J Am Oil Chem Soc (2017) 94:611–617
DOI 10.1007/s11746-017-2961-7

ORIGINAL PAPER

Assessment of Variation in Castor Genetic Resources for Oil Characteristics

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Received: 23 April 2016 / Revised: 30 January 2017 / Accepted: 30 January 2017 / Published online: 11 February 2017

Abstract Castor (*Ricinus communis* L.) oil is used in production of wide range of industrial products because of the presence of nearly 85% of ricinoleic acid in it. Any increase in the ricinoleic acid level would be great benefit to industry. None of the existing castor cultivars possess ≥90% ricinoleic acid because donors with this level of ricinoleic acid are not available to develop high ricinoleic type cultivars. In order to search for high ricinoleic acid genotypes, the present investigation was under taken. Fatty acid and oil content were assayed in 392 castor genotypes comprising 335 Indian and 57 non-Indian collections. Great variation was observed among the collections for oil content and fatty acid composition. Oil content ranged from 38.5 to 53.5% while ricinoleic acid was between 71.15 and 93.68%. Diversity analysis was done using K-means clustering which clustered the entire collection into 30 diverse groups by minimizing the dissimilarity within each cluster while maximizing the dissimilarity between clusters. Finally, 15 accessions having high oil (52–54%), high ricinoleic acid (91.12–93.68%) and high monounsaturates (92.8–94.95%) levels were identified. These would be of great value as donors to develop high oil, high ricinoleic type castor cultivars.

Keywords Castor · Diversity · Fatty acids · Germplasm · Oil

Electronic supplementary material The online version of this article (doi:10.1007/s11746-017-2961-7) contains supplementary material, which is available to authorized users.

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Introduction

Castor (*Ricinus communis* L. $2n = 20$), a multipurpose, drought resistant, perennial plant belonging to the *Euphorbiaceae* family is gaining a lot of importance in industry and for the production of biodiesel [1, 2]. Its seed contains approximately 48% oil. Castor oil is the only naturally available oil with very high concentration of ricinoleic acid (85%). The castor oil molecule has three types of functional groups viz, the carboxyl group, the hydroxyl group and carbon–carbon double bonds. Therefore, it has received extensive exploration as polyurethane building blocks, such as casting resins, elastomers, urethane foams, coatings, plastics, inks, cosmetics, pharmaceuticals, and in the production of macrolactones and polyesters, soaps, amine compounds, esters in cutting oils, industrial lubricants, emulsifiers, metal working compounds, thermosetting acrylics and non-drying plasticizing esters and interpenetrating networks [3]. Quaternary ammonium compounds based on ricinoleates and hydroxy stearates have been used in for cosmetics skin and hair care, personal products, germicides and textile processing agents. A new class of biodegradable polyanhydrides based on ricinoleic acid has been synthesized [4]. It serves as an excellent reactant for the synthesis of several different second and third generation derivatives which find application in a number of industrial uses. The most prominent second generation derivatives are ricinoleic acid, Turkey red oil, hydrogenated castor oil, 12-hydroxy stearic acid (12-HSA), dehydrated castor oil (commercial), sebacic acid and undecylenic acid. Third generation derivatives of castor oil are high specialty chemicals such as zinc ricinoleate. These derivatives, however, produced in smaller volumes individually than the second generation derivatives, though they command much

higher prices [4]. The global market for second generation derivatives is estimated at about US$ 300 million. Half of the second generation derivatives are converted into third generation derivatives, whose estimated market is close to US$350 million [5].

India alone has exported 135,513 MT of ricinoleic acid worth US$ 69,406,297 during 01, January 2014 to 14, November 2016 [6]. Ricinoleic acid is produced biochemically by the hydroxylation reaction that converts oleate to ricinoleate. Based on the hypothesis that the hydroxylation reaction is analogous to or the first step in the desaturation reaction, researchers proposed that the hydroxylase would share little sequence similarity with FA desaturases [7]. Using this approach attempts have been made by researchers to express the hydroxylase gene in transgenic *Arabidopsis thaliana*. However, so far transgenics have not produced more than 17% hydroxy fatty acid content in oil [8]. Any increase in ricinoleic acid content in castor beyond 88% would be beneficial to the industry. This would increase the value of castor oil. With the rising environmental concerns and the need for bio-based products to replace synthetic feed stocks, castor oil and castor oil oleochemicals will have higher potential in the future to be utilized in many newer industries.

The interest in castor cultivation has escalated in the past decade because of recognition of the immense potential of castor oil for demand growth. Therefore, the promotion of cultivation of castor has become a strategic choice in many countries. The world castor seed production increased from 580,010 tonnes in 1961 to 1,948,070 tonnes in 2014 [9]. The major castor producing countries are India, China and Brazil. India is the largest producer of castor seed and exporter of castor oil with an 82.75% share of the international trade in this commodity [10]. India has the largest castor germplasm resources in the world (4307) followed by China (2111) and USA (1390) [11].

Castor seed is a good feedstock for biodiesel production, although a serious concern is its high kinematic viscosity. Kinematic viscosity of castor oil is 14.51 cSt—a value considerably higher than the maximum of 5.5 cSt specified by the National Petroleum Agency for fuel used in diesel engines [12]. However, this problem can be reduced by converting castor oil into fatty acid methyl or ethyl esters by transesterification. The viscosity can be reduced 11.8-fold by transesterification [13]. The presence of hydroxyl groups in castor oil makes the oil soluble in alcohol in any proportion.

Though castor oil has been recognized worldwide as an important source of high ricinoleic acid for many industrial uses, no research efforts have been made globally to develop cultivars with high oil and ricinoleic acid levels. The characteristics of castor oil from other countries such as Mexico [14], Brazil [15], Nigeria [16], Malaysia [17] and USA [18] had been studied. In India, though it was achieved that there was a significant genetic improvement in castor with regard to maturity, disease and insect resistance and seed yield [19–21], no concerted efforts were made to develop high oil and high ricinoleic type cultivars. Only 15 Indian castor genotypes (released varieties, hybrids, male and female lines) have been evaluated so far for oil characteristics [22]. None of the released castor cultivars contain more than 88% ricinoleic acid content. Availability of genetic donors for high ricinoleic acid content is required to breed cultivars with ricinoleic acid levels beyond 88%. Germplasm is the primary resource for searching natural donors for traits of interest. The castor germplasm management unit (GMU) at the Indian Council of Agricultural Research-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India presently conserves around 3000 castor germplasm accessions. Since, germplasm exchange among countries has become a sensitive issue under the present international treaties, it is essential to search the available native germplasm for desirable traits. Hence, investigation of the vast Indian germplasm collection has become the crucial primary step for identifying genetic material with high oil content and appropriate fatty acid profile, especially high ricinoleic acid content, in order to develop high oil, high ricinoleic type castor cultivars. In this context, a preliminary screening of 392 castor genotypes, comprising 389 castor germplasm accessions maintained at ICAR-IIOR and three Indian commercial cultivars, DSH-519, DSH-177 and 48-1, was taken up with the objective of assessing the genetic variation for oil content and fatty acid composition in these genotypes and identify promising genotypes with ricinoleic levels beyond 88% and oil above an average level of 48%.

Materials and Methods

Plant Material

A total of 392 castor accessions was assessed in this investigation, of which 30 were introductions from Brazil, Hungary, Italy, Nigeria and the USA, 27 accessions were of unknown origin, 332 were Indian accessions, two were commercial hybrids, DCH-519 and DCH-177 and one was a commercial cultivar, 48–1 developed in India. The 332 Indian collections were collected from 18 provinces and Andaman and Nicobar Islands of India through conducting castor germplasm explorations [11]. The information on origin/source of each accession is given in an Electronic Supplementary Materials (ESM) document.

Oil Content Analysis

Oil content of each genotype was analyzed using a bench top pulsed nuclear magnetic resonance (NMR)—Oxford-MQC-5 analyzer (London, UK), supplied with preloaded 'easy cal' software, calibrated with known oil castor seed samples [23]. The calibration was performed with a 40 mm diameter sample probe, 5 MHz operating frequency, 4 scans, 1 s recycle delay and 40.00 magnetic box temperature. NMR room temperature was maintained at 25 °C ± 2. Before construction of calibration sample seeds were dried by keeping them at 80 °C for 8 h in a hot air oven.

Analysis of Fatty Acid Composition

Oil from seeds was extracted in hexane on a Soxhlet apparatus (Extraction unit, E-816, Buchi, Flawil, Switzerland). For complete extraction of oil, the extraction, rinsing and drying process was carried out for at least 80 (30 cycles), 60 and 30 min, respectively. Methyl esters were obtained by a two-step catalytic process according to a slightly modified method of Ghadge and Raheman (2005) [24]. Oil (100–150 mg) was treated with 2% sulfuric acid in methanol (5 ml) for 2 h at 60 °C. After the reaction, the mixture was allowed to settle for an hour and the methanol–water mixture that separated at the top was removed. The second step product at the bottom was transesterified using 2 ml of 13% methanolic KOH for 30 min at 55 °C. The organic phase was extracted with hexane and washed with water till neutral pH. The hexane was dried over anhydrous sodium sulfate and concentrated with nitrogen to get methyl esters.

Fatty acid composition was determined using an Agilent 7860A gas chromatograph (Santa Clara, California, USA) equipped with a flame ionization detector (FID) and an auto sampler. Peak separation was performed on a HP-1 capillary column (100% dimethyl polysiloxane, diameter-320 µm, length-30 m, film thickness-0.25 µm) from Agilent Technologies. The carrier gas was nitrogen set to a constant gas flow of 1.2 ml/min at 150 °C initial temperature. Then 0.2 µl of the sample was injected at a 20:1 split ratio into the column with the following temperature conditions: 150 °C for 2 min; raised from 150 to 300 °C at 10 °C/min. Both inlet and detector were set to 325 °C. The fatty acid composition was determined by identifying and calculating the relative peak area percentages by GC post run analysis EZChrom elite compact software.

Diversity and Statistical Analysis

The K-means clustering method was used for diversity analysis [25]. The number of clusters was determined by rerunning the analysis for different numbers of clusters until the square error was minimized to its lowest. Pearson's coefficient analysis was done to understand the correlations among different traits. K-means clustering analysis using Euclidian distance metric, Pearson's coefficient analysis, calculation of the mean and standard error of the mean (SEm±) were done using INDOSTAT statistical software, Indostat services, Hyderabad, India. Composition of each cluster formed by K-means clustering analysis was given as an ESM document.

Results and Discussion

Variation in Oil Content

The average seed oil content of 392 genotypes was 49.9% (SEm ± 0.11) with a range between 38.5 and 54% (Fig. 1). Germplasm accessions with 37–60% oil content have been reported earlier in castor [18]. Since seed oil content is affected by the environmental conditions, cultural practices, time of harvesting and moisture content in the seed, the 392 genotypes included in the present investigation were grown in 2013–14 at the research farm of the ICAR-Indian Institute of Oilseeds Research, Hyderabad, and seeds of these accessions were harvested at same stage of maturity. The seed moisture content was reduced to 7–8% by oven drying prior to analysis. The data showed no significant variation between accessions of Indian and non-Indian origins with respect to oil content. The highest oil content (54%) was observed in RG-2451 and RG-178 and the lowest (38%) was in RG-172 (PI 306724). RG-2451 (PI 258368) originated from South Africa, RG-178 was from Hungary and RG-172 was an introduction from United States Department of Agriculture (USDA). In addition, a non-Indian accession of unknown origin, RG-3283 and an Indian accession, RG-329 contained 53% oil content. Oil content exhibited non-significant negative correlations with fatty acids viz, palmitic acid ($r = -0.005$), linolenic acid ($r = -0.08$), linoleic acid ($r = -0.01$), oleic acid ($r = -0.07$), stearic acid ($r = -0.008$), gadoleic acid ($r = -0.05$) and ricinoleic acid ($r = -0.03$) with p values more than 0.0001.

Variation in Fatty Acid Profile

The ideal plant genotype for industrial applications should have not only high oil content but also an appropriate fatty acid composition since the fatty acid profile influences the oil properties [26]. Ricinoleic acid which is the predominant fatty acid in castor oil possesses a hydroxyl group that adds extra stability to the oil and its derivatives by preventing the formation of hydroperoxides [27]. In the tested castor genotypes, ricinoleic acid ranged from 71.15 to 93.68% with a mean value of 85.68% (SEm ± 0.17) (Fig. 1). Other fatty

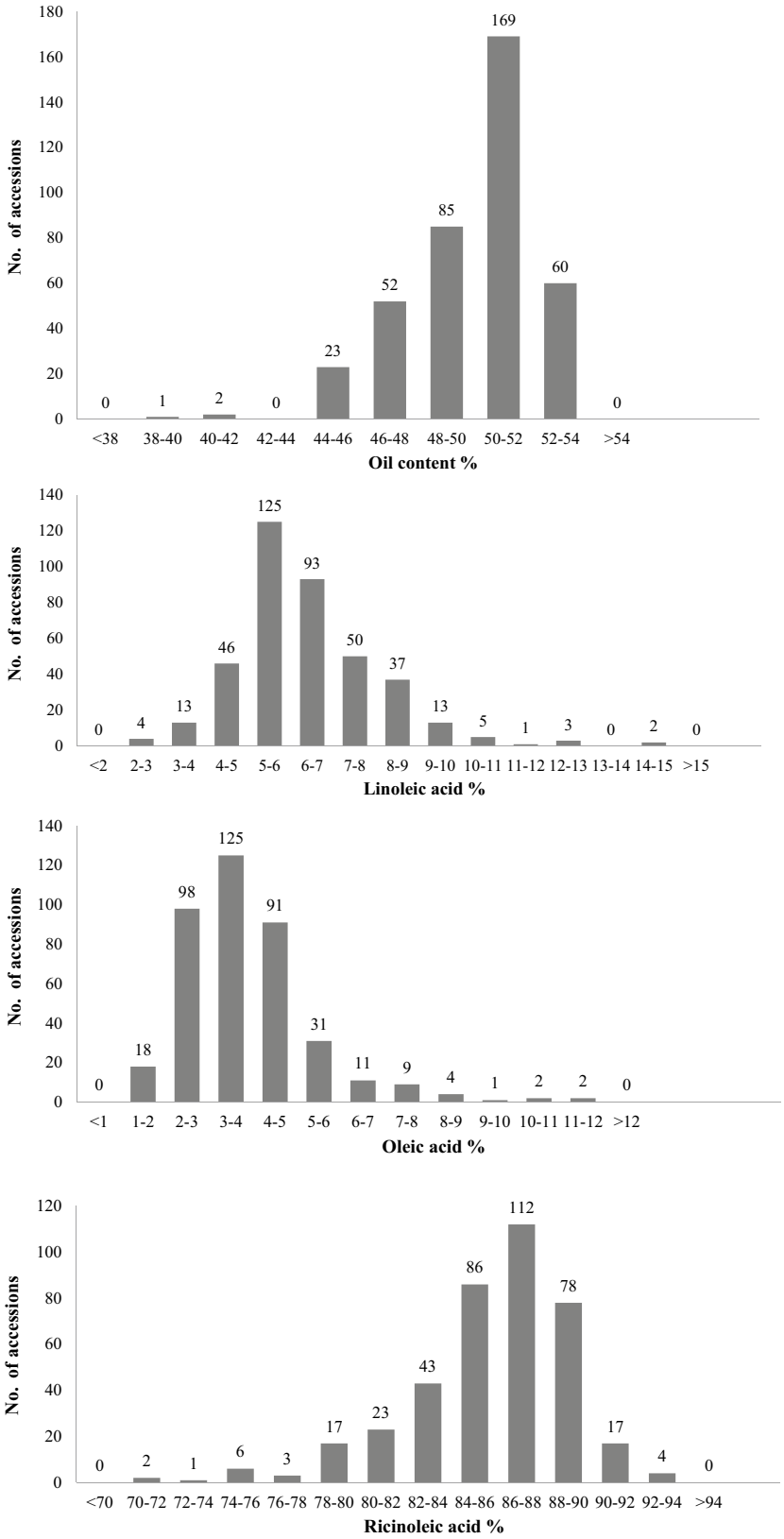

Fig. 1 Frequency distribution of oil content, oleic acid, linoleic acid and ricinoleic acid (%) among 392 castor accessions

Table 1 Cluster means for oleic acid, linoleic acid, ricinoleic acid and oil contents

Cluster	*n*	SS	Oleic acid	Linoleic acid	Ricinoleic acid	Oil content (%)
1	20	6.3	2.91	6.04	87.02	48.5
2	16	9.2	4.91	8.01	82.69	51.2
3	10	5.9	3.19	7.64	84.11	50.6
4	15	9.9	4.92	6.16	84.70	51.3
5	12	5	4.20	5.91	85.56	49.0
6	17	5.8	3.62	5.29	87.12	49.9
7	24	6.5	3.91	6.43	85.72	51.0
8	6	1.2	2.42	6.33	86.92	51.5
9	14	3.1	4.08	5.62	86.54	51.6
10	12	3.6	2.59	5.44	88.64	46.4
11	17	5.2	2.67	5.02	88.83	49.0
12	14	3.2	2.79	5.82	87.88	50.9
13	25	8.2	4.05	7.40	83.96	51.6
14	19	4.5	3.34	5.80	86.96	51.7
15	19	8.0	3.67	5.94	86.52	46.1
16	12	6.6	4.49	6.72	84.20	46.6
17	16	5.0	2.92	4.65	89.10	51.0
18	13	5.9	4.07	7.04	84.65	47.7
19	22	4.2	2.68	5.31	88.45	51.9
20	6	5.7	4.81	3.09	88.72	50.8
21	12	5.8	1.96	4.45	90.40	51.5
22	6	4.6	1.94	4.27	90.59	45.7
23	7	4.7	1.58	3.49	92.03	51.9
24	15	16.9	4.94	8.52	81.50	47.6
25	7	14.2	6.22	9.56	78.28	47.1
26	9	11.3	7.64	7.48	80.50	51.4
27	10	7.2	5.20	8.56	80.31	51.5
28	8	17.2	6.37	11.18	77.08	51.7
29	3	1.2	2.74	5.24	88.65	39.8
30	6	26	10.03	10.96	74.18	50.6

n number of accessions in a cluster, *SS* within cluster sum of square

acids present were palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, gadoleic acid and dihydroxy stearic acid (DHSA) with an average of 1.56% (SEm ± 0.02), 1.38% (SEm ± 0.02), 3.87% (SEm ± 0.07), 6.37% (SEm ± 0.08), 0.15% (SEm ± 0.007), 0.45% (SEm ± 0.01) and 0.54% (SEm ± 0.007), respectively. The data indicated the presence of considerable variation for ricinoleic acid among 392 accessions. Very low levels of linoleic acid and linolenic acid were observed among castor germplasm accessions. The range of linoleic acid content was from 2.57 to 14.96, and of linolenic acid content was from 0.01 to 0.89. Linolenic acid content below 0.1% was recorded in 196 accessions. The lowest linolenic acid content (0.01%) was recorded in an Indian accession, RG-243, followed by another Indian accession, RG-348 with 0.02% level.

The commercial hybrids, DCH-519 and DCH-177 have recorded 86.38% and 87.46% ricinoleic acid content, respectively and the variety, 48-1 had 85.92% ricinoleic acid. Among the germplasm collections, above 92% ricinoleic acid content was observed in four accessions viz, RG-66 (93.68%), RG-3477 (93.56%), RG-57 (92.54%) and RG-226 (92.12%), and less than 75% ricinoleic acid levels were observed in five accessions viz, RG-43 (71.15%), RG-260 (71.90%), RG-196 (73.88%), RG-3053 (74.38%) and RG-280 (74.62%). All these accessions were of Indian origin. The ricinoleic acid content varying from 58 to 92% had been reported in castor by earlier studies [22, 27, 28].

Monounsaturates

High monounsaturation can be altered to give various value added products such as polymerized oils, hydroxystearates, epoxidized oils, halogenated oils, factice and sulfonated oils [29]. Monounsaturates (ricinoleic + oleic) combines

good ignition quality with adequate cold flow performance of biodiesel [30]. In the present study, castor oil had on an average of 89.51% monounsaturates (85.68% ricinoleic acid + 3.83% oleic acid). Monounsaturates in 392 genotypes varied from 79.53 to 95.03%. Four accessions, RG-57, RG-66, RG-336, and RG-3477, contained high monounsaturates (94–95%) and 11 had 93.06–93.98%.

Diversity Analysis

K-means clustering is simple and fast, and proved to be effective in many practical applications [31, 32]. This method is one of the most widely used algorithms for clustering large sets of data. Hence, diversity analysis using K-means clustering was done to partition the 392 castor genotypes into different groups so as to identify similar and dissimilar accessions for traits of interest. The data on linoleic acid, oleic acid, ricinoleic acid and oil contents of 392 genotypes were subjected to K-means clustering. K-means algorithm was run with several different initial number of clusters (K) to choose ideal K. Thus, the ideal K chosen was 30 as it had given the smallest values of within clusters sum of squares (Table 1). The clustering method minimized the dissimilarity of the accessions within each cluster while maximizing the dissimilarity of different clusters. Cluster means for different traits Table 1.

Among the 30 clusters, cluster-13 was composed of the highest number of accessions (25) followed by cluster-7 possessing 24 accessions. Cluster-29 included the lowest number of accessions (3) followed by cluster-8, 20, 22, and 30 each having 6 accessions. The commercial hybrids, DSH-519 and DCH-177 were included in cluster-1 and the variety, 48-1 was placed in cluster-7. Cluster-23 comprising seven accessions exhibited the highest mean ricinoleic acid content (92.03%) and the second highest oil content (51.92%) whereas the highest oil content (51.94%) was observed in cluster-19 having 22 accessions with 88.45% mean ricinoleic acid content. Cluster-30 showed the lowest mean ricinoleic acid content (74.18%) and cluster-29 had the lowest mean oil content (39.83%). K-means clustering facilitated an easy approach for classifying similar accessions and identifying a group of accessions having optimal levels of traits of interest.

Optimum Combination of Oil Content, Fatty Acid Composition

The germplasm accessions need to have high oil content coupled with high monounsaturates for using them as parents in breeding programs aiming to develop cultivars with high monounsaturates for industrial uses. From the results on oil content, fatty acids, the clusters 21 and 23 were found to be promising (Table 2). It appears that the accessions viz, RG-226, RG-2451 and RG-3477 belonging to cluster-23 and RG-380 belonging to cluster-21, are the promising ones to use as parents as they exhibited high oil content (52 and 54%), high ricinoleic acid (91.12–93.56%) and monounsaturates (92.8–94.95%) contents. In addition, eight accessions viz, RG-370, RG-3467, RG-408, RG-63, RG-358, RG-357 and RG-3233 in cluster-21 and three accessions, viz, RG-329, RG-311 and RG-2685 in cluster-23 were found to be promising.

Conclusions

The results revealed the existence of great genetic diversity among castor germplasm for oil content and fatty acids. Till now castor has been bred for increased seed yield across the world with little attention to genetic improvement for oil

Table 2 Linoleic acid, oleic acid, ricinoleic acid and oil contents of promising accessions identified using K-mean cluster analysis

Cluster	Accession	Linoleic acid (%)	Oleic acid (%)	Ricinoleic acid (%)	Oil content (%)
Cluster-21	RG-63	3.73	2.07	91.44	51.0
	RG-357	4.21	1.67	91.39	50.2
	RG-358	3.13	1.52	90.9	51.8
	RG-370	4.32	2.59	90.01	52.6
	RG-380	4.08	2.19	91.12	52.1
	RG-408	4.91	1.54	90.50	51.6
	RG-3233	4.33	1.73	91.86	51.7
	RG-3467	4.95	1.92	90.56	50.4
Cluster-23	RG-66	3.02	1.35	93.68	50.1
	RG-226	3.56	1.83	92.12	52.4
	RG-311	3.97	1.62	91.01	51.5
	RG-329	3.69	2.26	90.54	52.9
	RG-2451	3.95	1.29	91.59	54.0
	RG-2685	3.35	1.36	91.72	51.6
	RG-3477	2.95	1.39	93.56	52.1

and ricinoleic acid levels. Any further increase in the level of oil content and ricinoleic acid would be beneficial to the industry. This preliminary screening could identify castor germplasm accessions with high oil contents and favorable fatty acid compositions. These promising accessions would be of great value to castor plant breeders for their use as donors of genes conferring high oil and high ricinoleic acid or high monounsaturates traits in castor breeding programs for developing cultivars with more enhanced levels of these traits than the existing ones.

References

1. Bello EI, Makanju A (2011) Production, characterization and evaluation of castor oil biodiesel as alternative fuel for diesel engines. J Emerg Trends Eng Appl Sci 2:525–530
2. Asmare M, Gabbiye N (2014) Synthesis and characterization of biodiesel from castor bean as alternative fuel for diesel engine. Am J Energy Eng 2:1–15
3. Patel VR, Dumancas GG, Viswanath LCK, Maples R, Subong BJJ (2016) Castor Oil: properties, uses, and optimization of processing parameters in commercial production. Lipid Insights 9:1–12
4. Castoroil.in (accessed on December, 2016). Comprehensive castor oil report. http://www.castoroil.in/reference/report/report.html
5. Energy Alternatives India (2016) Production and uses of key castor oil oleochemicals. http://www.consult.eai.in/wp-content/uploads/2016/05/Production-and-Uses-of-Key-Castor-Oil-Oleochemicals.pdf. Accessed Dec 2016
6. eximpluse.com (2016) https://www.eximpulse.com/export-product-Castor.htm. Accessed Dec 2016
7. Bafor M, Smith MA, Jonsson L, Stobart K, Stymne S (1991) Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (*Ricinus communis* L.) endosperm. Biochem J 280:507–514
8. Broun P, Somerville C (1997) Accumulation of ricinoleic, lesquerolic, and densipolic acids in seeds of transgenic arabidopsis plants that express a fatty acyl hydroxylase cDNA from castor bean. Plant Physiol 113:933–942
9. Food and Agriculture Organization of the United Nations (2016) Food and agriculture data. http://www.fao.org/faostat/en/#data/QC. Accessed Dec 2016
10. Inditrade.com (2016) http://www.inditrade.com/static/commodity.aspx. Accessed Dec 2016
11. Anjani K (2012) Castor genetic resources: a primary gene pool for exploitation. Ind Crop Prod 35:1–14
12. Monteiro LA, Junior GP, Velasquez JA, Rocha DS, Bueno AV (2013) Performance impact of the application of castor oil biodiesel in diesel engines. Eng Agric 33:100–6916
13. Jeong GT, Park DH (2009) Optimization of biodiesel production from castor oil using response surface methodology. Appl Biochem Biotechnol 156:431–441
14. Perdomo FA, Acosta-Osorio AA, Herrera G, Vasco-Leal JF, Mosquera-Artamonov JD, Millan-Malo B, Rodriguez-Garcia ME (2013) Physicochemical characterization of seven Mexican *Ricinus communis* L. seeds & oil contents. Biomass Bioenergy 48:17–24
15. Ramos LCD, Tango JS, Savi A, Leal NR (1984) Variability for oil and fatty acid composition in castor bean varieties. J Am Oil Chem Sci 61:1841–1843
16. Nangbes JG, Nvau JB, Buba MW, Zukdimma AN (2013) Extraction and characterization of castor (*Ricinus communis* L.) seed oil. Int J Eng Sci 2:105–109
17. Salimon Noor DAM, Nazrizawati AT, Firdaus MYM, Noraishah A (2010) Fatty acid composition and physicochemical properties of Malaysian castor bean *Ricinus communis* L. seed oil. Sains Malays 39:761–764
18. Wang ML, Morris JB, Tonnis B, Pinnow D, Davis J, Raymer P, Pederson GA (2011) Screening of the entire USDA castor germplasm collection for oil content and fatty acid composition for optimum biodiesel production. J Agric Food Chem 59:9250–9256
19. Anjani K (2010) Extra-early maturing germplasm for utilization in castor improvement. Ind Crop Prod 31:139–144
20. Anjani K (2006) RG2819 (IC346591;INGR 06010) castor (*Ricinus communis* L.) germplasm resistant to Macrophomina root rot and Fusarium wilt. Indian J Genet Resour 19:298
21. Anjani K, Palliva M, Sudhakara Babu SN (2010) Biochemical basis of resistance to leaf miner in castor (*Ricinus communis* L). Ind Crop Prod 31:192–196
22. Lavanya C, Murthy IYLN, Nagaraj G, Mukta N (2012) Prospects of castor (*Ricinus communis* L.) genotypes for biodiesel production in India. Biomass Bioenergy 39:204–209
23. Yadav P, Murthy IYLN (2016) Calibration of NMR Spectroscopy for accurate estimation of oil content in sunflower, safflower and castor seeds. Curr Sci 110:73–76
24. Ghadge SV, Raheman H (2005) Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids. Biomass Bioenergy 28:601–605
25. Indostat (2016) http://www.indostat.org/agriculture.html. Accessed Dec 2016
26. Knothe G (2008) "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuels 22:1358–1364
27. Ogunniyi DS (2006) Castor oil: a vital industrial raw material. Bioresour Technol 97:1086–1091
28. Ramos LCD, Tango JS, Savi A, Leal NR (1984) Variability for oil and fatty acid composition in castor bean varieties. J Am Oil Chem Soc 61:1841–1843
29. Bhardwaj HL, Mohamed AI, Webber CL III, Lovell GR (1996) In: Janick J (ed) Evaluation of castor germplasm for agronomic and oil characteristics. Progress in New Crops, ASHS Press, Alexandria
30. Serdari A, Lois E, Stournas S (1999) Impact of esters of mono- and dicarboxylic acids on diesel fuel quality. Ind Eng Chem Res 38:3543–3548
31. Singh N, Singh D (2012) Performance evaluation of K-means and hierarchal clustering in terms of accuracy and running time. Int J Comput Sci Inf Technol 3:4119–4121
32. Kaur M, Kaur N (2013) Web document clustering approaches using k-means algorithm. Int J Adv Res Comput Sci Softw Eng 3:861–864