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GENETICS AND PLANT BREEDING

Oil Content and Fatty Acid Composition in Castor (*Ricinus communis* L.) Genotypes

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ABSTRACT

Castor (*Ricinus communis* L.) is an important non-edible oilseed crop with diversified industrial usage. In the present study, twenty-six genotypes of castor were analyzed for total seed oil content using Nuclear Magnetic Resonance (NMR) spectroscopy and fatty acid profiling through gas chromatography with flame-ionization detector (GC-FID) to update the database of this important industrial crop. In the present study, the highest seed oil content was determined in SHB-1019 genotype which was 45.86 %. Total saturated fatty acid, steric acid and palmitic acid per cent was recorded higher in SKI-370 genotype which was 3.38 %, 1.92 % and 1.46 %, respectively. Among the all genotypes, the higher per cent of total unsaturated fatty acid, ricinoleic acid and linolenic acid was observed higher in genotype VI-9 whereas, mono unsaturated fatty acid (oleic acid) and poly unsaturated fatty acid (linoleic) was found higher in genotype GEETA. The present study can be very important in the selection of male parents for the development of high ricinoleic acid castor hybrid. This will ultimately help in increasing the production and quality of industrially important oil.

HIGHLIGHTS

- Twenty six genotypes of castor were analyzed for total seed oil content through NMR spectroscopy and fatty acid composition through GC-FID.
- The highest seed oil content (45.86 %) was determined in SHB-1019 genotype.
- The highest oleic acid/linoleic acid ratio was 0.536 in SH-72 genotype indicating higher stability of castor oil for industrial applications.

Keywords: Castor, *Ricinus communis* (L.), Castor oil, Fatty acid composition, GC-FID

Castor (*Ricinus communis* L. 2n = 2X = 20) resides one of the most important non-edible oilseed crop (Dharajiya *et al*. 2020). It is hails to mono specific genus *Ricinus* of Euphorbiaceae family (Chaudhari *et al*. 2019). It has cross pollination up to the extent of 50 per cent due to its monoecious nature. Castor seed contains 38 to 54 per cent oil (Román-Figueroa *et al*. 2020; Yadav *et al*. 2017). The castor oil has extra properties than other vegetable oils, as it does not freeze even under -12ºC to -18ºC temperature. It is therefore, considered as one of the best lubricating agent particularly for high speed engine and aero planes. Owing to its unique physico-chemical properties, castor oil is used as raw material for

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numerous and varied industrial applications, such as manufactures of polymers, coatings, cosmetics and production of biodiesel (Dharajiya *et al*. 2020). Due to commercial use and advancement of industrialization, the demand of raw castor oil has been increased all over the world. The refined oil has a good domestic market (Jeong and Park 2009).

Fatty acid of castor oil contains ricinolenic acid, oleic acid, linoleic acid, α-linolenic acid, stearic acid, and palmitic acid (Shah *et al*. 2017). Ricinoleic acid is the major component of castor oil and is normally occupies over 89 per cent of the total fatty acid of the oil (Shah *et al*. 2017). Fatty acids in castor seeds have been studied since 1970s (Cooper 1971; Lin and Arcinas 2007; Shah *et al*. 2015). The castor seed are studied much for oil content, but limited attempt has been made for fatty acid profiling of castor oil. Castor industry expects low variability in the fatty acid profile of oil, grown at either different locations or in different years (da Silva Ramos *et al*. 1984; Ogunniyi 2006; Mutlu and Meier 2010). Therefore, assessing fatty acid profile of as many germplasm as possible is very useful in industrial level to generate database and to set benchmark. By considering these facts, this study was aimed to analyze the total seed oil through Nuclear Magnetic Resonance (NMR) spectroscopy and absolute contents of fatty acids in castor seeds by gas chromatography to update the database of this important industrial crop.

MATERIALS AND METHODS

Twenty six castor genotypes including eleven hybrids (GAUCH-1, GCH-2, GCH-4, GCH-5, GCH-6, GCH-7, SHB-1005, SHB-1018, SHB-1019, SHB-1029 and GNCH-1), four pistillate lines (VP-1, GEETA, JP-65 and SKP-84) and eleven inbred lines (VI-9, JI-35, 48-1, SH-72, JI-96, SKI-215, SKI-352, SKI-370, SKI-372, SKI-373 and DCS-94) were grown at Agricultural Research Station, S. D. Agricultural University, Kholwada, Gujarat, India during *kharif-rabi* 2016-17 with spacing of 120 cm × 60 cm. Standard uniform agronomic practices were followed to raise the crop. Biochemical analysis was carried out at Main Castor-Mustard Research Station, SDAU, Sardarkrushinagar, Gujarat, India.

Seed oil content (%) determination

Random sample of 45 grams seeds was obtained from the bulk produced from each genotype from each replication the oil content of seeds was obtained by Bench top NMR Analyzer (Oxford instruments, USA).

Fatty acid profiling of seed oil

Approximately 60 g dried seeds were collected from each genotype and crushed into uniform texture using mortar-pestle. The crushed seeds were loaded into Soxhlet to extract oil using petroleum ether (60-80°C) as solvent. The oil was used for fatty acid profiling using gas chromatography with flame-ionization detector (GC-FID). The oil was converted in to fatty acid methyl ester (FAME) using alkaline catalysed transesterification with 0.5 ml 4 % KOH solution in methanol using standard protocol. The instrument (Thermo-Trace GC Ultra-Al 3000 auto sampler) (Thermo Scientific, USA) was programmed as inlet temperature 210°C and FID detector temperature was 260°C. Oven was kept at 160°C for two minutes. After that an increase of 10°C/min. was applied to raise oven temperature up to 200°C, where it was kept at isotherm for ten minutes. The gas flow rate in column was 1.5 ml/min. and the sample injection volume was 0.2 μl. The capillary was TR FAME-30 m × 0.25 mm, ID × 0.25 μm film. Fatty acids composition of each treatment sample was estimated in percentage by using Chrom-card software associated with GC.

Data analysis

The data were analysed by Microsoft Excel 2010 to derive different ratios among fatty acids as suggested by Shah *et al*. (2017). Pearson's correlation coefficients (r) was calculated using PAST 3.22 software (Hammer *et al*. 2001) to determine significance of correlation among fatty acids at $P<0.05$.

RESULT AND DISCUSSION

Seed oil content (%) determination

Castor oil has great value in industry, pharmaceutical and agricultural sectors. The variability in seed oil content ranged from 38.25 to 45.86 % with an average of 42.93 %. Among the genotypes studied, SHB-1019 (45.86 %) was significantly higher for oil content imitated by genotypes namely, GCH-7 (45.67%) and GEETA (45.65 %). The genotype, SKI-372 recorded the lowest oil content (38.25 %) (Table 1).

Fatty acid profiling of seed oil

Total saturated fatty acid content in all castor genotype was ranged from 2.27 to 3.38 % with a mean of 2.70 %. It was observed that genotype SKI-370 (3.38 %) had higher per cent of total saturated fatty acid followed by genotypes GEETA (2.98 %), SKI-372 (2.97%), and SH-72 (2.96 %). The genotypes VI-9 (2.27 %) had low per cent of total saturated fatty acid. Total saturated fatty acid content was categorized in two type of acids viz., steric acid (C16:0) and palmitic acid (C18:0) (Table 1). The amount of steric acid in all castor genotypes was ranged from 1.05 to 1.46 % with a mean of 1.26%. Among all the accessions, SKI-370 (1.46 %) contained highest amount of steric acid, followed by genotypes namely, SHB-1005 (1.14 %), GEETA (1.14 %), SKI-373 (1.14 %), and SH-72 (1.40 %). The genotypes, VI-9 (1.05 %) contained lowest amount of steric acid. Palmitic acid content in all castor

Table 1: Seed oil content and fatty acid profile of castor genotypes

Genotype	Oil content (%)	Fatty acid composition (%)							
		SFA			UFA				
					MUFA	PUFA		RA	Total
		C16:0	C18:0	Total	C18:1	C18:2	C18:3	C18:1 (OH)	
GAUCH-1	43.13	1.18	1.36	2.54	3.26	4.71	0.5	88.98	97.45
GCH-2	44.93	1.24	1.52	2.76	3.95	5.1	0.57	87.62	97.24
GCH-4	43.67	1.20	1.35	2.55	3.1	4.91	0.61	88.83	97.45
GCH-5	43.03	1.28	1.4	2.68	3.28	4.95	0.53	88.55	97.31
GCH-6	44.09	1.28	1.38	2.66	3.01	4.78	0.59	88.96	97.34
GCH-7	45.67	1.15	1.24	2.39	2.67	4.71	0.61	89.62	97.61
SHB-1005	44.21	1.41	1.49	2.9	3.87	5.34	0.64	87.25	97.1
SHB-1018	42.54	1.20	1.25	2.45	2.65	4.85	0.57	89.48	97.55
SHB-1019	45.86	1.20	1.43	2.63	3.81	5.04	0.57	87.96	97.38
SHB-1029	44.52	1.23	1.41	2.64	3.36	4.88	0.62	88.5	97.36
GNCH-1	42.46	1.16	1.35	2.51	3.31	4.75	0.56	88.87	97.49
VP-1	40.04	1.27	1.46	2.73	3.00	5.15	0.65	88.48	97.28
GEETA	45.65	1.41	1.57	2.98	4.26	5.82	0.55	86.39	97.02
JP-65	40.50	1.26	1.49	2.75	3.44	4.89	0.61	88.33	97.27
SKP-84	40.20	1.11	1.35	2.46	3.47	4.74	0.55	88.8	97.56
VI-9	44.50	1.05	1.22	2.27	2.1	3.87	0.89	91.29	98.15
JI-35	43.73	1.22	1.68	2.9	4.06	4.98	0.52	87.56	97.12
48-1	42.00	1.26	1.61	2.87	3.43	5.25	0.56	87.9	97.14
SH-72	41.23	1.40	1.56	2.96	2.76	5.15	0.65	88.49	97.05
JI-96	43.99	1.37	1.56	2.93	3.73	4.75	0.52	88.07	97.07
SKI-215	45.50	1.30	1.45	2.75	2.92	5.38	0.6	88.35	97.25
SKI-352	42.20	1.35	1.6	2.95	3.87	4.82	0.52	87.85	97.06
SKI-370	41.17	1.46	1.92	3.38	3.97	5.66	0.64	86.37	96.64
SKI-372	38.25	1.14	1.3	2.44	2.97	4.69	0.59	89.32	97.57
SKI-373	41.50	1.41	1.56	2.97	3.18	5.41	0.62	87.83	97.04
DCS-94	41.51	1.13	1.42	2.55	3.07	4.46	0.46	89.47	97.46
Mean	42.93	1.26	1.46	2.70	3.33	4.96	0.59	88.41	97.30
Maximum	45.86	1.46	1.92	3.38	4.26	5.82	0.89	91.29	98.15
Minimum	38.25	1.05	1.22	2.27	2.10	3.87	0.46	86.37	96.64

C16:0: Steric acid; C18:0: Palmitic acid; C18:1: Oleic acid; C18:1(OH): Ricinoleic acid; C18:2: Linoleic acid; C18:3: Linolenic acid; MUFA: Mono Unsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid; RA: Ricinoleic acid; SFA: Saturated Fatty Acid; UFA: Unsaturated Fatty Acid.

genotypes was ranged from 1.22 % (VI-9) to 1.92 % (SKI-370) with a mean (1.46 %). From the perusal of data genotype SKI-370 (1.92 %) found the highest amount of palmitic acid, followed by genotypes *viz.,* JI-35 (1.68 %) and 48-1 (1.61 %).

Total unsaturated fatty acid content in all castor genotypes was ranged from 96.64 % (SKI-370) to 98.15 % (VI-9) with a mean 97.30 % (Table 1). Mono unsaturated fatty acid (MUFA) oleic acid (C18:1) content in all castor genotype was ranged from 2.10 % (VI-9) to 4.26 % (GEETA) with a mean of 3.33 %. It was observed that genotype GEETA (4.26 %) had higher per cent of oleic acid followed by genotypes *i.e.* JI-35 (4.06 %) and SKI-370 (3.97 %) (Table 1). Poly unsaturated fatty acid (PUFA) in castor oil contained linoleic acid (C18:2) and linolenic acid (C18:3) (Table 1). Linoleic acid content ranged from 3.87 to 5.82 % with a mean of 4.96 %. Among the all genotypes the highest per cent of linoleic acid was recorded in 5.82 % for genotype GEETA followed by genotypes, SKI-370 (5.66 %) and SKI-373 (5.41 %). The lowest per cent of linoleic acid was recorded in genotype VI-9 (3.87 %). The values for linolenic acid in castor genotypes ranged from 0.46 (DCS-94) to 0.89 % (VI-9) with a mean of 0.59 % (Table 1).

Ricinoleic acid, the predominant mono unsaturated fatty acid varied among castor genotypes from 86.37 to 91.29 % with a mean. Among all the genotypes the higher per cent of ricinolenic acid was recorded in genotype *viz.,* VI-9 (91.29 %) followed by genotype namely, GCH-7 (89.62 %) and SHB-1018 (89.48 %). The lowest per cent of ricinolenic acid was recorded in genotypes SKI-370 (86.37 %) (Table 1). The results of the present study are in agreement with the results reported by Huang *et al.* (2015), Shah *et al.* (2015), Omari *et al.* (2015), Omohu and Omale (2017) and Shah *et al.* (2017). Linolenic acid and oleic acid in castor oil are very much favorable to human health and have been used in the treatments of some human diseases like skin cancer, diabetes, heart attack, high blood pressure, high cholesterol level, renal disease, and lupus (Ganesan *et al*. 2018). Linoleic acid, palmitic acid, and stearic acid contain certain beneficial properties for the skin, hence utilized in cosmetic industries (Ganesan *et al*. 2018). Esters of stearic acid: glycol stearate, glycol distearate, and ethylene glycol have been utilized in manufacturing various cosmetic products and in shampoos to increase the pearly effect. The presence of such fatty acids in castor oil offers the industrial and nutritional benefits of castor (Yeboah *et al*. 2020). The amount of different fatty acids present in castor oil is represented in Fig. 1. Based on the amount of different beneficial fatty acids in the oil, genotypes can be utilized in further breeding programs.

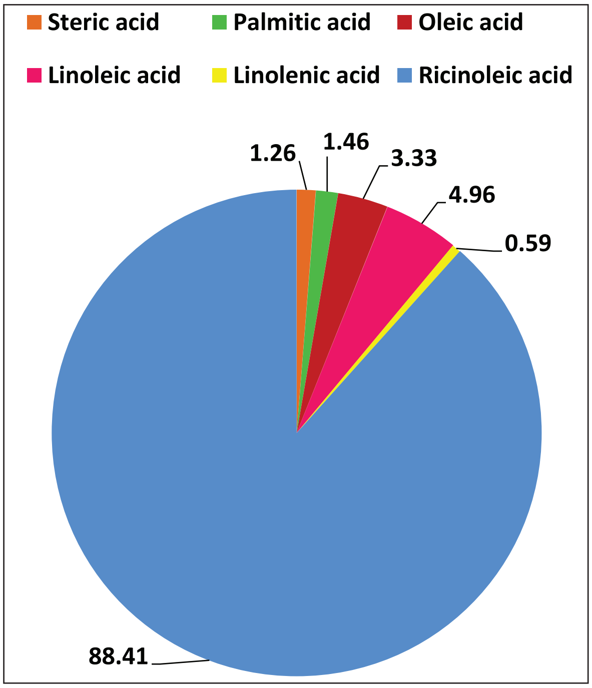

Fig. 1: Contribution of different fatty acids in castor seed oil (Based on data collected from 26 genotypes)

Significant positive correlation was observed between palmitic acid and stearic acid (r = 0.750*). The oleic acid showed significantly positive correlation with palmitic acid (r = 0.691*) and stearic acid (r = 0.498*) (Table 2). These results were resembled with the findings of Shah *et al*., (2017). The linoleic acid has significantly positive correlation with oleic acid (r = 0.582*), palmitic acid (r=0.670*) and stearic acid (r = 0.794*). However, the linolenic acid has negative correlation with stearic acid (r =-0.088), palmitic acid (r = -0.193), oleic acid (r = -0.509*) and linoleic acid (r = -0.211). In the present study, the ricinoleic acid content was significantly negatively correlated with stearic acid (r = -0.793*), palmitic acid (r = -0.836*), oleic acid (r = - 0.876*) and linoleic acid (r = -0.884*) and positive and non-significant correlation with linolenic acid (r = 0.360*) (Fig. 2). These results were in agreement with the findings of Shah *et al*. (2017).

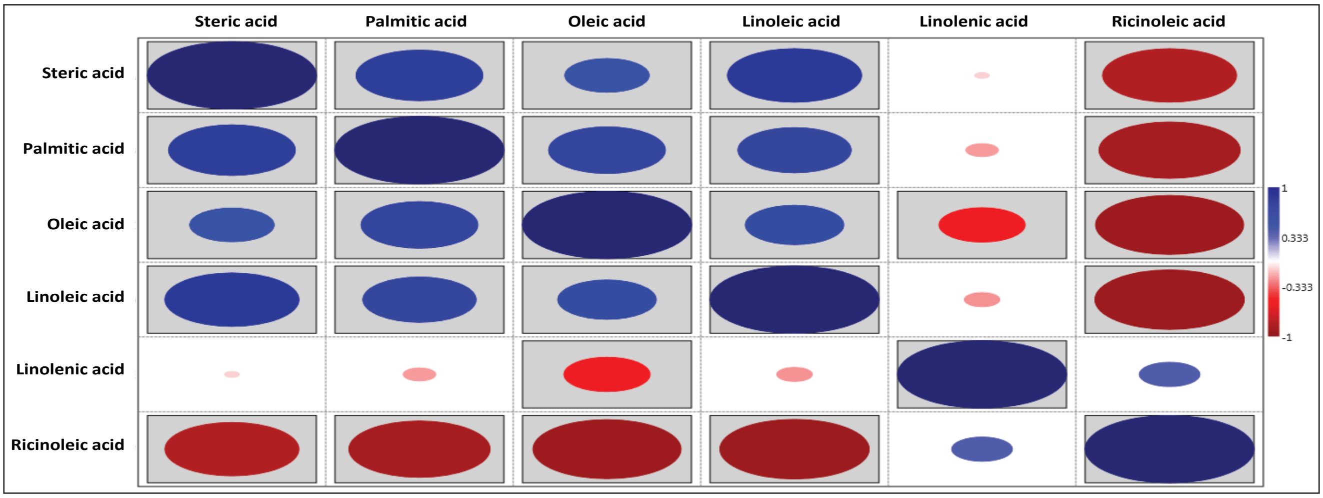

Fig. 2: Correlation analysis among different fatty acids in castor oil (Grey boxes represent significance at P<0.05)

Table 2: Correlation coefficients among different fatty acids in castor oil

	Steric acid	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid
Palmitic acid	0.750*				
Oleic acid	0.498*	0.691*			
Linoleic acid	0.794*	0.670*	0.582*		
Linolenic acid	-0.088	-0.193	-0.509*	-0.211	
Ricinoleic acid	-0.793*	-0.836*	-0.876*	-0.884*	0.360

** Represents significance at P<0.05.*

Different ratios among fatty acids of castor genotypes are shown in Table 3. The Oleic acid/ricinoleic acid ratio among the all the genotypes ranged from 0.023 (VI-9) to 0.049 (GEETA). High oleic acid and low ricinoleic acid content in castor oil is preferable for industrial applications demanding high oxidative stability like bio-fuel requiring lower ricinoleic acid content than the standard castor oil (Rojas-Barros *et al*. 2004). The oleic acid/linoleic acid (O/L) ratio among all the genotypes ranged from 0.536 (SH-72) to 0.815 (JI-35). Higher O/L ratio showed higher stability of castor oil (Shah *et al*. 2015). For the improvement of character through selection, it is necessary to understand the extent of diversity existing for each character in germplasm (Parita *et al*. 2018). This will enhance the development of new varieties using plant breeding (Gelotar *et al*. 2019). The estimation of seed oil content and fatty acid profiling can be utilized for selection of parents for population development and improvement in oil quantity as well as quality.

Table 3: Different ratios among fatty acids of castor genotypes

Genotypes	Oleic acid/ Linoleic acid (O/L)	MUFA/ PUFA	Oleic acid/ Ricinoleic acid (MUFA/RA)
GAUCH-1	0.693	0.626	0.037
GCH-2	0.774	0.696	0.045
GCH-4	0.630	0.561	0.035
GCH-5	0.663	0.599	0.037
GCH-6	0.629	0.560	0.034
GCH-7	0.565	0.500	0.030
SHB-1005	0.724	0.646	0.044
SHB-1018	0.547	0.490	0.030
SHB-1019	0.755	0.678	0.043
SHB-1029	0.689	0.611	0.038
GNCH-1	0.696	0.622	0.037
VP-1	0.582	0.517	0.034
GEETA	0.732	0.669	0.049
JP-65	0.704	0.626	0.039
SKP-84	0.732	0.656	0.039
VI-9	0.543	0.441	0.023
JI-35	0.815	0.738	0.046
48-1	0.653	0.590	0.039
SH-72	0.536	0.476	0.031
JI-96	0.786	0.708	0.042
SKI-215	0.543	0.488	0.033
SKI-352	0.803	0.724	0.044
SKI-370	0.701	0.630	0.046

SKI-372	0.633	0.562	0.033
SKI-373	0.588	0.527	0.036
DCS-94	0.689	0.625	0.034
Mean	0.669	0.599	0.038
Max	0.815	0.738	0.049
Min	0.536	0.441	0.023

CONCLUSION

On the basis of the results obtained, it was concluded that among the genotypes evaluated, the maximum oil content was 45.86 % in seeds of SHB-1019. The maximum amount of ricinoleic acid (91.29 %) was recorded in VI-9. The present study can be very important in the selection of male parents for the development of high ricinoleic acid castor hybrid.

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