd}	1.47 ± 0.04 ^{cd}	5.73 ± 0.33 ^{c}	0.27 ± 0.03 ^{d}
18	Tongbima8	Tongliao (China)	12.84 ± 0.56 ^{cd}	8.46 ± 0.37 ^{c}	1.52 ± 0.10 ^{c}	6.47 ± 0.25 ^{bc}	0.34 ± 0.06 ^{cd}
19	Tongbima9	Tongliao	13.23 ± 0.58 ^{cd}	8.09 ± 0.49 ^{c}	1.64 ± 0.04 ^{b}	6.13 ± 0.26 ^{bc}	0.32 ± 0.06 ^{d}
20	Tongbima10	Tongliao (China)	12.31 ± 0.37 ^{d}	8.59 ± 0.29 ^{bc}	1.43 ± 0.02 ^{d}	6.29 ± 0.25 ^{bc}	0.33 ± 0.04 ^{d}
21	ZHE10	Shanxi (China)	14.14 ± 0.34 ^{c}	8.80 ± 0.41 ^{bc}	1.61 ± 0.10 ^{b}	6.07 ± 0.21 ^{bc}	0.36 ± 0.04 ^{cd}
22	SL42-30	Shanxi (China)	12.47 ± 0.34 ^{d}	8.83 ± 0.16 ^{bc}	1.41 ± 0.04 ^{d}	6.20 ± 0.25 ^{bc}	0.36 ± 0.02 ^{cd}
23	SL17-5	Shanxi (China)	13.22 ± 0.28 ^{c}	8.85 ± 0.27 ^{bc}	1.49 ± 0.03 ^{cd}	5.99 ± 0.13 ^{c}	0.37 ± 0.03 ^{cd}
24	SYUN10-18	Shanxi (China)	13.24 ± 0.19 ^{c}	8.88 ± 0.25 ^{bc}	1.49 ± 0.03 ^{cd}	6.03 ± 0.12 ^{bc}	0.38 ± 0.01 ^{cd}
25	SYUN10-24	Shanxi (China)	14.43 ± 0.30 ^{c}	9.40 ± 0.10 ^{bc}	1.54 ± 0.04 ^{c}	6.14 ± 0.05 ^{bc}	0.43 ± 0.02 ^{c}
26	20111116	Tongliao (China)	12.55 ± 0.43 ^{cd}	8.60 ± 0.10 ^{bc}	1.46 ± 0.04 ^{cd}	6.55 ± 0.09 ^{b}	0.34 ± 0.04 ^{cd}
27	20082227	Tongliao (China)	12.49 ± 0.54 ^{d}	8.38 ± 0.44 ^{c}	1.49 ± 0.05 ^{cd}	6.21 ± 0.33 ^{bc}	0.27 ± 0.06 ^{d}
28	20102189	Tongliao (China)	12.25 ± 0.52 ^{d}	8.83 ± 0.17 ^{bc}	1.39 ± 0.05 ^{de}	6.38 ± 0.34 ^{bc}	0.32 ± 0.06 ^{d}
29	20111064	Tongliao (China)	13.19 ± 0.12 ^{cd}	9.07 ± 0.10 ^{bc}	1.45 ± 0.02 ^{cd}	6.83 ± 0.26 ^{b}	0.29 ± 0.06 ^{d}
30	20111066	Tongliao (China)	12.23 ± 0.65 ^{d}	8.68 ± 0.14 ^{bc}	1.41 ± 0.05 ^{d}	6.33 ± 0.22 ^{bc}	0.29 ± 0.04 ^{d}
31	CSR181	France	9.72 ± 0.40 ^{g}	7.98 ± 0.15 ^{cd}	1.22 ± 0.02 ^{f}	6.17 ± 0.16 ^{bc}	0.28 ± 0.06 ^{d}
32	20111149	Tongliao (China)	12.90 ± 0.74 ^{cd}	8.19 ± 0.26 ^{c}	1.58 ± 0.10 ^{bc}	6.08 ± 0.09 ^{bc}	0.27 ± 0.15 ^{d}
33	20071113	Tongliao (China)	10.91 ± 0.48 ^{ef}	8.17 ± 0.29 ^{c}	1.34 ± 0.06 ^{e}	6.08 ± 0.19 ^{bc}	0.20 ± 0.04 ^{de}
34	20091078	Tongliao (China)	14.27 ± 0.21 ^{c}	8.71 ± 0.15 ^{bc}	1.64 ± 0.05 ^{b}	6.87 ± 0.14 ^{b}	0.33 ± 0.03 ^{d}
35	almAB1	Tongliao (China)	11.22 ± 0.13 ^{e}	8.51 ± 0.17 ^{bc}	1.32 ± 0.04 ^{e}	6.51 ± 0.27 ^{b}	0.30 ± 0.02 ^{d}
36	almAB2	Tongliao (China)	11.47 ± 0.05 ^{e}	7.87 ± 0.16 ^{cd}	1.46 ± 0.03 ^{cd}	6.75 ± 0.19 ^{b}	0.30 ± 0.03 ^{d}
37	almAB3	Tongliao (China)	11.86 ± 0.34 ^{de}	8.40 ± 0.53 ^{c}	1.41 ± 0.10 ^{d}	6.41 ± 0.30 ^{bc}	0.22 ± 0.03 ^{de}
38	almAB4	Tongliao (China)	11.24 ± 0.48 ^{e}	8.37 ± 0.17 ^{c}	1.34 ± 0.06 ^{e}	6.11 ± 0.08 ^{bc}	0.28 ± 0.06 ^{d}
39	Red Caster 1	Zibo (China)	12.68 ± 0.08 ^{cd}	8.21 ± 0.05 ^{c}	1.54 ± 0.02 ^{c}	6.94 ± 0.01 ^{b}	0.34 ± 0.01 ^{cd}
40	Red Caster 2	Zibo (China)	13.97 ± 0.09 ^{c}	8.88 ± 0.12 ^{bc}	1.57 ± 0.06 ^{bc}	6.41 ± 0.09 ^{bc}	0.35 ± 0.03 ^{cd}
41	Red Caster 3	Zibo (China)	12.83 ± 0.08 ^{cd}	8.41 ± 0.14 ^{c}	1.53 ± 0.02 ^{c}	5.85 ± 0.12 ^{c}	0.30 ± 0.01 ^{d}
42	Yellow Caster 1	Zibo (China)	12.53 ± 0.07 ^{cd}	8.16 ± 0.25 ^{c}	1.54 ± 0.03 ^{c}	5.82 ± 0.15 ^{c}	0.30 ± 0.02 ^{d}
43	Yellow Caster 2	Zibo (China)	13.77 ± 0.08 ^{c}	9.04 ± 0.33 ^{bc}	1.52 ± 0.04 ^{c}	6.52 ± 0.08 ^{b}	0.38 ± 0.01 ^{cd}
44	Hongwuci	Shaanxi (China)	12.87 ± 0.04 ^{cd}	8.95 ± 0.34 ^{bc}	1.44 ± 0.03 ^{d}	6.50 ± 0.22 ^{b}	0.37 ± 0.02 ^{cd}
45	T5	Zibo (China)	11.52 ± 0.05 ^{de}	7.82 ± 0.23 ^{cd}	1.47 ± 0.02 ^{cd}	6.64 ± 0.24 ^{b}	0.23 ± 0.01 ^{de}
46	Vietnam Castor	Vietnam	15.58 ± 0.24 ^{b}	9.34 ± 0.31 ^{bc}	1.69 ± 0.10 ^{ab}	6.55 ± 0.19 ^{b}	0.44 ± 0.03 ^{c}
47	Gua1	Pakistan	13.58 ± 0.14 ^{c}	8.63 ± 0.14 ^{bc}	1.57 ± 0.02 ^{bc}	6.12 ± 0.15 ^{bc}	0.39 ± 0.02 ^{cd}
48	Gua2	Pakistan	12.03 ± 0.09 ^{d}	7.63 ± 0.18 ^{cd}	1.58 ± 0.02 ^{bc}	5.71 ± 0.14 ^{c}	0.28 ± 0.08 ^{d}
49	Paraguay Castor	Paraguay	15.59 ± 0.33 ^{b}	9.54 ± 0.27 ^{b}	1.63 ± 0.03 ^{b}	6.68 ± 0.31 ^{b}	0.48 ± 0.04 ^{bc}
50	Malaysia Castor	Malaysia	11.18 ± 0.12 ^{e}	6.56 ± 0.09 ^{de}	1.70 ± 0.10 ^{a}	4.80 ± 0.11 ^{d}	0.18 ± 0.03 ^{de}

Table 1. *Cont.*

No.	Variety	Location	Length (mm)	Width (mm)	L/W Ratio	Thickness (mm)	Weight (g)
51	Ethiopia Castor	Ethiopia	18.24 ± 0.21^{a}	15.11 ± 0.24^{a}	1.21 ± 0.01^{f}	7.83 ± 0.22^{a}	1.06 ± 0.02^{a}
52	Indonesia Castor	Indonesia	15.80 ± 0.18^{b}	8.97 ± 0.18^{bc}	1.76 ± 0.03^{a}	6.08 ± 0.18^{bc}	0.43 ± 0.03^{c}
53	Indonesia Castor	Indonesia	10.19 ± 0.09^{f}	6.57 ± 0.27^{de}	1.55 ± 0.02^{bc}	4.95 ± 0.09^{d}	0.18 ± 0.01^{de}
54	Nigeria Castor	Nigeria	12.55 ± 0.17^{cd}	7.91 ± 0.38^{cd}	1.59 ± 0.05^{bc}	5.57 ± 0.14^{c}	0.23 ± 0.02^{de}

Each value represents the mean ± standard error (n = 10). The different superscript letters a-d indicate the significant difference based on Duncan's Multiple Range Test ($p \leq 0.05$).

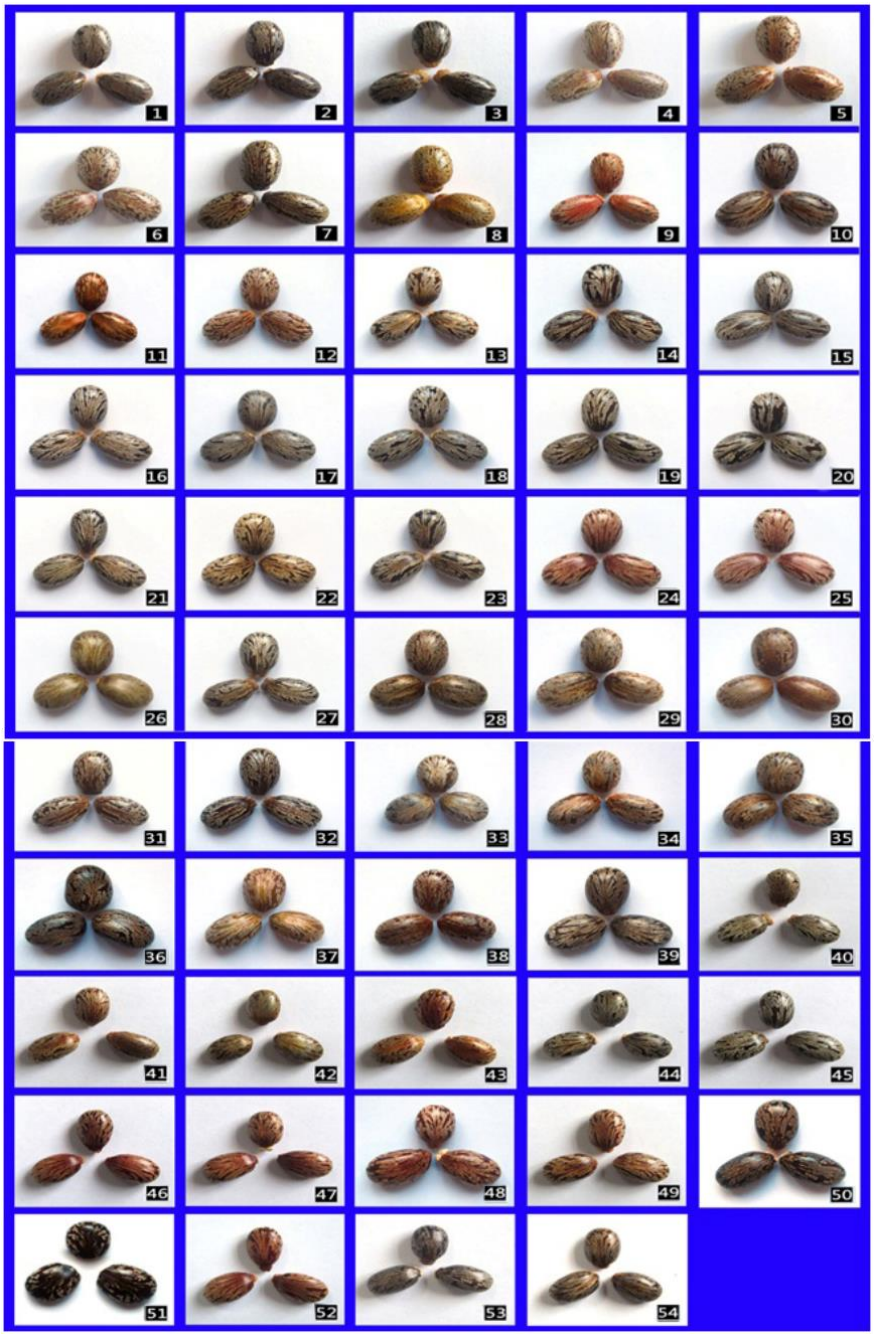

Figure 1. The seed patterns of 54 *R. communis* L. samples were collected from different locations in the world. See Table 1 for abbreviations of the varieties.

2.2. DNA Extraction

The castor seeds were first peeled and split in half, then were put into plastic bags and kept frozen in the temperature of –80 °C. DNA was extracted from seed tissue (~0.1 g) ground in liquid nitrogen by using Plant Genomic DNA kit (EP309, Beijing Zoman Biotechnology Co., Ltd., Beijing, China).

The extracted DNA was examined through electrophoresis on 1.0% agarose gel and quantified by a spectrophotometer (BioRad, Germany). Then, the DNA samples were diluted into 50 ng/μL and stored at –20 °C for PCR amplification.

2.3. PCR Amplification of ISSR Marker

A total of 100 ISSR primers (University of British Columbia, Vancouver, BC, Canada) were tested with nine (UBC #808, #824, #827, #836, #841, #842, #847, #857, and #873) showing high reproducibility and accuracy selected for PCR amplification. The PCR reaction was performed as described in literature with a minor modification [36]. The optimal reaction condition of ISSR-PCR amplification contained 2.5 μL template DNA, 2 μL 10× PCR buffer, 1.5 μL dNTPs, 0.2 μL Taq polymerase, 0.5 μL primer, 13.3 μL ddH_2O to make the total volume of 20 μL.

The cycling conditions were as follows: 4 min at 94 °C for an initiation step, followed by 40 cycles of 30 s at 94 °C, 30 s at a primer-appropriate temperature, and 2 min at 72 °C, and finalized at a final cycle of 10 min at 72 °C. PCR products were detected by electrophoresis on a 2.0% agarose gel stained with 0.1 μL/mL of DNAgreen (UV) (Tiandz, China) and run in 1× TAE buffer at 100 V for 40 min. PCR products were photographed by the Bio-image System (BioRad, Germany). Each PCR reaction was repeated three times.

2.4. PCR Amplification of RAPD Marker

The RAPD analysis was performed using nine primers as previously described by Powell et al. [37]. The composition of the PCR reaction mixture contained 2.2 μL of DNA template, 2 μL 10×PCR Buffer, 1.5 μL dNTPs, 0.2 μL Taq polymerase, 0.5 μL Primer, and 13.6 μL ddH2O. The cycling conditions were as follows: 4 min at 94 °C for an initiation step, followed by 40 cycles of 30 s at 94 °C, 30 s at a primer-appropriate temperature, and 2 min at 72 °C, and a final cycle of 10 min at 72 °C. Amplified products were separated on 2% agarose gel in 1× TAE buffer by electrophoresis at 100 V for 40 min and photographed by Bio-image System (BioRad, CA, USA).

2.5. Data Analysis

SPSS 19.0 was used for one-way analysis of variance (ANOVA) and differences between means were assessed by Duncan's multiple range test ($p \leq 0.05$). Each band amplified with ISSR and RAPD primers was manually scored as present (1) or absence (0). Only clear and reproducible bands were counted (Table S1). The genetic relationship between 54 castor varieties were evaluated by UPGMA analyses [25]. The genetic similarity of the castor varieties based on Jaccard's index [38]. The principle coordinate analysis (PcoA) was performed by NTSYS-pc version 2.10 software for three-dimensional distribution analysis [39].

3. Results

Generally, morphological characters (such as seed size and seed coat color) are an important factor in classification of castor varieties and evaluation of genetic diversity. In Table 1, the 51-Eth variety was superior to other varieties in seed length, width, thickness, and weight. The seed coat color pattern of the 54 varieties is given in Figure 1. Cluster analysis was carried out based on the morphological characteristics of seed size and color. Results showed that the 54 castor samples could be divided into three main groups (i.e., Clusters I, II, and III) with the genetic similarity coefficient ranging from 0.02 to 3.78 with an average of 1.90 (Figure 2). There were a total of 6, 32, and 15 varieties grouped in Clusers 1, 2, and 3, respectively, with variety 51-Eth in its own group. The genetic similarity

coefficient of the 51-Eth variety was evidently different from those of Clusters I, II, and III. Most of the seed color was brown or dark brown, and some varieties were red-brown (9-Jor, 11-Yun, 24-Sha, 25-Sha, 30-Ton, 34-Ton, 35-Ton, 41-Zi, 43-Zi, 46-Vie, 47-Pak, 48-Pak, 49-Par, and 52-Indo), while three varieties (4-Ind, 6-Ind, and 37) were light brown, and two varieties (8-Fra and 26-Ton) were yellow-brown.

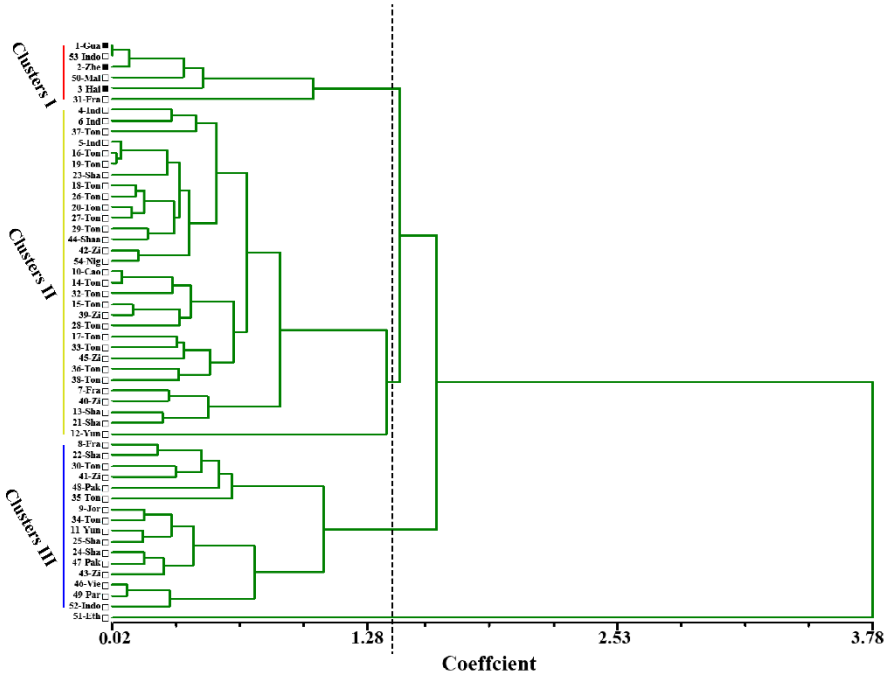

Figure 2. Classification of 54 castor samples based on six morphological traits.

3.1. ISSR Analysis

A total of 9 out of 100 primers that produced clear and reproducible bands were selected in the present study. Those primers produced 83 bands in total and the lengths of the amplified fragments ranged from 200 bp to 2700 bp (Table 2).

Table 2. Amplification of ISSR markers in 54 samples of castor seeds.

Primer Code	Sequence (5′–3′)	Tm (°C)	TNB	NPB	PPB (%)	Range of the Band Size (bp)
UBC-808	$(AG)_8$-C	52	9	9	100.00	600~2500
UBC-827	$(AC)_8$-G	52	9	5	55.55	450~2400
UBC-836	$(AG)_8$-YA	50	11	6	54.55	250~2400
UBC-841	$(GA)_8$-YC	52	7	6	85.71	300~1800
UBC-842	$(GA)_8$-YG	52	12	9	75.00	200~2700
UBC-847	$(CA)_8$-RC	52	5	4	80.00	2300~500
UBC-856	$(AC)_8$-YA	50	10	7	70.00	400~2200
UBC-857	$(AC)_8$-YG	52	10	9	90.00	550~2600
UBC-873	$(GACA)_4$	50	10	6	60.00	450~2550
Mean			9.2	6.8	74.53	

Note: TNB, total number of bands; NPB, number of polymorphic bands; PPB (%), percentage of polymorphic bands; R represents either A or G and Y represents either C or T.

The percentage of polymorphic bands per primer ranged from 54.55% (UBC-836) to 100% (UBC-808) with an average of 74.53%. As a representative, the ISSR profile of 54 castor samples using UBC-842 primer was shown in Figure 3.

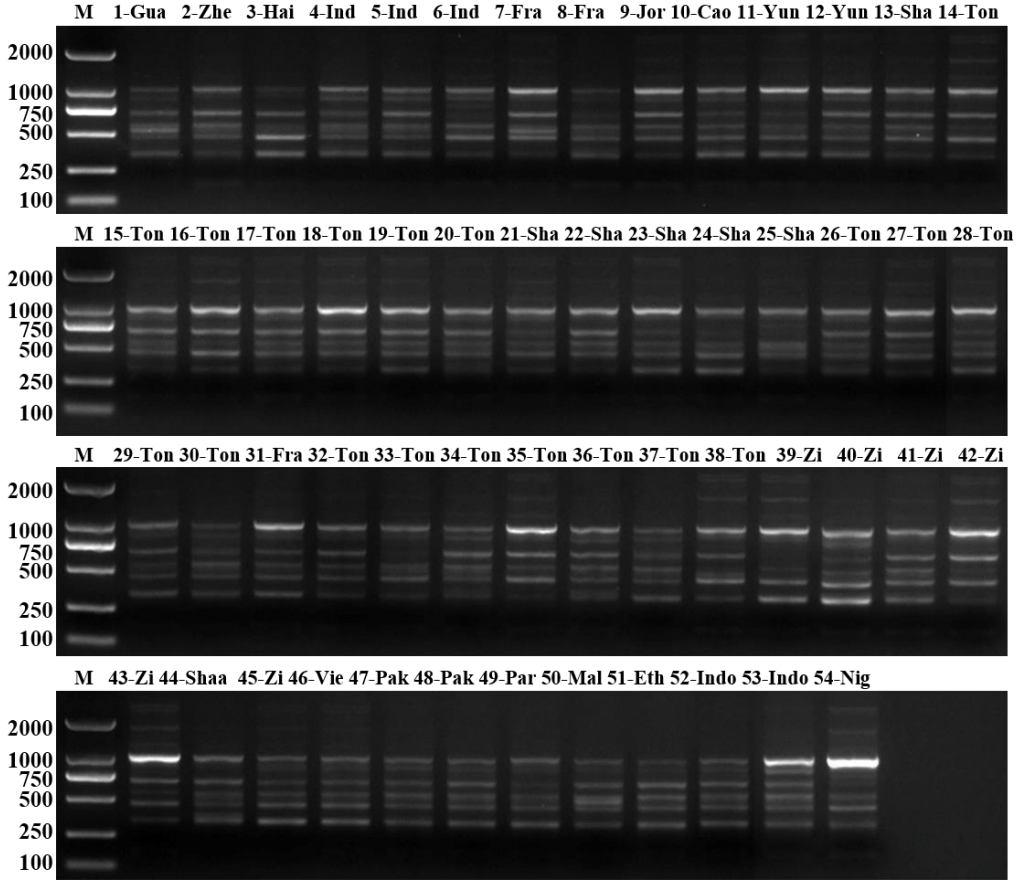

Figure 3. ISSR profiles of 54 *R. communis* L. samples amplified using primer UBC 842.

The UPGMA dendrogram using ISSR data based on the genetic similarity coefficient showed two main clusters with the genetic similarity coefficient ranging from 0.74 to 0.96 with an average of 0.86 (Figure 4).

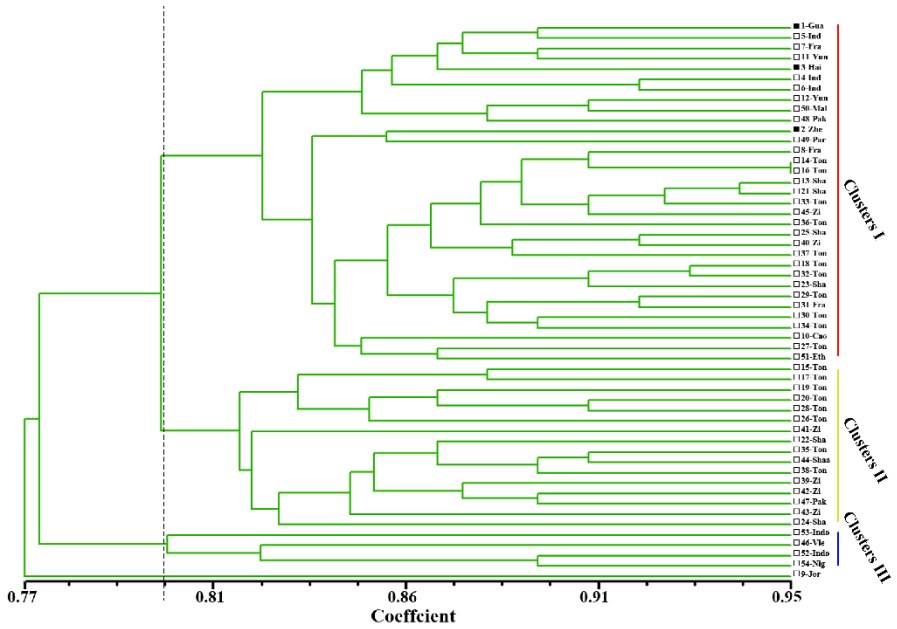

Figure 4. Dendrogram of 54 *R. communis* L. seed samples based on the UPGMA cluster analysis of ISSR data.

Two varieties (14-Ton and 16-Ton) showed the highest similarity coefficient (0.97). Clusters I and II included most of the 54 varieties, and Clusters III includes 46-Vie, 52-Indo, 53-Indo and 54-Nig varieties. However, variety 9-Jor was obviously different from those of Cluster I and II, III.

The results of the three-dimensional plot based on the PCoA analysis (Figure 5) were largely consistent with those revealed on the UPGMA clustering (Figure 4).

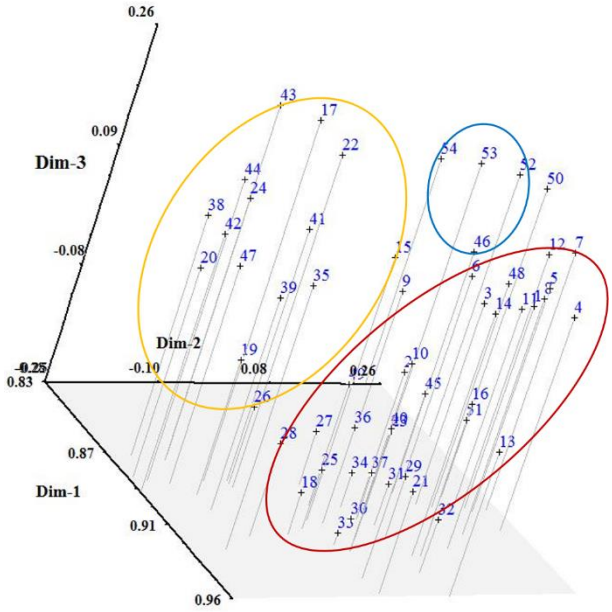

Figure 5. Three-dimensional plot of 54 *R. communis* L. seed samples (Table 1) based on the principal coordinate analysis using Jaccard similarity coefficient of the ISSR markers.

3.2. RAPD Analysis

Genetic diversity analysis was performed on 11 out of 20 RAPD primers that were reproducible and provided a unique polymorphism products. These 11 primers produced a total of 90 bands with the lengths of the amplified fragments ranging from 250 bp to 3000 bp (Table 3).

Table 3. Amplification of 11 RAPD markers in 54 castor seed samples.

Primer Code	Sequence (5′–3′)	Tm (°C)	TNB	NPB	PPB (%)	Range of the Bang Size (bp)
B2	ACAGGGCTCT	52	6	2	33.33	500~2500
B7	ATCCTGCCTG	52	4	1	25	650~1400
B9	ATCGGGTCGA	50	10	8	80	250~3000
B17	CCGGCCTTAG	52	9	7	77.78	500~2450
B19	CCGGCCTTCC	52	11	6	54.55	250~2350
B21	CCGGGGAAAC	52	11	10	90.91	250~2350
B23	CCGGGGTTTG	50	12	8	75	350~2700
F3	CCTGGGCTGG	52	7	4	5714	450~2400
F4	CCTGGGCTAT	50	6	5	83.33	750~2700
OPA-02	TGCCGAGCTG	50	8	2	25	450~1950
OPA-10	GTGATCGCAG	50	6	2	33.33	500~2400
Mean			8.2	5	60.98	

Note: TNB, total number of bands; NPB, number of polymorphic bands; PPB (%), percentage of polymorphic bands.

The percentage of polymorphic bands per primer ranged from 25% (B7 and OPA-02) to 90.91% (B21) with an average of 60.98%. As a representative, the RAPD profile of 54 castor varieties using B21 primer was shown in Figure 6.

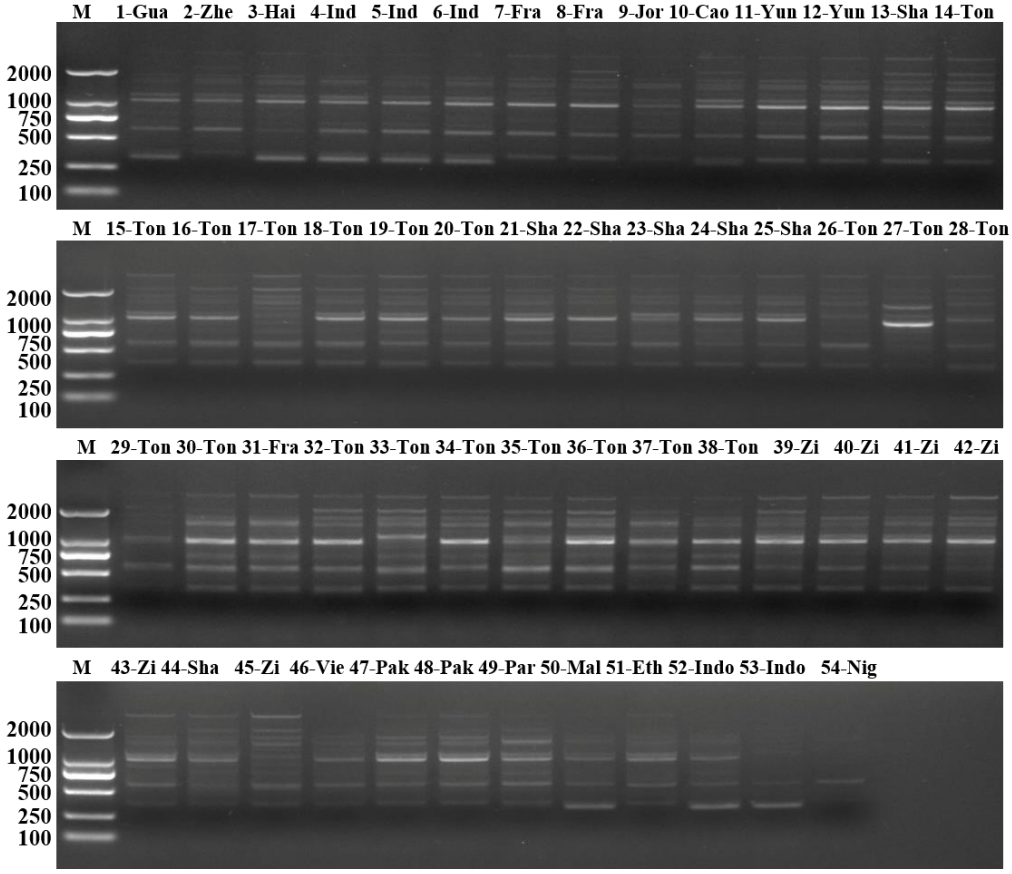

Figure 6. RAPD profiles of 54 *R. communis* L. seed samples amplified using primer B21.

See Table 1 for the abbreviations of these 54 accessions.

A dendrogram based on UPGMA analysis using RAPD data was shown in Figure 7. The genetic similarity coefficient ranged from 0.75 to 0.99 with an average of 0.85.

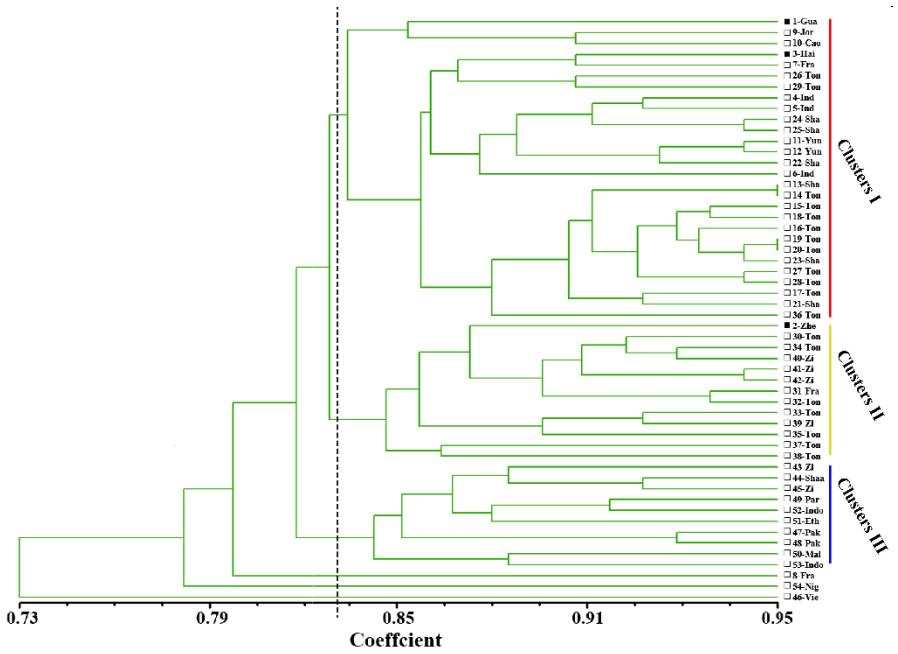

Figure 7. Dendrogram of 54 *R. communis* L. seed samples generated by the UPGMA cluster analysis based on RAPD markers.

The 13-Sha and 14-Ton as well as 19-Ton and 20-Ton showed the highest similarity coefficient (0.95), while the lowest similarity coefficient (0.75) was revealed between 4-Ind and 46-Vie. UPGMA cluster analysis based on RAPD data grouped 54 samples into three major Clusters. Like the ISSR analysis, Cluster 1 contained most varieties, while Cluster II comprised 2-Zhe, 30-Ton, 34-Ton, 40-Zi, 41-Zi, 42-Zi, 31-Fra, 32-Ton, 33-Ton, 39-Zi, 35-Ton, 37-Ton and 38-Ton. Cluster III contained ten varieties (i.e., 43-Zi, 44-Sha, 45-Zi, 49-Par, 52-Indo, 51-Eth, 47-Pak, 48-Pak, 50-Mal and 53-Indo). The results of PCoA were comparable to those of cluster analysis with insignificant deviations (Figure 8).

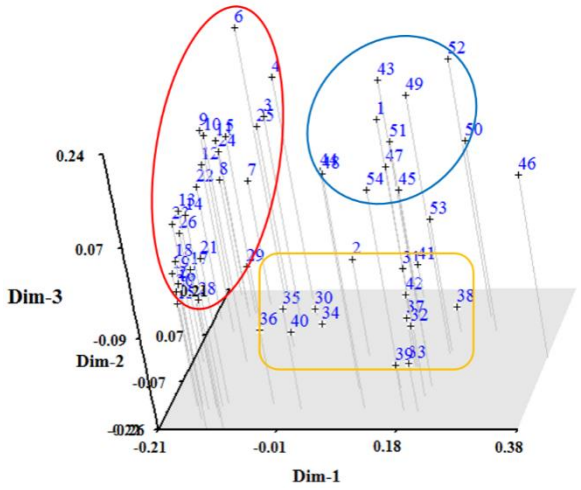

Figure 8. Three-dimensional plot of the PCoA analysis based on RAPD markers of 54 *R. communis* L. seed samples.

4. Discussion

Castor bean seeds were used by people dating from about 4000 BC and were thought to have originated in Eastern Africa, particularly Ethiopia [40]. The application of castor in India, Saudi Arabia, Greece, and Rome has been documented as having anti-inflammatory, antiasthmatic, and cathartic effects [41]. Castor seeds have also been used in China for centuries as a traditional medicine [42]. It is reasonable to assume that castor might have been introduced to China many centuries ago. However, genetic diversity of varieties cultivated in China has not been systematically studied.

In this study, the analysis of genetic diversity of 54 castor samples collected in India, Jordan, France, Vietnam, Pakistan, Paraguay, Malaysia, Ethiopia, Indonesia, Nigeria, and China was carried out based on morphological characters of the castor seed and two molecular markers (RAPD and ISSRs). For a long time, genetic diversity analysis has been done based on the morphological characteristics. Morphological traits display a continuous phenotypic range, and as quantitative traits, they are dominantly controlled by multi genes [43]. Qualitative traits are reported as equally effective in diversity assessment compared with molecular markers in plants [44]. In particular, the size and weight of seeds contribute to the oil content of seeds and are important morphological indicators. The length-to-width ratio of the castor seeds in different varieties ranged from 1.76 (52-Ind) to 1.21 (51-Eth) with an average of 1.51. This ratio was not significantly different from the ratio reported in previous studies [45]. The 54 castor seeds were divided into five groups (brown, light brown, dark brown, red-brown, and yellow-brown) based on stripe color and background color. The stripe pattern of seed is an important factor in characterizing genetic diversity, but it was difficult to investigate this trait due to its great variability. The 54 castor varieties were clustered into three groups according to six morphological characteristics. This result was different from that of the clustering analysis based on molecular markers. Similar results have been reported in previous studies, indicating that genetic diversity analysis based on combined RAPD and ISSR markers may be a more useful tool for the identification of Brahmi than morphological characters [46]. Morphological and

geographical patterns may also reflect genetic characteristics due to the environmental influences during the stages of plant development and reproduction. In contrast, both RAPD and ISSR technology are useful tools for the classification and genetic analysis of plant varieties compared to morphological analysis [47,48]. Both RAPD and ISSR markers show high polymorphism and are simple and cheap to generate. Therefore, they are widely used for the preservation studies of varieties and phylogenetic analysis [49,50].

Results of the genetic diversity of 54 castor varieties demonstrated that the ISSR and RAPD markers showed high polymorphism with an average of 74.59% and 60.98%, respectively. These values are comparable with 68.08% and 80.2% but much higher than 38% and 54% as previously reported by Gajera et al. [51] and Kallamadi et al. [5], respectively. Our results show that the genetic diversity among the 54 varieties of castor collected in different regions in the world is quite large. These results are similar to those reported in the previous studies showing the ample diversity among the castor genotypes [52]. Some different topological arrangements of these 54 varieties were revealed in the UPGMA dendrograms based on ISSR and RAPD markers. For example, the variety 9-Jor was revealed in its own group in the ISSR dendrogram but grouped into cluster I in the RAPD dendrogram. Furthermore, the variety 54-Nig was grouped in Clusters III and II in ISSR and RAPD dendrograms, respectively. These differences shown in UPGMA dendrograms are probably due to the polymorphism in the different regions of the castor genome. By using two molecular markers, the genetic background of 54 castor samples can be identified. The numbers of bands amplified by primers of ISSR and RAPD markers are different, ranging from the minimum of 4 or 5 to the maximum of 10 to 12. The different DNA amplification bands revealed in different varieties reflect the differences in genetic background.

Worldwide, Parzies et al. (2008) have categorized varieties of castor into two groups with Group a containing varieties collected from Asian countries (i.e., India, China, Pakistan, Indonesia, Jordan, and Paraguay), while Group b contains varieties collected from Americas (i.e., USA, Brazil, Cuba, and Argentina) [24]. In our study, out of 54 genotypes, 17 varieties were collected from areas outside China and 37 were distributed in China. Cluster I contains a large number of cultivars collected from China closely related to three wild accessions (1-Gua, 2-Zhe, and 3-Hai), indicating that these wild germplasms could be the putative progenitors of the cultivars of Cluster I (Figures 4 and 7). Furthermore, varieties collected from Vietnam, Indonesia, and Nigeria were identified in Cluster III (Figure 4), together with varieties collected from Vietnam, Malaysia, Pakistan, Paraguay, Ethiopia, and Indonesia (Figure 7), indicating that these varieties share similar genetic backgrounds. The studies of Kallamadi et al. [5], based on the combined data of the three-marker system, showed similar results with these accessions classified into three major clusters, while the Indian and Nigerian accessions were clustered in two large groups, respectively. These results indicated that gene flow occurred frequently worldwide, which may be attributed to castor being either self-pollinated or cross-pollinated by wind, instead of using outcrossing as a predominant mode of reproduction [53]. However, the alternative hypothesis states that castor oil was not dispersed widely after its initial introduction. Therefore, the dispersal pattern appears to be dependent on human introduction or physical transport, owing to the heavy seeds [54].

The results of PCoA used to determine the spatial representation of genetic distances among the castor varieties were consistent with the results of genetic differentiation based on cluster analysis. The results of PCoA based on ISSR and RAPD markers differed slightly from those of the UPGMA dendrogram. The results of genetic diversity analyses based on both morphological and molecular data showed that three varieties (9-Jor, 46-Vie, and 51-Eth) showed larger genetic differences compared to other varieties. Therefore, the larger genetic distance of these three varieties may provide rich genetic resources to satisfy breeding requirements. Indeed, our results showed that we can effectively discriminate the genetic difference and phylogenetic relationship among castor varieties based on ISSR and RAPD markers. Furthermore, these results help establish the theoretical foundation for selection, genetic preservation, and breeding of the castor varieties. Our results demonstrate

the utility of wild germplasm to exploit the unique caster breeding resources. Moreover, the castor resources in China showed a narrow genetic base. Therefore, we recommend that more castor resources be introduced to China to enrich the local breeding materials.

5. Conclusions

In the present study, we combined ISSR and RAPD markers to assess the genetic relationship of 54 *R. communis* samples collected worldwide (wild or cultivated varieties). Cluster analysis based on morphological data (seed length, width, thickness, weight, length to width ratio, and seed color) grouped all 54 castor samples into three main clusters. The results of PCoA and UPGMA cluster analysis were consistent with each other. The present study showed genetic divergence between the China elite local lines and foreign sources but the overall genetic variation was not extremely large despite the geographical distance. These results are important references for further resource exploitation and conservation of the genetic resources of castor.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/3/457/s1, Figure S1: Global distribution of the 54 R. communis L. seed accessions used in this study. The sample numbers are listed in Table 1, Table S1: Scoring of amplified bands of 54 samples of castor seeds based on ISSR and RAPD markers.

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References

1. Muraguri, S.; Xu, W.; Chapman, M.; Muchugi, A.; Oluwaniyi, A.; Oyebanji, O.; Liu, A.Z. Intraspecific variation within Castor bean (*Ricinus communis* L.) based on chloroplast genomes. *Ind. Crop. Prod.* **2020**, *155*, 112779. [CrossRef]
2. Santos, C.M.D.; Endres, L.; Ferreira, V.M.; Silva, J.V.; Rolim, E.V.; Wanderley, H.C.L.F. Photosynthetic capacity and water use efficiency in *Ricinus communis* (L.) under drought stress in semi-humid and semi-arid areas. *Acad. Bras. Cienc.* **2017**, *89*, 3015–3029. [CrossRef]
3. Sausen, T.L.; Luis Mauro, G.R. Growth and carbon assimilation limitations in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions. *Acta Bot. Bras.* **2010**, *24*, 648–654. [CrossRef]
4. Thatikunta, R.; Siva Sankar, A.; Sreelakshmi, J.; Palle, G.; Leela, C.; Durga Rani, C.V.; Gouri Shankar, V.; Lavanya, B.; Narayana Reddy, P.; Dudhe, M.Y. Utilization of in silico EST-SSR markers for diversity studies in castor (*Ricinus communis* L.). *Physiol. Mol. Biol. Plants* **2016**, *22*, 535–545. [CrossRef] [PubMed]

5. Kallamadi, P.R.; Nadigatla, V.P.R.G.R.; Mulpuri, S. Molecular diversity in castor (*Ricinus communis* L.). *Ind. Crop. Prod.* **2015**, *66*, 271–281. [CrossRef]
6. Lei, P.; Liu, Z.; Hu, Y.; Kim, H.; Liu, S.; Liu, J.; Xu, L.; Li, J.; Zhao, Y.; Yu, Z.; et al. Transcriptome analysis of salt stress responsiveness in the seedlings of wild and cultivated *Ricinus communis* L. *J. Biotechnol.* **2021**, *327*, 106–116. [CrossRef] [PubMed]
7. Jeong, G.T.; Park, D.H. Optimization of biodiesel production from castor oil using response surface methodology. *Appl. Biochem. Biotechnol.* **2009**, *156*, 1–11. [CrossRef]
8. Severino, L.S.; Auld, D.L.; Baldanzi, M.; Cândido, M.J.D.; Chen, G.; Crosby, W.; Tan, D.; He, X.; Lakshmamma, P.; Lavanya, C.; et al. A Review on the Challenges for Increased Production of Castor. *Agron. J.* **2012**, *104*, 853. [CrossRef]
9. Roetheli, J.C.; Roetheli, L.K.; Roetheli, B.D. *Castor: Assessing the Feasibility of US Production: Summary of Workshop Held at Plainview*; Texas: Growing Industrial Material Series; FAO: Rome, Italy, 1991.
10. Yang, T.; Lu, J.; Yeboah, A.; Gu, S.; Li, D.; Shi, Y.; Yin, X. Construction of castor functional markers fingerprint and analysis of genetic diversity. *Biocell* **2020**, *44*, 381–388. [CrossRef]
11. Wang, M.L.; Dzievit, M.; Chen, Z.; Morris, J.B.; Norris, J.E.; Barkley, N.A.; Tonnis, B.; Pederson, G.A.; Yu, J. Genetic diversity and population structure of castor (*Ricinus communis* L.) germplasm within the US collection assessed with EST-SSR markers. *Genome* **2017**, *60*, 193–200. [CrossRef]
12. Agyenim-Boateng, K.G.; Lu, J.; Shi, Y.; Zhang, D.; Yin, X. SRAP analysis of the genetic diversity of wild castor (*Ricinus communis* L.) in South China. *PLoS ONE* **2019**, *14*, e0219667. [CrossRef] [PubMed]
13. Ahn, Y.-J.; Vang, L.; McKeon, T.A.; Chen, G.Q. High-frequency plant regeneration through adventitious shoot formation in castor (*Ricinus commuinis* L.). *In Vitro Cell. Dev. Biol.-Plant* **2007**, *43*, 9–15. [CrossRef]
14. Yu, A.; Li, F.; Liu, A. Comparative proteomic and transcriptomic analyses provide new insight into the formation of seed size in castor bean. *BMC Plant Biol.* **2020**, *20*, 48. [CrossRef] [PubMed]
15. Sanchez-Alvarez, A.; Ruiz-Lopez, N.; Javier Moreno-Perez, A.; Martinez-Force, E.; Garces, R.; Salas, J.J. Agrobacterium-Mediated Transient Gene Expression in Developing *Ricinus communis* Seeds: A First Step in Making the Castor Oil Plant a Chemical Biofactory. *Front. Plant Sci.* **2019**, *10*, 1410. [CrossRef]
16. Fan, W.; Lu, J.; Pan, C.; Tan, M.; Lin, Q.; Liu, W.; Li, D.; Wang, L.; Hu, L.; Wang, L.; et al. Sequencing of Chinese castor lines reveals genetic signatures of selection and yield-associated loci. *Nat. Commun.* **2019**, *10*, 3418. [CrossRef] [PubMed]
17. Menssen, M.; Linde, M.; Omondi, E.O.; Abukutsa-Onyango, M.; Dinssa, F.F.; Winkelmann, T. Genetic and morphological diversity of cowpea (*Vigna unguiculata* (L.) Walp.) entries from East Africa. *Sci. Hortic.-Amst.* **2017**, *226*, 268–276. [CrossRef]
18. Bird, C.; Schweizer, M.; Roberts, A.; Austin, W.E.N.; Knudsen, K.L.; Evans, K.M.; Filipsson, H.L.; Sayer, M.D.J.; Geslin, E.; Darling, K.F. The genetic diversity, morphology, biogeography, and taxonomic designations of Ammonia (Foraminifera) in the Northeast Atlantic. *Mar. Micropaleontol.* **2020**, *155*, 101726. [CrossRef]
19. Worku, N.; Heslop-Harrison, J.S.; Adugna, W. Diversity in 198 Ethiopian linseed (*Linum usitatissimum*) accessions based on morphological characterization and seed oil characteristics. *Genet. Resour. Crop Evol.* **2015**, *62*, 1037–1053. [CrossRef]
20. Godwin, I.D.; Aitken, E.A.B.; Smith, L.W. Application of intersimple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis* **1997**, *18*, 1524–1528. [CrossRef]
21. Linos, A.; Nikoloudakis, N.; Katsiotis, A.; Hagidimitriou, M. Genetic structure of the Greek olive germplasm revealed by RAPD, ISSR and SSR markers. *Sci. Hortic.-Amst.* **2014**, *175*, 33–43. [CrossRef]
22. Pecina-Quintero, V.; Anaya-López, J.L.; Núñez-Colín, C.A.; Zamarripa-Colmenero, A.; Montes-García, N.; Solís-Bonilla, J.L.; Aguilar-Rangel, M.R. Assessing the genetic diversity of castor bean from Chiapas, México using SSR and AFLP markers. *Ind. Crop. Prod.* **2013**, *41*, 134–143. [CrossRef]
23. Reddy, M.P.; Sarla, N.; Siddiq, E.A. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* **2002**, *128*, 9–17. [CrossRef]
24. Allan, G.; Williams, A.; Rabinowicz, P.D.; Chan, A.P.; Ravel, J.; Keim, P. Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLPs and SSRs. *Genet. Resour. Crop Evol.* **2007**, *55*, 365–378. [CrossRef]
25. Liu, S.; Yin, X.G.; Lu, J.N.; Liu, C.; Bi, C.; Zhu, H.B.; Shi, Y.Z.; Zhang, D.; Wen, D.Y.; Zheng, J.; et al. The first genetic linkage map of *Ricinus communis* L. based on genome-SSR markers. *Ind. Crop. Prod.* **2016**, *89*, 103–108. [CrossRef]
26. Vijayan, K.; Chatterjee, S.N. ISSR profiling of Indian cultivars of mulberry (Morus spp.) and its relevance to breeding programs. *Euphytica* **2003**, *131*, 53–63. [CrossRef]
27. Noor Camellia, N.A.; Lee, A.T.; Abdullah, N.A.P. Genetic relationships and diversity of Jatropha curcas accessions in Malaysia. *Afr. J. Biotechnol.* **2012**, *11*, 3048–3054. [CrossRef]
28. Chowdhury, M.A.; Vandenberg, B.; Warkentin, T. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). *Euphytica* **2002**, *127*, 317–325. [CrossRef]
29. Mahar, K.S.; Rana, T.S.; Ranade, S.A.; Meena, B. Genetic variability and population structure in Sapindus emarginatus Vahl from India. *Gene* **2011**, *485*, 32–39. [CrossRef]
30. Ganie, S.H.; Upadhyay, P.; Das, S.; Prasad Sharma, M. Authentication of medicinal plants by DNA markers. *Plant Gene* **2015**, *4*, 83–99. [CrossRef] [PubMed]
31. Singh, M.K.; Singh, N.B.; Thakur, S.; Naik, P.K. Molecular Evaluations of Thirty One Clones of Poplar Based on Rapd and Ssr Molecular Markers. *Genetika* **2014**, *46*, 985–1001. [CrossRef]

32. Patel, H.K.; Fougat, R.S.; Kumar, S.; Mistry, J.G.; Kumar, M. Detection of genetic variation in Ocimum species using RAPD and ISSR markers. *3 Biotech* **2015**, *5*, 697–707. [CrossRef] [PubMed]
33. Pessina, D.; Gentili, R.; Barcaccia, G.; Nicole, S.; Rossi, G.; Barbesti, S.; Sgorbati, S. DNA content, morphometric and molecular marker analyses of *Citrus limonimedica*, *C. limon* and *C. medica* for the determination of their variability and genetic relationships within the genus *Citrus*. *Sci. Hortic.-Amst.* **2011**, *129*, 663–673. [CrossRef]
34. Onamu, R.; Solano, J.P.L.; Castellanos, J.S.; Nieto, J.P. Analysis of morphological and molecular markers in potato (*Solanum tuberosum* L.). *Rev. Fitotec. Mex.* **2012**, *35*, 267–277.
35. Joshi, D.C.; Shrotria, P.K.; Singh, R.; Srivastava, M.K.; Chawla, H.S. Assessment of RAPD and ISSR marker systems for establishing distinctiveness of forage Sorghum (*Sorghum bicolor* L. Moench) varieties as additional descriptors for plant variety protection. *Indian J. Genet. Plant Breed.* **2011**, *71*, 25–36.
36. Xu, F.; Lei, P.; Jiang, M.; Sang, L.; Guan, F.; Meng, F.; Quan, H. Genetic diversity of Herpetospermum caudigerum (Ser.) Baill using AFLP and chloroplast microsatellites. *Biotechnol. Biotechnol. Equip.* **2019**, *33*, 1260–1268. [CrossRef]
37. Powell, W.; Morgante, M.; Andre, C. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* **1996**, *2*, 225–238. [CrossRef]
38. Titilayo, O.A.; Samuel, A.O.; David, O.O.; Adewunmi, T.I. Genetic variations among three major ethnic groups in Nigeria using RAPD. *Mol. Biol. Res. Commun.* **2018**, *7*, 51–58.
39. Dey, S.S.; Singh, A.K.; Chandel, D.; Behera, T.K. Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. *Sci. Hortic.-Amst.* **2006**, *109*, 21–28. [CrossRef]
40. Qiu, L.; Yang, C.; Tian, B.; Yang, J.B.; Liu, A. Exploiting EST databases for the development and characterization of EST-SSR markers in castor bean (*Ricinus communis* L.). *BMC Plant Biol.* **2010**, *10*, 278. [CrossRef]
41. Ribeiro, P.R.; de Castro, R.D.; Fernandez, L.G. Chemical constituents of the oilseed crop *Ricinus communis* and their pharmacological activities: A review. *Ind. Crop. Prod.* **2016**, *91*, 358–376. [CrossRef]
42. Hajrah, N.H.; Abdul, W.M.; Al-Garni, S.M.; Sheikh, A.; Ahmed, M.M.M.; Hall, N.; Saini, K.S.; Mohammad Sabir, J.S.; Bora, R.S. Gene expression profiling to elucidate the pharmacological and toxicological effects of *Ricinus communis* L. leaf extract in mammalian cells. *Biotechnol. Biotechnol. Equip.* **2019**, *33*, 397–407. [CrossRef]
43. Chen, L.Q.; Lu, D.F.; Chen, Z.Y. Study on wild *Chimonanthus praecox* in Hubei province. *J. Chin. Landsc. Arch.* **1990**, *6*, 24–26.
44. Patel, M.P.; Parmar, D.J.; Kalola, A.D.; Kumar, S. Morphological and molecular diversity patterns in castor germplasm accessions. *Ind. Crop. Prod.* **2017**, *97*, 316–323.
45. Kushwah, P.; Singh, K.P. Morphological variation in castor (Ricinus communis L.). *Int. J. Inst. Pharm. Life Sci. Space Res.* **2012**, *2*, 128–134.
46. Tripathi, N.; Chouhan, D.S.; Saini, N.; Tiwari, S. Assessment of genetic variations among highly endangered medicinal plant Bacopa monnieri (L.) from Central India using RAPD and ISSR analysis. *3 Biotech* **2012**, *2*, 327–336. [CrossRef]
47. Luo, Q.; Ma, D.; Wang, Y. ISSR Identification of genetic diversity in Aconitum carmichaeli. *Chin. Tradit. Herb. Drugs* **2006**, *37*, 1554–1557.
48. Zhao, F.; Nie, J.; Chen, M.; Wu, G. Assessment of genetic characteristics of Aconitum germplasms in Xinjiang Province (China) by RAPD and ISSR markers. *Biotechnol. Biotechnol. Equip.* **2015**, *29*, 309–314. [CrossRef]
49. Jing, Z.B.; Wang, X.P. Genetic relationship between Chinese wild Vitis species and American and European Cultivars based on ISSR markers. *Biochem. Syst. Ecol.* **2013**, *46*, 120–126. [CrossRef]
50. PCRRAPD. Genetic diversity analysis of Morchella sp.by RAPD. *Mol. Biol. Res. Commun.* **2017**, *6*, 27–31.
51. Gajera, B.B.; Kumar, N.; Singh, A.S.; Punvar, B.S.; Ravikiran, R.; Subhash, N.; Jadeja, G.C. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Ind. Crop. Prod.* **2010**, *32*, 491–498. [CrossRef]
52. Rukam, S.T.; Parakhia, M.V.; Kavani, R.H.; Dobariya, K.L.; Thakkar, J.R.; Rathod, V.M.; Dhingani, R.M.; Golakiya, B.A. Characterization of castor (*Ricinus communis* L.) genotypes using different markers. *Res. J. Biotechnol.* **2014**, *9*, 6–13.
53. Kanti, M.; Anjani, K.; Kiran, B.U.; Vivekananda, K. Agro-morphological and Molecular Diversity in Castor (*Ricinus communis* L.) Germplasm Collected from Andaman and Nicobar Islands, India. *Czech J. Genet. Plant Breed.* **2015**, *51*, 96–109. [CrossRef]
54. Foster, J.T.; Allan, G.J.; Chan, A.P.; Rabinowicz, P.D.; Ravel, J.; Jackson, P.J.; Keim, P. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biol.* **2010**, *10*, 13. [CrossRef] [PubMed]