doi: 10.17221/205/2014-CJGPB

Agro-morphological and Molecular Diversity in Castor (*Ricinus communis* L.) Germplasm Collected from Andaman and Nicobar Islands, India

Meena KANTI[1], Kammili ANJANI[2], Betha USHA KIRAN[2] and K. VIVEKANANDA[2]

[1]Central Research Institute for Jute and Allied Fibres, Barrackpore, India;
[2]Directorate of Oilseeds Research, Hyderabad, India

Abstract

Kanti M., Anjani K., Usha Kiran B., Vivekananda K. (2015): Agro-morphological and molecular diversity in castor (*Ricinus communis* L.) germplasm collected from Andaman and Nicobar Islands, India. Czech J. Genet. Plant Breed., 51: 96–109.

Castor (*Ricinus communis* L.) is an industrial oilseed crop grown worldwide. Its oil with more than 80% ricinoleic acid makes it a chief raw material for numerous industrial applications and biofuel production. Castor grows in a wild form across India including Andaman & Nicobar Islands, which are geographically quite isolated from mainland India. Thirty-three accessions growing in isolation in these Islands were used in the present study. Genetic diversity among these accessions was assessed using 18 agro-morphological traits and 29 EST-SSR markers. High agro-morphological and molecular variability was observed among these accessions. Both agro-morphological traits and EST-SSR markers effectively discriminated the accessions. However, EST-SSRs separated the accessions into more groups than did agro-morphological data, implying high efficiency and resolution of EST-SSR markers in genetic analysis of castor germplasm from Andaman & Nicobar. The diverse accessions identified in the present investigation would serve as genetically diverse sources in castor breeding programmes.

Keywords: agro-morphological traits; EST-SSRs; genetic diversity; polymorphism

Castor (*Ricinus communis* L., $2n = 2x = 20$, Euphorbiaceae) is an industrially important non-edible oilseed crop widely cultivated in the arid and semi-arid regions of the world (Govaerts *et al.* 2000). It is believed to have four centres of origin. The Ethiopian-East African region is considered to be the most probable site of origin because of the presence of high diversity in Ethiopia (Moshkin 1986). The plants can be self- or cross-pollinated by wind, with outcrossing as a predominant mode of reproduction (Meinders & Jones 1950; Brigham 1967). Castor oil, which has a long history of use for medicinal purposes (Gaginella *et al.* 1998), has been considered a promising raw material for the production of renewable energy in tropical countries. Besides, castor bean has been traditionally cultivated for the production of lubricants and paints (Ogunniyi 2006; Scholz & Silva 2008; Berman *et al.* 2011). Castor seed contains more than 45% oil, which is rich (80–90%) in an unusual hydroxyl fatty acid, ricinoleic acid (Jeong & Park 2009). A high viscosity of castor oil is the main reason which restricts the use of pure castor bean diesel in the engines (Pinzi *et al.* 2009). However, blended biodiesel with petrol can be exploited in regions with severe winter. It has many advantages associated like low freezing point and lubricant power, which makes it perfect for utilization for renewable energy resources (Ogunniyi 2006; Demirbas 2007; Pinzi *et al.* 2009; Berman *et al.* 2011; Singh 2011). Unique chemical and physical properties of the oil make it a raw material for numerous and varied industrial applications, such as manufacture of polymers, coatings, lubricants for aircrafts, cosmetics, hydraulic fluids, plastics, artificial leather, manufacture of fibre optics, bulletproof glass and bone prostheses and as antifreeze

doi: 10.17221/205/2014-CJGPB

for fuels and lubricants utilized in aircraft and space rockets and for the production of biodiesel farming (Ogunniyi 2006; Jeong & Park 2009), and medicines (Allan *et al.* 2008).

India is the largest producer of castor in the world and also one of the centres of castor diversity, where great diversity exists across the country in varied ecosystems (Anjani 2012). It is usually cultivated as a hybrid in India, which gives significantly greater yields than pure lines or varieties (Birchler *et al.* 2003; Reif *et al.* 2007). Genetic diversity assessment prior to developing hybrids can help in better exploitation of heterosis (Santalla *et al.* 1998). Diversity analyses require a large number of polymorphic markers to measure genetic relationships and genetic diversity in a reliable manner. Lack of adequate diversity in castor (in terms of isozymes), limited number of morphological and biochemical markers (Soltis *et al.* 1992) or environment factors limit (Esselman *et al.* 2000) their use for diversity study. Molecular markers appear to be an attractive alternative to the conventional diversity analyses and in management and conservation of biodiversity (Lowrey & Crawford 1985). They are ideal tools for germplasm characterization and phylogenetic studies. Unlike other important oilseed crops, such as soybean (*Glycine max*), sunflower (*Helianthus annuus*), oil palm (*Elaeis guineensis*) and some species of the family Euphorbiaceae, especially cassava and rubber tree, diversity is still poorly characterized by means of molecular marker systems for castor bean (Feng *et al.* 2009; Talia *et al.* 2010; Sayama *et al.* 2011). This species had been neglected until the late 2000s, the first publication on the genetic diversity of germplasm collections was reported by Allan *et al.* (2008) using AFLP markers. It is the first member of the family Euphorbiaceae with the whole genome published (Chan *et al.* 2010), which was used to assemble the chloroplast and mitochondrion genomes to identify the SNPs for phylogenetic analysis and reported a broad geographic distribution (Rivarola *et al.* 2011).

Diversity in castor germplasm was initiated in the early days using protein based markers (isozymes) (Athma *et al.* 1982; Sathaiah & Reddy 1983, 1984, 1988). Limitation of these markers has shifted the castor molecular research towards the use of DNA based markers. Molecular markers like AFLP (Allan *et al.* 2008), gSSR (Allan *et al.* 2008; Bajay *et al.* 2009) – only few gSSR markers have been exploited, RAPD markers and agro-morphological traits and quantitative phenotypic traits (Figueiredo Neto *et al.* 2004; Costa *et al.* 2006; Milani *et al.* 2009), RAPD and ISSR (Gajera *et al.* 2010), RAPD (Vivodík *et al.* 2014), SNPs (Foster *et al.* 2010) and recently eSSRs (Qiu *et al.* 2010; Kanti *et al.* 2014) have been investigated. However, very weak geographic structuration among castor bean populations with narrow genetic diversity have been reported, regardless of a marker system used (Allan *et al.* 2008; Bajay *et al.* 2009, 2011; Milani *et al.* 2009; Foster *et al.* 2010; Gajera *et al.* 2010; Qiu *et al.* 2010; Kanti *et al.* 2014; Vivodík *et al.* 2014). In contrast, a certain degree of geographically structured clusters was reported among the accessions used by Qiu *et al.* (2010). Sequencing of the castor bean genome (Chan *et al.* 2010) has just opened a wide range of possibilities in analysing the genetic diversity of this economically important species. Despite the recent publication of the castor bean genome little is known about the actual genetic diversity of this species.

The Andaman & Nicobar Union territory of India is a group of picturesque islands lying approximately 414 km in the South-Eastern part of the Bay of Bengal (6° to 14°N and 92° to 94°E). These islands are isolated from the mainland of India and are different in ecosystem. Castor is not a cultivated crop in these islands, but wild-type castor is found growing in different islands at a very low frequency. Castor in these islands might be growing on its own and have been introduced by the inhabitants who migrated from mainland India. Castor in Andaman & Nicobar Islands might have evolved on its own in isolation and adapted itself to the ecosystem of these islands. Wild-type castor may have the ability to endure climatic changes. *In-situ* conservation of these types in Andaman & Nicobar Islands is not practicable as castor is not a crop over there and no sustainable income comes from wild-type castor. Therefore, wild-type castor accessions were collected through an exploration (Anjani 2001a, b) and are maintained *ex-situ* at the Directorate of Oilseeds Research, Hyderabad, India.

In this investigation, these collections of castor were analysed for genetic diversity and evaluated for 18 agro-morphological traits at the Directorate. Data sets of agro-morphological traits and EST-SSR (Expressed Sequence Tag-Simple Sequence Repeat) markers were used to study genetic diversity.

MATERIAL AND METHODS

Plant material. Seed sources from thirty-three accessions of castor germplasm collected from different parts of Andaman and Nicobar Islands and

doi: 10.17221/205/2014-CJGPB

maintained in the Germplasm Maintenance Unit at the Directorate of Oilseeds Research (DOR), Hyderabad, have been used for genetic diversity study. The detailed collection site of 33 castor accessions is presented (Table 1). Prior to evaluation, all the accessions underwent eight generations of self-pollination at a DOR farm under controlled pollination conditions. All the accessions exhibited high within accession uniformity with respect to 23 morphological descriptors developed at DOR, Hyderabad (Anjani 2001a, b).

Agro-morphological and divergence study. Thirty-three accessions collected from Andaman and Nicobar Islands, India, were evaluated for 18 agro-morphological traits in a randomized complete block design with three replications at the Directorate of Oilseeds Research, Hyderabad, India (17.366°N and 78.478°E) in 2009 and 2010. Each plot consisted of three rows of 5 m in length with 45 × 90 cm spacing. The crop was grown under rainfed conditions in red sandy soil and planted in the second fortnight of June in each year. The total rainfall was 595 mm during the crop growth period (15 June to 15 February) in 2009 and 1125 mm in 2010. The maximum temperature was between 28°C and 34°C and the minimum was between 15°C and 24°C during the crop growth period in both years. Recommended dose of N, P_2O_5 and K_2O (40:40:0 kg/ha) was applied to experimental plots.

The data on 18 agro-morphological traits, *viz.* plant height (cm), number of nodes on the main stem, total length of primary raceme (cm), length of primary raceme covered with capsules (cm), length of primary raceme covered with male flowers, number of secondary racemes/plant, number of tertiary racemes/plant, number of higher order racemes/plant, total number of productive racemes/plant, days to 50% flowering, days to maturity,100-seed weight (g), seed yield/plant at 120, 150, 180 and 210 days after sowing, total yield/plant, and oil content (%) were recorded on 15 plants from each replication in each year. For divergence studies principal component analysis (PCA) and Ward's minimum variance cluster analysis (Ward 1963) were used. Pearson

Table 1. List of Andaman and Nicobar accession collections

No.	Germplasm	Collection site	No.	Germplasm	Collection site
1.	RG2694	Phoenix Bay	18	RG2718	Lakshmipur, Digilipur, North Andaman
2.	RG2697	Dundas point, South Andaman	19	RG2720	Milangram, Digilipur, North Andaman
3.	RG2698	Mudranad Marine, South Andaman	20	RG2722	Radhanagar, Digilipur, North Andaman
4.	RG2699	Mudranad Marine, South Andaman	21	RG2723	Ramnagar, Digilipur, North Andaman
5.	RG2701	Mazarphad, Port blair, South Andaman	22	RG2724	Subhashgram, Digilipur, North Andaman
6	RG2702	New Wandoor	23	RG2725	Subhashgram, Digilipur, North Andaman
7	RG2704	Bitnabath, South Andaman	24	RG2727	Ramakrishnapuram, Hut Bay, Little Andaman
8	RG2706	Austinbad, South Andaman	25	RG2728	Ramakrishnapuram, Hut Bay, Little Andaman
9	RG2707	Mau Canbycove, South Andaman	26	RG2729	Netajinagar, Little Andaman
10	RG2710	Rangat, Mid. Andaman	27	RG2730	Netajinagar, Hut Bay, Little Andaman
11	RG2711	Rampur, Rangat, Middle Andaman	28	RG2731	Break Water, Hut Bay, Little Andaman
12	RG2712	Nimbutala, Rangat Bay, Middle Andaman	29	RG2732	Harbinder Bay, Little Andaman
13	RG2713	Amkang, Rangat, Middle Andaman	30	RG2733	Harbinder Bay, Little Andaman
14	RG2714	Panchavati, Middle Andaman	31	RG2734	Harbinder Bay, Nicobary settlement, Little Andaman
15	RG2715	Dasaratpur, Rangat, Middle Andaman	32	RG2735	Govindanagar, Havelock
16	RG2716	Bastinapur, Mayabundar, Middle Andaman	33	RG2736	Radhanagar, Havelock
17	RG2717	Shivpur, Digilipur, North Andaman			

doi: 10.17221/205/2014-CJGPB

correlation coefficients (*r*) were calculated between 18 agro-morphological traits. Two-year mean data on 18 traits of 33 accessions were used for multivariate statistical algorithms and for deriving Pearson correlation coefficients. INDOSTAT statistical software, INDOSTAT Services, India (http://indostat.software.informer.com/) was used for PCA and cluster analysis and for estimating *r* values.

EST-SSR analysis. The genomic DNA was isolated from young leaf samples by cetyl-trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990) with minor modifications as per the requirement. DNA was quantified electrophoretically using λ-DNA on 0.8% agarose gel. Twenty-nine EST-SSR markers of castor developed at DOR, Hyderabad, were used for Polymerase Chain Reaction (PCR) amplification. Reactions were carried out in a volume of 20 µl containing ~ 25 ng of template DNA, 0.08mM dNTPs, 1× reaction buffer (containing 1.5mM $MgCl_2$) (Bangalore, Genei), 5 Pmoles of each forward and reverse primer and 1U of Taq DNA polymerase. Amplification was performed using the Eppendorf Mastercycler gradient version 2.1, programmed according to Williams *et al.* (1990) with minor modifications. The PCR cycle profile followed had initial denaturation at 94°C for 5 min and then 30 cycles of 30 s denaturation at 94°C, 30 s primer annealing at 56°C and 30 s extension at 72°C and 10 min at 72°C for the final product extension.

Amplified products were stored at 4°C until further use. The PCR reaction mixture was size fractionated on 3.0 % agarose gel containing ethidium bromide in 1× TAE buffer at 120 volts using a horizontal gel electrophoresis system. The 100 bp DNA ladder ran along the sides of the amplified product to determine their approximate size. The amplified fragments were visualized under ultraviolet light and photographed with gel documentation system (Gene Flash Syngene Bioimaging, Syngene, Cambridge, UK). The amplified PCR products were further subjected onto 6% polyacrylamide gel on a Sequi-Gen (Bio-Rad, Hercules, USA) sequencing cell in 1× TBE buffer at 100 W, 50 mA at 55°C for 2 h. The gel was stained with silver stain for 30 min.

Polymorphic bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the presence and absence of bands in each accession, respectively. Molecular weights of the bands were estimated by using 100 bp DNA ladder as standards. For individual primer/primer combination, confusion probability (*C*) and limit of discriminating power (*DL*) were calculated according to Tessier *et al*. (1999). Polymorphism information content (PIC) values were calculated based on Anderson *et al.* (1993), in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. The binary data scoring was used to construct a radial tree using Darwin's (5.0) software (Perrier *et al.* 2003). Genetic similarity values were calculated according to the Dice similarity coefficient. Polymorphism information content (PIC) was used to identify primers that would distinguish genotypes more efficiently.

RESULTS AND DISCUSSION

Agro-morphological study. The analysis of variance showed variations among the 33 accessions from Andaman and Nicobar Islands for all the 18 traits studied. The two-year mean values of 18 traits for each accession are given in Table 2. Most of the Andaman and Nicobar accessions were tall with a high number of nodes on the main stem, medium to late duration and moderate seed yielding capacity. However, the mean oil content of these accessions was high (51%). Great diversity was observed for all the 18 agro-morphological traits among 33 accessions. Plant height ranged from 44 to 163 cm, number of nodes on the main stem varied from 12 to 27. The number of nodes gives an indication of days to flowering and maturity in castor. Accordingly, days to 50% flowering ranged from 58 to 91 days; days to maturity varied from 98 to 138 days. Primary raceme in castor is the major yield contributing raceme, its total length varied from 19 to 44 cm. Great variation was observed among the accessions for primary raceme lengths covered with male flowers (13–35 cm) and by female flowers (4–23 cm). Considerable variation was observed for number of secondary racemes (1–4), tertiary racemes (2–5), higher order racemes (2–6) and total racemes/plant (6–13). Oil content ranged from 48 to 56% and the 100-seed weight varied from 12 to 46 g. Seed yield/plant at different pickings after planting varied greatly, it was 5 to 47 g/plant at 120 days after planting (DAP), 3 to 51 g/plant at 150 DAP (1–51 g/plant), 4 to 33 g/plant at 180 DAP (4–33 g/plant) and 5 to 33 g/plant at 210 DAP (5–33 g/plant). Total seed yield/plant also varied greatly with a range from 37 to 107 g under rainfed conditions.

The accessions RG2733, collected from Harbinder Bay Island in Little Andaman (10.9°N, 97.57°E), and

doi: 10.17221/205/2014-CJGPB

Table 2. Means of 18 agro-morphological traits in 33 castor accessions collected from Andaman and Nicobar Islands, India

Accession	PH	NN	TLP	LPM	LPC	SR	TR	HR	PR	DF	DM	SW	OC	Seed yield/plant at different pickings (g)				TY
														120 DAP	150 DAP	180 DAP	210 DAP	
RG-2694	70	18	36	23	17	3	3	3	9	62	122	15	52	34	9	17	9	68
RG-2697	118	25	45	35	10	2	3	3	8	90	129	24	52	12	16	11	6	45
RG-2698	63	17	36	20	16	4	3	4	13	62	123	17	51	33	14	10	15	73
RG-2699	71	16	29	21	17	4	3	3	13	62	121	16	51	47	10	16	20	92
RG-2701	125	21	36	13	23	1	2	3	8	62	124	12	50	25	11	10	21	67
RG-2702	83	21	39	20	10	2	3	4	10	62	125	18	50	29	19	17	11	75
RG-2704	69	17	35	19	16	1	4	4	9	72	121	26	51	21	14	14	5	55
RG-2706	78	17	33	21	13	1	3	4	9	72	121	26	56	17	8	15	5	45
RG-2707	121	19	38	20	18	3	2	3	7	79	124	16	50	23	10	17	11	61
RG-2710	86	21	37	23	14	4	3	4	11	72	124	17	51	16	12	15	11	54
RG-2711	97	20	37	23	16	4	3	5	10	72	124	17	51	20	7	12	13	52
RG-2712	65	17	33	19	15	4	3	3	11	62	121	17	50	15	6	13	14	48
RG-2713	137	22	37	24	7	1	3	6	6	79	124	46	52	11	7	26	6	51
RG-2714	141	22	30	26	4	1	3	3	9	90	125	33	50	11	51	4	8	74
RG-2715	140	24	19	14	5	1	3	5	9	90	126	23	48	11	7	13	7	37
RG-2716	135	25	25	20	7	1	3	2	8	90	126	36	51	10	14	5	9	38
RG-2717	120	24	26	20	5	3	3	4	12	72	126	32	49	13	18	32	19	82
RG-2718	48	12	43	19	23	3	3	3	10	58	98	17	51	10	4	10	15	39
RG-2720	154	20	19	14	6	2	3	5	6	90	138	41	48	6	12	10	8	37
RG-2722	161	25	27	21	6	2	3	2	6	91	125	35	50	5	27	10	11	53
RG-2723	156	27	32	26	5	2	2	5	7	91	138	43	50	45	25	19	18	107
RG-2724	152	25	38	25	12	2	3	2	8	88	124	16	50	15	15	5	11	47
RG-2725	79	17	27	16	9	3	3	3	6	79	122	22	52	32	8	14	33	86
RG-2727	70	18	33	19	14	4	4	3	6	62	123	17	51	30	5	11	16	62
RG-2728	120	22	31	21	12	3	4	6	12	79	125	22	53	37	26	33	11	107
RG-2729	73	17	39	25	12	4	4	3	9	62	121	15	52	31	7	14	12	64
RG-2730	90	19	36	24	14	3	3	4	8	62	123	15	51	28	3	16	13	60
RG-2731	74	18	38	25	16	3	3	3	9	72	121	16	52	24	10	11	10	55
RG-2732	51	12	40	21	19	3	5	3	9	59	98	12	50	20	5	22	12	59
RG-2733	44	12	28	16	21	3		3	8	58	98	12	51	16	1	11	14	42
RG-2734	101	19	43	28	15	3	4	4	10	72	123	17	50	22	6	14	21	63
RG-2735	163	23	23	20	5	3	4	3	10	78	122	17	49	44	7	9	17	76
RG-2736	140	21	29	25	5	3	5	3	10	88	122	14	51	11	6	7	27	51
Mean	103	20	33	21	12	3	3	3	9	74	122	22	51	22	12	14	13	61
SD	15.4	1.5	4.6	3.3	1.9	0.2	0.3	0.2	1.2	5.9	14.6	0.8	1.0	3.7	2.1	2.2	2.4	10.9
CV%	15	7.8	14	16	16	9	11	8	14	8	12	4	2	17	18	16	19	18
CD ($P = 0.05$)	13	1.2	3.2	3.0	2.5	1	1	1	1.5	3.2	4.5	1.2	0.7	4.2	2.1	3.2	2.0	13

PH – plant height (cm); NN – No. of nodes on thr main stem; TLP – total length of primary raceme (cm); LPM – length of primary raceme covered with male flowers (cm); LPC – length of primary raceme covered with capsules (cm); SR – No. of secondary racemes/plant; TR – No. of tertiary racemes/plant; HR – No. of high order racemes/plant; PR – total number of productive racemes/plant; DF – days to 50% flowering; DM – days to maturity; SW – 100-seed weight (g); OC – oil content (%); DAP – days after planting; TY – total seed yield/plant (g)

doi: 10.17221/205/2014-CJGPB

RG2718 from Digilipur Island in North Andaman (13.2°N, 92.9°E) were the earliest flowering (58 days) accessions. These two accessions also had a low number of nodes on the main stem (12); low node number is an indicator of early flowering in castor (Anjani 2010). RG2697 had the longest primary raceme (45 cm). The accessions RG2701 and RG2718 had the longest primary racemes covered with capsules (23 cm). A high number of productive racemes (13/plant) was recorded in RG2698 and RG2699. The high total seed yield (107 g/plant) was realized from RG 2723 and RG2728. These two accessions also yielded high at the first two pickings taken up at 120 DAP (45 and 37 g/plant) and 150 DAP (25 and 26 g/plant). RG2723 was a collection from Rangat Island in North Adaman (13.04°N, 92.95°E) and RG2728 was from Hut Bay Island in Little Andaman. The accession RG 2706 recorded the highest oil content (56%) followed by RG 2728 (53.64%). RG 2706 was collected from Austinbad in South Andaman (11.37°N, 97.73°E) and RG2728 was form Hut Bay Island in Little Andaman (10.42°N, 92.57°E).

Correlations. Genotypic correlations between 18 agro-morphological traits are shown in Table 3. Plant height exhibited high significant positive associations with the number of nodes on the main stem. Both plant height and number of nodes on the main stem had significantly high positive associations with days to 50% flowering, days to maturity, 100-seed weight and seed yield/plant at 150 days after planting, and had high negative associations with the length of primary raceme covered with capsules and number of secondary racemes/plant. Days to 50% flowering exhibited significant high positive association with days to maturity; days to 50% flowering and days to maturity showed high significant positive associations with 100-seed weight, seed yield at 150 days after planting. Days to maturity had a low positive significant association with total seed yield/plant and days to 50% flowering had a significant negative association with seed yield at 120 days after planting. Correlations between total seed yield/plant and seed yield/plant at 120, 150, 180 and 210 days after planting were significant and positive. Oil content had no significant associations with seed weight, days to 50% flowering and days to maturity.

Diversity analysis. Cluster analysis based on agro-morphological traits grouped the 33 accessions into two major clusters (I and II), which were further grouped into 16 precisely diverse clusters at the decreased phenon levels (Figure 1). Geographical distribution of the accessions did not account for clustering of most of the accessions. However, a few accessions, namely RG2730, RG2731, RG2729 and RG2727, collected from Hut Bay Island were placed in one group at a reduced phenon level. Similarly, RG2698 and RG2699 collected from Mudranad Marine in South Andaman formed one group and three accessions (RG 2710, RG2711 and RG2712) collected from Rangat Island in Middle Andaman were grouped together.

The principle component analysis (PCA) performed on 18 agro-morphological characters of 33 accessions showed that the first four components explained 67.83% of total variation (Table 4). The first four components were retained as per Kaiser criterion (Kaiser 1960). This criterion retains only the components that have eigenvalues greater than 1 for interpretation. The first component (PC1) accounted for 31.75% of the variance, the second component (PC2) accounted for 15.58% of total variation while the third component (PC3) and fourth component (PC4) accounted for 10.98% and 9.51% of total variation, respectively. The most effective traits in the first component were plant height, nodes on the main stem, length of primary raceme covered with capsules, days to 50% flowering, days to maturity, 100-seed weight, number of secondary racemes/plant. Seed yield/plant at 120 and 180 days after planting (DAP), and total seed yield/plant were the most effective traits in the second component. In the third component, total length of primary raceme, length of primary raceme covered with male flowers, oil

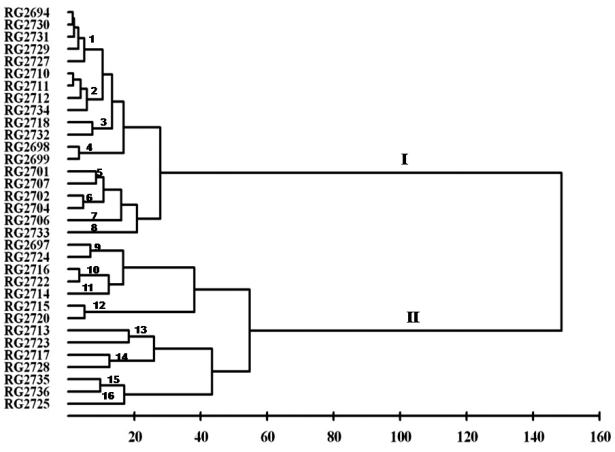

Figure 1. Clustering of 33 castor accessions collected from Andaman and Nicobar Islands, India, using Ward's minimum variance method based on agro-morphological data set

Czech J. Genet. Plant Breed., 51, 2015 (3): 96–109

doi: 10.17221/205/2014-CJGPB

Table 3. Genotypic correlations among 18 agro-morphological traits in 33 accessions collected from Andaman and Nicobar Islands, India

	NN	TLP	LPM	LPC	SR	TR	HR	PR	DF	DM	SW	OC	SY-120	SY-150	SY-180	SY-210	TY
PH	0.86*, **	–0.44*, **	0.11	–0.73*, **	–0.49*, **	0.21	0.12	–0.31**	0.84*, **	0.65*, **	0.56*, **	–0.4*, **	–0.19	0.45*, **	–0.10	–0.05	0.06
NN	–	–0.27**	0.28**	–0.73*, **	–0.42*, **	0.25**	0.14	–0.17	0.77*, **	0.77*, **	0.54*, **	–0.27**	0.10	0.50*, **	0.005	0.12	0.15
TLP	–	–	0.56*, **	0.57*, **	0.20	0.14	–0.08	0.1	–0.41*, **	–0.28**	–0.38*, **	0.36*	0.06	–0.14	0.07	–0.12	–0.04
LPM	–	–	–	–0.18	0.08	0.25**	–0.05	0.07	0.23**	0.17	0.05	0.29**	0.02	0.24**	–0.005	–0.15	0.08
LPC	–	–	–	–	0.34**	–0.26**	–0.19	0.19	–0.76*, **	–0.62*, **	–0.66*, **	0.25**	0.16	–0.46*, **	–0.009	0.02	–0.12
SR	–	–	–	–	–	0.01	–0.10	0.43*, **	–0.51*,**	–0.23**	–0.56*,**	0.02	0.39**	0.37*	0.12	0.41*, **	0.23
TR	–	–	–	–	–	–	0.07	0.14	0.19	0.35**	0.08	–0.001	0.08	0.13	0.09	0.01	0.18
HR	–	–	–	–	–	–	–	0.11	0.08	0.31**	0.37**	0.04	0.10	0.02	0.59*, **	0.19	0.23**
PR									–0.36**	–0.16	–0.39**	0.05	0.27**	0.03	0.22	0.11	0.29**
DF									–	0.60*, **	0.63*, **	–0.21	–0.34**	0.49*, **	0.20	0.14	0.07
DM									–	–	0.52*, **	–0.16	0.10	0.40*, **	0.01	–0.07	0.25**
SW									–	–	–	–0.09	0.19	0.18	0.16	0.19	0.21
OC									–	–	–	–	0.15	–0.10	0.16	–0.13	0.05
SY-120									–	–	–	–	–	0.06	0.15	0.19	0.78*, **
SY-150									–	–	–	–	–	–	0.01	0.01	0.40*, **
SY-180									–	–	–	–	–	–	–	0.02	0.49*, **
SY-210									–	–	–	–	–	–	–	–	0.42*, **

*,**level of significance at 1 and 5% probability; PH – plant height (cm); NN – No. of nodes on the main stem; TLP – total length of primary raceme (cm); LPM – length of primary raceme covered with male flowers (cm); LPC – length of primary raceme covered with capsules (cm); SR – No. of secondary racemes/plant; TR – No. of tertiary racemes/plant; HR – No. of high order racemes/plant; PR – total number of productive racemes/plant; DF – days to 50% flowering; DM – days to maturity; SW – 100-seed weight (g); OC – oil content (%); SY-120 – seed yield/plant at 120 days after planting; SY-150 – seed yield/plant at 150 days after planting; SY-180 – seed yield/plant at 180 days after planting; SY-210 – seed yield/plant at 210 days after planting; TY – total seed yield/plant (g)

doi: 10.17221/205/2014-CJGPB

content and seed yield/plant at 210 DAP were the most effective traits while the number of higher order racemes/plant was the most effective one in the fourth component. Number of tertiary racemes/plant and total number of productive racemes/plant were little discriminative while total seed yield/plant was the least discriminating trait among the 18 traits. The principal component plot depicted a wide spread of 33 accessions across the plot (Figure 2), indicating the existence of great diversity among the accessions based on 18 agro-morphological traits.

EST-SSR study. To assess the genetic variation among 33 castor accessions collected from Andaman and Nicobar, islands of India, twenty-nine different castor eSSRs were applied. It was found that twenty-five primer pairs produced polymorphic products with a polymorphic percentage of 86.3 and 4 primer pairs produced monomorphic products. A total of 69 alleles were amplified using 29 primer pairs among 33 genotypes with an average frequency of 2.37 bands per primer. Fifteen eSSRs amplified a maximum number of three alleles, while 10 markers amplified a minimum number of two alleles (Table 5).

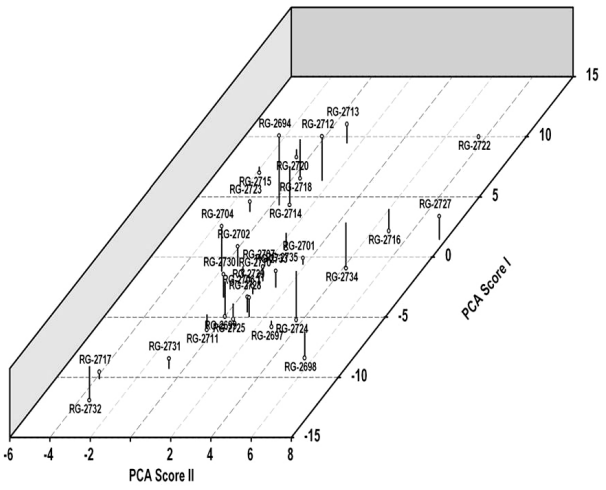

Figure 2. PCA analysis for the castor germplasm collection of Andaman and Nicobar Islands

Molecular size of the bands ranged from 120 bp with primer CES02 to 310 bp with primer CES35. Confusion probabilities for EST-SSR primers ranged from 0.11 to 0.65 with a mean of 0.45, which was

Table 4. Principal component analysis for 18 agro-morphological traits observed in 33 castor germplasm accessions collected from Andaman and Nicobar Islands, India

Trait	PC1	PC2	PC3	PC4
Plant height	0.898	0.033	0.123	0.164
Number of nodes on main stem	0.868	0.176	–0.0677	0.167
Total length of primary raceme	–0.494	0.010	–0.697	0.204
Length of primary raceme covered by male flowers	0.128	0.195	–0.751	0.500
Length of primary raceme covered by capsules	–0.853	–0.173	–0.082	–0.119
Number of secondary racemes/plant	–0.617	0.378	0.165	0.281
Number of tertiary racemes/plant	–0.011	0.254	–0.187	0.351
Number of high order racemes/plant	0.223	0.428	–0.167	–0.676
Total number of productive racemes/plant	–0.372	0.474	–0.027	0.091
Days to 50% flowering	0.904	–0.097	–0.042	0.186
Days to maturity	0.741	0.329	–0.021	0.072
100-seed weight	0.796	0.002	–0.125	–0.357
Oil content	–0.309	0.094	–0.592	–0.115
Seed yield/plat at 120 days after planting	–0.291	0.749	0.160	0.085
Seed yield/plat at 150 days after planting	0.598	0.233	–0.194	0.123
Seed yield/plat at 180 days after planting	–0.063	0.612	–0.183	–0.576
Seed yield/plat at 210 days after planting	–0.226	0.361	0.574	0.366
Total seed yield/plant	0.033	0.915	0.116	0.027
Eigenvalues	5.7105	2.7992	1.9750	1.69725
Variance (%)	31.758	15.581	10.968	9.511
Cumulative variance (%)	31.758	47.340	58.325	67.836

doi: 10.17221/205/2014-CJGPB

almost on par with (0.40) the report of Kanti *et al.* (2014). The limit of discriminating power (*DL*) was used to measure the efficiency of SSRs. *DL* value ranged from 0.355 to 0.88 with a mean of 0.55, which was almost similar (0.59) as reported by Kanti *et al.* (2014). In castor being a cross-pollinated crop, heterozygosity is obvious. Though all 33 accessions underwent eight generations of self-pollination in *ex-situ* to bring in within accession homozygosity, the EST-SSR profile indicated the existence of heterozygosity in three accessions (Figure 2). These three accessions (RG2722, RG2728, RG2736) should further undergo some more generations of self-pollination to fix the residual heterozygosity. In the remaining accessions, phenotypic uniformity, brought in within each accession through eight generations of self-pollination, was corresponding to the genotypic homozygosity as depicted by EST-SSR profile. The average expected heterozygosity in the germplasm collection was 0.58, which was higher in comparison with Foster *et al.* (2010) (0.21) with SNP markers and Allan *et al.* (2008) with SSR and AFLP markers. Differences in the seed sources of the same germplasm contribute a potentially important source of genetic variation. Establishing the level of heterozygosity in seed is critical because it improves the usefulness

Table 5. Calculations for No. of polymorphic bands (*n*), confusion probability (*C*), limit of discriminating power (*DL*) and heterozygosity (*He*) for EST-SSR primers

S. No	Primers	Expected size (bp)	Observed size (bp)	No. of alleles (*n*)	PIC	*C*	*DL*	*He*
1	CES02	230	120–150	3	0.56	0.65	0.35	0.63
2	CES05	195	190–260	3	0.53	0.55	0.45	0.55
3	CES06	190	170–250	3	0.59	0.61	0.39	0.61
4	CES10	217	210–260	3	0.49	0.49	0.51	0.50
5	CES20	207	190–220	2	0.11	0.12	0.88	0.22
6	CES30	220	190–230	2	0.48	0.50	0.50	0.49
7	CES34	212	190–230	3	0.62	0.64	0.36	0.60
8	CES35	178	290–310	2	0.41	0.42	0.58	0.42
9	CES36	197	180–225	3	0.20	0.26	0.74	0.21
10	CES44	213	230	1	0	0	0	0
11	CES39	250	250–265	2	0.42	0. 44	0.56	0.42
12	CES49	209	130–270	3	0.51	0. 56	0.44	0.51
13	CES51	169	140–180	3	0.51	0.53	0.47	0.51
14	CES56	247	210–250	3	0.42	0.51	0.49	0.42
15	CES58	210	155–190	3	0.53	0.53	0.47	0.53
16	CES59	190	240–300	2	0.16	0.18	0.82	0.18
17	CES62	213	200–220	2	0.42	0.47	0.53	0.42
18	CES65	245	220–285	2	0.30	0.36	0.64	0.35
19	CES72	273	250–275	2	0.49	0.50	0.50	0.49
20	CES73	226	225	1	0	0	0	
21	CES79	242	239–289	3	0.27	0.24	0.76	0.28
22	CES80	189	185–200	3	0.43	0.45	0.55	0.43
23	CES125	172	180	1	0	0	0	0
24	CES126	190	195	1	0	0	0	0
25	CES128	242	210–250	2	0.27	0.32	0.68	0.27
26	CES132	232	240–280	3	0.53	0.58	0.42	0.54
27	CES137	200	195–210	3	0.46	0.51	0.49	0.46
28	CES143	224	210–230	2	0.41	0.43	0.57	0.41
29	CES146	215	190–240	3	0.47	0.51	0.49	0.47
Total No. of alleles				69	0.37	0.45	0.55	0.42

doi: 10.17221/205/2014-CJGPB

Table 6. Detailed information obtained with EST-SSR markers in Andaman and Nicobar castor germplasm

No.	Indexes	EST-SSR
1	Number of assay units, U	29.0
2	Number of polymorphic bands, n_p	65.0
3	Number of monomorphic bands, n_{np}	4.0
4	Average Number of polymorphic bands/ assay unit, n_p/U	2.24
5	Number of loci, L	29.0
6	Number of loci/assay unit, n_u	1.0
7	Average confusion probability, C	0.45
8	Average limit of discriminating power, D_L	0.55
9	Effective number of patterns/assay unit, P	2.2
10	Average number of alleles per locus, n_{av}	2.4
11	Fraction of polymorphic loci, B	0.94
12	Expected heterozygosity, H_e	0.42
13	Effective number of alleles per locus, n_e	1.65
14	Total number of effective alleles, N_e	46.21
15	Assay efficiency index, A_i	1.59
16	Effective multiple ratio, E	0.94
17	Marker index, MI	0.52

of data to other breeders. It also ensures uniformity and stability of any materials developed from them. The effective number of patterns per assay unit (P), assay efficiency index (A_i), marker index (MI) were calculated (Table 6). The extent of polymorphism obtained among the 33 accessions as revealed by eSSR primer on 6% polyacrylamide gel is represented in Figure 3.

PIC value is indicative of the effectiveness of SSR loci information and measures the information about a given marker locus for the pool of genotypes (Kupper *et al.* 2011). The PIC value ranged from 0.12 (CES20) to 0.35 (CES34) with an average PIC value of 0.37 (Table 4), indicating that the loci were potent enough. The increase in the allele number did not affect the PIC value very much. The average PIC value in the current investigation was similar to 0.38 reported by Kanti *et al.* (2014), but was higher compared to 0.21 reported with SNPs (Foster *et al.* 2010). In contrast, the value was lower than 0.40 with gSSR and AFLP in worldwide germplasm genotyping (Allan *et al.* 2008) and 0.82, 0.88 with RAPD & ISSR, respectively, with the breeding material (Gajera *et al.* 2010). Genetic diversity of 72 castor accessions of north-east based on quantitative and qualitative methods was studied and reported seven most promising accessions based on the performance of yield attributing traits (Gogoi *et al.* 2011). An allele is considered to be rare when it is revealed in less than 5% of the genotypes under analysis (Jain *et al.* 2004) and null alleles are observed whenever an amplification product is not detected, in our study five primers generated eight rare alleles and sixteen primers generated null alleles, respectively.

With the aid of 69 allelic information dice the similarity coefficient of UPGMA cluster analysis was used to construct a dendrogram from DARWin 5.0, which illustrated the overall genetic relationship among the 33 accessions studied. Based on the dendrogram, the radial diagram grouped 33 castor accessions into three major clusters. Although there were three distinct clusters (I, II and III), only one cluster (III) was dominant. Among the three major clusters, cluster III grouped the largest number of accessions (18). Major cluster III generated 2 minor clusters IIIa (11 accessions) and IIIb (7 accessions). The other two major clusters (I and II) grouped 8 and 7 accessions, respectively. However, 22 minor clusters were formed among 33 accessions at the maximum reduced clad level (Figure 4). In conclusion, our results showed significant diversity among castor collections from Andaman and Nicobar at a phenotypic and molecular level. The results also indicate that EST-SSRs can be effective and promising molecular markers for identifying diversity in castor.

The EST-SSR marker system revealed a good amount of genetic variation among the castor accessions collected from Andaman and Nicobar, islands of India. This is contradictory to the results of Foster *et al.* (2010) and Allan *et al.* (2008) with worldwide genotyping and Kanti *et al.* (2014) with north-east castor accessions. This may be due to the presence of Andaman and Nicobar Islands situated at a far distance from the mainland. Similarly, genetic variability was also observed among the accessions

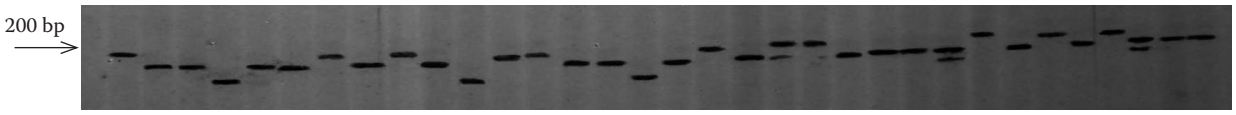

Figure 3. EST- SSR profile for thirty-three accessions of the Andaman and Nicobar collection on 6% polyacrylamide gel with CES49 primer

doi: 10.17221/205/2014-CJGPB

with the agro-morphological traits. The 33 accessions were separated into three major clusters (A, B, C) and 22 precise minor groups based on EST-SSR data set. However, agro-morphological data separated the accessions into 2 major clusters (I and II). Clustering of accessions based on EST-SSR data set did not match entirely with that based on agro-morphological traits. However, there were some similarities between both clustering approaches in grouping the accessions. Twelve of the 15 accessions placed in two major clusters (I and II) by EST-SSRs were located in major cluster I formed by cluster analysis based on agro-morphological data. Similarly, 12 out of the 18 accessions of major cluster III formed by EST-SSRs were located in cluster B of cluster analysis. At the maximum reduced clad level, RG2702 and RG2704 were placed together in one minor cluster, RG2713 and RG2723 were located in one minor cluster, and RG2694 and RG2727 were grouped in one minor cluster by both clustering approaches.

Both AFLP and SSR markers divided 41 castor accessions collected from 5 continents and 35 countries of the world into 2 major clusters as reported by Allan *et al.* (2008). However, one of them was dominating with maximum accessions with both the marker systems, indicating low diversity. Similarly, Gajera *et al.* (2010) used 30 RAPD and 5 ISSR markers to study diversity in 22 castor genotypes and showed that both the markers divided the genotypes into 2 major clusters dominated by one of the clusters. Though Foster *et al.* (2010) reported five clusters involving 152 accessions with 48 SNPs, molecular variance was observed within the populations followed by among the populations and continents with low diversity and narrow geographical distribution, showing a mixture of genotypes in most of the geographical regions studied. Similarly, Qiu *et al.* (2010) also reported 5 clusters with 118 EST-SSRs among 24 castor samples, but with low geographical distribution. Based on the sequence of mitochondria and chloroplast genome sequence of castor, SNPs were identified for phylogenetic analysis (Rivarola *et al.* 2011). They divided the accessions into two distinct subclades, which confirmed the previously observed low levels of genetic diversity in worldwide germplasm and showed a broad geographic distribution of each subclade.

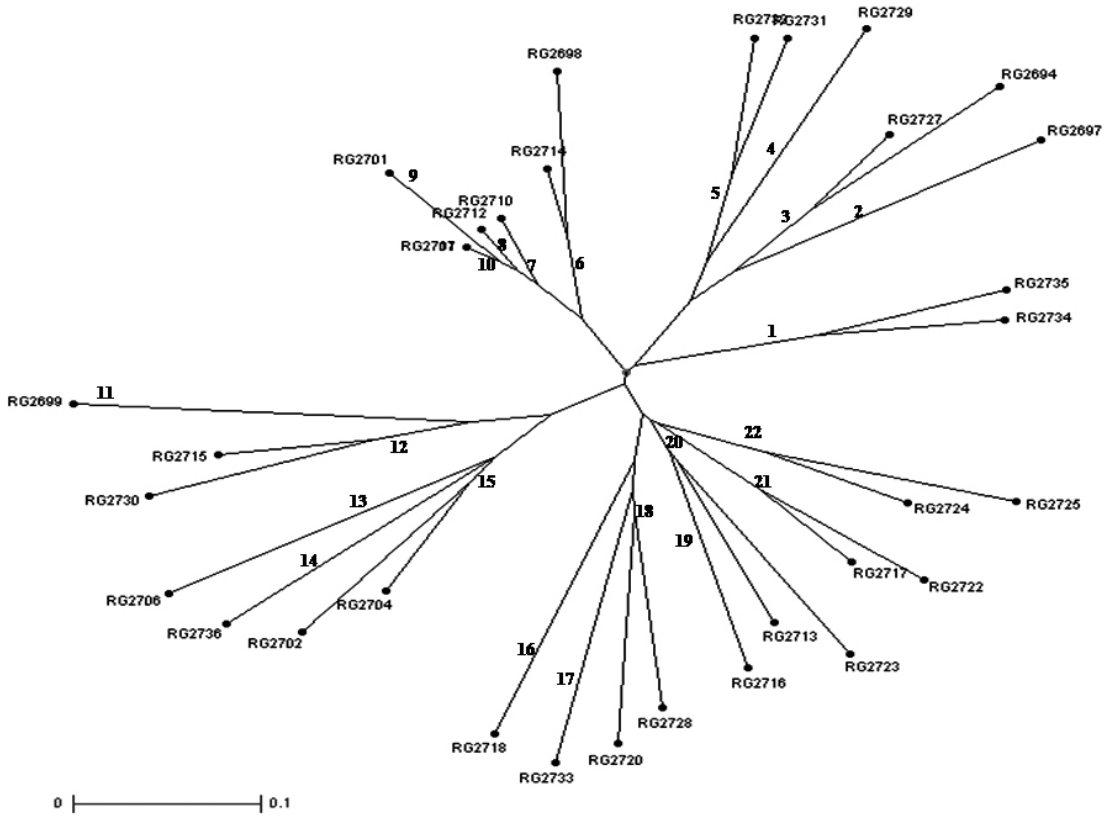

Figure 4. Radial tree diagram created by Darwin5 software depicting the clustering of 33 castor accessions collected from Andaman & Nicobar Islands, India, based on EST-SSR data set

doi: 10.17221/205/2014-CJGPB

From a cluster diagram generated from eSSR data it is found that the accessions collected from different regions of North Andaman clustered together in cluster C, indicating high similarity at a molecular level, whereas other accessions from North, South and Little Andaman were scattered only between 2 clusters (I and II), showing some variations. The accession RG2694 from Phoenix Bay and RG2702 from New Wandoor were grouped with the members of cluster A and C, respectively. The accessions RG2735 and RG2736 from different regions of Havelock were scattered between cluster A and C, showing similarity to the members of the cluster. The cluster C grouped a large number of accessions from different collection sites indicating high diversity. Similarly, from the agro-morphological data it is found that the accessions were scattered between cluster I and II almost equally. The accessions RG2694 and RG2702 were grouped with the members of cluster I and RG2735 and RG2736 were grouped together in cluster A and C, showing similarity to them. The accessions from different regions of North, Little and South Andaman did not show much difference at the agro-morphological level. This indicates that both clustering approaches have efficiently discriminated the accessions. However, EST-SSRs separated the accessions in a higher number of groups as compared to the agro-morphological data set, implying high efficiency and resolution of EST-SSR markers in genetic analysis of castor germplasm from Andaman and Nicobar Islands.

CONCLUSION

Considerable agro-morphological diversity was observed among 33 accessions collected from Andaman and Nicobar Islands. The correlation study clearly demonstrated the existence of associations among many agro-morphological traits. Ward's minimum variance was effective in categorizing 33 accessions into diverse groups based on agro-morphological traits. PCA could identify the most discriminating traits among the 18 traits evaluated. EST-SSR markers were efficient for the genetic diversity analysis of castor germplasm as well in identifying heterozygous and homozygous accessions. The primers showing PIC > 0.5 can also be useful for a wide range of genetic investigations such as association mapping, linkage map. Highly diverse accessions identified based on agro-morphological and molecular studies can be utilized in hybrid development programmes.

Acknowledgements. The authors gratefully acknowledge Dr. V. Dinesh Kumar, Principal Scientist, Directorate of Oilseeds Research, Rajendranagar, Hyderabad, for kindly providing the castor EST-SSR used in the study.

References

Allan G., Williams A., Rabinowicz P.D., Chan A.P., Ravel J., Keim P. (2008): Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLP and SSRs. Genetic Resources and Crop Evolution, 55: 365–378.

Anderson J.A., Churchill G.A., Autroque J.E., Tanksley S.D., Swells M.E. (1993): Optimizing selection for plant linkage map. Genome, 36: 181–186.

Anjani K. (2001a): Exploration of castor (*Ricinus communis* L.) germplasm in Andaman and Nicobar Islands. IPGRI Newsletter for Asia, the Pacific and Oceania, 34: 10.

Anjani K. (2001b): Catalogue of Castor (*Ricinus communis* L.) (1990–2000). Vol. 1. Hyderabad, Germaplasm Management Unit (Castor), Directorate of Oilseeds Research.

Anjani K. (2010): Extra-early maturing germplasm for utilization in castor improvement. Industrial Crops and Products, 31: 139–144

Anjani K. (2012): Castor genetic resources: A primary gene pool for exploitation. Industrial Crops and Products, 35: 1–4.

Athma P., Vaidyanath K., Reddy T.P. (1982): Peroxidase isoenzyme studies in certain varieties of *Ricinus communis* L. Indian Journal of Botany, 5: 178–182.

Bajay M.M., Pinheiro J.D., Batista C.E.A., Nobrega M.B.M., Zucchi M.I. (2009): Development and characterization of microsatellite markers for castor (*Ricinus communis* L.), an important oleaginous species for biodiesel production. Conservation Genetics and Resources, 1: 237–239.

Bajay M.M., Zucchi M.I., Kiihl T.A.M., Batista C.E.A., Monteiro M., Pinheiro J.B. (2011): Development of a novel set of microsatellite markers for Castor bean, *Ricinus communis* (Euphorbiaceae). American Journal of Botany, 98: E87–E89.

Berman P., Nizri S., Wiesman Z. (2011): Castor oil biodiesel and its blends as alternative fuel. Biomass and Bioenergy, 35: 2861–2866.

Birchler J.A., Auger D.L., Riddle N.C. (2003): In search of the molecular basis of heterosis. Plant Cell, 15: 2236–2239.

Brigham R.D. (1967): Natural out crossing in dwarf internode castor, *Ricinus communis* L. Crop Science, 7: 353–355.

Chan A.P., Crabtree J., Zhao Q., Lorenzi H., Orvis J., Puiu D., Melake-Berhan A., Jones K.M., Redman J., Chen G., Cahoon E.B., Gedil M., Stanke M., Haas B.J., Wortman J.R., Fraser-Liggett C.M., Ravel J., Rabinowicz P.D. (2010): Draftgenome sequence of the oilseed species *Ricinus communis*. Nature Biotechnology, 28: 951–956.

doi: 10.17221/205/2014-CJGPB

Costa M.N., Pereira W.E., Bruno R.L.A, Freire E.C., Noberega M.B.M., Milani M., Oliveira A.P. (2006): Genetic divergence between castor bean accessions and cultivars through multivariate statistics. Pesquisa Agropecuária Brasileira, 41: 1617–1622.

Demirbas A. (2007): Importance of biodiesel as transportation fuel. Energy Policy, 35: 4661–4670.

Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. Focus, 12: 13–15.

Esselman E.J., Crawford D.J., Brauner S., Stuessy T.F., Anderson G.J., Silva O.M. (2000): RAPD marker diversity within and divergence among species of *Dendroseris* (Asteraceae: Lactuceae). American Journal of Botany, 87: 591–596.

Feng S., Li W., Huang H., Wang J., Wu Y. (2009): Development, characterization and cross-species/genera transferability of EST-SSR markers for rubber tree (*Hevea brasiliensis*). Molecular Breeding, 23: 85–97.

Figueiredo Neto A., Almeida F.A.C., Gouveia Jpg D.E., Nobrega M.B.M., Carneiro R.M., Pedroza J.P. (2004): Genetic divergence in accessions of castor bean (*Ricinus communis* L.) based on characteristics of seed. Journal of Biology and Earth Sciences, 4: 1–10.

Foster J.T., Allan G.J., Chan A.P., Rabinowicz P.D., Ravel J., Jackson P.J., Keim P. (2010): Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). BMC Plant Biology, 10: 13–22.

Gaginella T.S., Capasso F., Mascolo N., Perilli S. (1998): Castor oil: new lessons from an ancient oil. Phytotherapy Research, 12(S1): S128–S130.

Gajera B.B., Kumar N., Amritpal S.S., Bhupendra S.P., Ravikiran R., Subhash N., Jadeja G.C. (2010): Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Industrial Crops and Products, 32: 491–498.

Gogoi S.N., Sarma R.N., Rajan R.K. (2011): Genetic diversity of castor (*Ricinus communis* L.) germplasm in North-eastern region of India. Indian Journal of Sericulture, 50: 51–57.

Govaerts R., Frodin D.G, Radcliffe-Smith A. (2000): World Cheklist and Bibliography of Euphorbiaceae (with Pandaceae). Trowbridge, Redwood Books, Ltd.

Jain S., Jain R.K., Mccouch S.R. (2004): Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theoretical and Applied Genetics, 109: 965–977.

Jeong G.T., Park D.H. (2009): Optimization of biodiesel production from castor oil using response surface methodology. Applied Biochemistry and Biotechnology, 156: 431–441.

Kaiser H.F. (1960): The application of electronic computers to factor analysis. Educational and Psychological Measurement, 20: 141–151.

Kanti M., Anjani K., Venkatramya K. (2014): Molecular Diversity in Castor Germplasm Collection Originated from North-Eastern Hill Province of India. International Journal Research for Scientific Innovations, 1: 1–6.

Kupper C.P.J.M., Chiotatto A., Zucchi M., Colombo C., Carbonell S., Costa M.J.M., Gazaffi R., Franco G.A.A., De Campos T., De Souza A. (2011): Genetic diversity in cultivated carioca common beans based on molecular marker analysis. Genetics and Molecular Biology, 34: 88.

Lowrey T.K., Crawford D.J. (1985): Allozyme divergence and evolution in *Tertramolopium* (Compositae: Astereae) on the Hawaiian Islands. Systematic Botany, 10: 64–72.

Meinders H.C., Jones M.D. (1950): Pollen shedding and dispersalin the castor plant *Ricinus communis* L. Journal of Agronomy, 4: 206–209.

Milani M., Dantas F.V., Martins W.F.S. (2009): Genetic divergence among castor bean genotypes by morphologic and molecular characters. Revista Brasileira de Oleaginosas e Fibrosas, 13: 61–71.

Moshkin V.A (ed.) (1986): Castor. New Delhi, Amerind Publishing Co., Pvt. Ltd.

Ogunniyi D.S. (2006): Castor oil: a vital industrial raw material. Bioresource Technology, 97: 1086–1091.

Perrier X., Flori A., Bonnet F. (2003): Data analysis methods. In: Hamon P., Seguin M., Perrier X., Glaszmann J.C. (eds): Genetic Diversity of Cultivated Tropical Plants. Montpellier, Enfield, Science Publisher: 43–76.

Pinzi S., Garcia I.L., Lopez-Gimenez F.J., Castro M.D.L., Dorado G., Dorado M.P. (2009): The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. Energy Fuels, 23: 2325–2341.

Qiu L., Yang C., Tian B., Yang J.B., Liu A. (2010): Exploiting EST database for the development and characterization of EST-SSR markers in castor bean (*Ricinus communis* L.) BMC Plant Biology, 10: 278–287.

Reif J.C., Gumpert F.M., Fischer S., Melchinger A.E. (2007): Impact of inter population divergence on additive and dominance variance in hybrid populations. Genetics, 176: 1931–1934.

Rivarola M., Foster J.T., Chan A.P., Williams A.L., Rice D.W., Liu X., Melake-Berhan A., Creasy H.H., Puiu D., Rosovitz M.J., Khouri H.M., Beckstrom-Sternberg S.M., Allan G.J., Keim P., Ravel J., Rabinowicz P.D. (2011): Castor bean organelle genome sequencing and worldwide genetic diversity analysis. PLoS ONE, 6: e21743.

Santalla M., Power J.B., Davey M.R. (1998): Genetic diversity in mung bean germplasm revealed by RAPD markers. Plant Breeding, 117: 473–478.

Sathaiah V., Reddy T.P. (1983): Inheritance of isoperoxidases in castor. In: 15th Int. Congress of Genetics, Dec 12–21, 1983, New Delhi, Abstracts Part 1: 387.

Sathaiah V., Reddy T.P. (1984): Qualitative and quantitative analysis of peroxidase and esterase enzymes in

doi: 10.17221/205/2014-CJGPB

castor (*Ricinus communis* L.). Indian Journal of Botany, 7: 68–73.

Sathaiah V., Reddy T.P. (1988): Esterase isozyme patterns of nine parental diallel set of castor (*Ricinus communis* L.). In: Proc. Conf. Cytology Genetics, 1: 240–246.

Sayama T., Hwang T.Y., Komatsu K., Takada Y., Takahashi M., Kato S., Sasama H., Higashi A., Nakamoto Y., Funatsuki H., Ishimoto M. (2011): Development and application of a whole-genome simple sequence repeat panel for high-through put genotyping in soybean. DNA Research, 18: 107–115.

Scholz V., Silva J.N. (2008): Prospects and risks of the use of castor oil as a fuel. Biomass and Bioenergy, 32: 95–100.

Singh A.K. (2011). Castor oil-based lubricant reduces smoke emission in two-stroke engines. Industrial Crops and Products, 33: 287–295.

Soltis P.S., Soltis D.E., Tucker T.L., Lang A. (1992): Allozyme variability is absent in the narrow endemic Bensoniellaoregona (Saifragacear). Conservation Biology, 6: 131–134.

Talia P., Nishinakamasu V., Hopp H.E., Heinz R.A. Paniego N. (2010): Genetic mapping of EST-SSRs, SSR and InDels to improve saturation of genomic regions in a previously developed sunflower map. Electronic Journal of Biotechnology, 13: 6.

Tessier C., David J., This P., Boursiquot J.M., Charrier A. (1999): Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theoretical and Applied Genetics, 98: 171–177.

Vivodík M., Balážová Z., Gálová Z. (2014): RAPD analysis of genetic diversity of castor bean. International Journal of Biological, Veterinary, Agricultural and Food Engineering, 8: 615–618.

Ward J.H. Jr. (1963): Hierarchical grouping to optimize an objective function. Journal of American Statistical Association, 58: 236–244.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalsky A., Tingey S.V. (1990): DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531–6535.

Received for publication October 13, 2014
Accepted after corrections May 22, 2015

Corresponding author:

Meena Kanti, Central Research Institute for Jute and Allied Fibres, Barrackpore-700 120, India;
e-mail: dhiru_dm@yahoo.co.in