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STUDIES ON GENETIC PURITY ASSESSMENT IN CASTOR (*Ricinus communis* L.) GENOTYPES USING MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR MARKERS

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ABSTRACT

Sixteen genotypes of castor (*Ricinus communis* L.) genetic purity were evaluated in Randomized Block Design (RBD) during late kharif season of 2020-21 with the objective to assess the genetic purity of castor genotypes based on morphological characters and assess the genetic purity of genotype based on biochemical and molecular markers. Results showed that based on seed caruncle, the genotypes were grouped into small and big genotypes. Based on seed mottling, the genotypes were grouped into high and low genotypes. Based on the variations in anthocyanin colour on hypocotyl as well as leaf: anthocyanin pigmentation of young emerging leaves, the genotypes were grouped as present and absent except for capsule spininess which showed absent, sparse and dense genotypes. Types of bloom, the genotypes were grouped as double and triple bloom. Variations in stem colour, the genotypes were grouped as mahogany, red and green stem colour. Based on the variations in types of internodes, the genotypes showed elongated and condensed genotypes. The length of 4th leaf from top ranged from 22.37 (PCS 124) to 36.90 cm (48-1) with a mean of 31.17 cm. All the genotypes showed shallow with leaf lascination and smooth for petiole surface respectively. The number of leaf lobes varied significantly among the genotypes ranged from 8.0 (SKI 215) to 11.33 (GCH 9) with a mean of 9.60. The petiole length as well as capsule length varied significantly among the genotypes, ranged from 25.13 (GCH 9) to 37.83 (DCS 89) with a mean of 32.26 cm and from 1.70 cm (DCS 94) to 2.8 cm (GCH 9) with a mean of 2.17 cm respectively. The genotypes with location of branches were grouped as basal and top in twelve and four genotypes respectively. On the basis of chemical tests, genotypes were classified into different groups. In potassium hydroxide (KOH) bleach test, the genotypes were grouped as colourless, orange and dark orange red colour genotypes. Based on peroxidase test as well as grain coloration with phenol, the genotypes were grouped as light brown, brown, dark brown and no colour. The moisture content ranged from JP 96 (3.90 per cent) to SKP 84 (4.91 per cent) with a mean seed germination percentage of 4.43 per cent. The oil content ranged from SKI 215 (45.00 per cent) to DCS 89 (48.67 per cent) with a mean seed germination percentage of 47.25 per cent. On the basis of total protein there was no variation observed in the bands under SDS PAGE. SSR analyses of eighteen alleles were generated from ten primers with an average of 1.8 alleles per loci. The amplified fragmented ranged from 115 bp (with primer Castor_SSR_102) to 635 bp (with primer Castor_SSR_123) suggested presence of sufficient genetic diversity in the number of repeats between the different alleles. The PIC values of these SSR markers varied from 0.22 (primer Castor_SSR_270) to 0.57 (primer Castor_SSR_102) with a mean of 0.29. Jaccards's similarity coefficient values for sixteen genotypes ranged from 0.35 to 1.0.

Keywords **:** Genetic purity, morphological, biochemical and molecular markers

Introduction

Castor (*Ricinus communis* L.), a member of the *Euphorbiaceae* family with a chromosome number of 2n = 2x = 20, is an economically significant non-edible oilseed crop predominantly cultivated in arid and semi-arid regions. Castor is grown widely in India. Castor is indigenous to South Eastern Mediterranean Basin, Eastern Africa and India. The crop is wide spread

throughout the tropical land and also grown at many places as ornamental plant. (Phillip and Martyn, 1999). It has gained importance as a potential source for bulk biodiesel production and for its medicinal applications. Castor oil possesses extensive industrial value, making it a versatile raw material (Govaerts *et al*., 2000).

India, Brazil and China are major castor growing countries in the world and they contribute the 90 per cent of the world export. India is the biggest exporter of castor oil and its derivatives with 88 per cent share in the international trade. Total area, production and productivity of castor in our country are 770 (000' ha), 1127 (000't) and 1520 kg/ha respectively. The same for Gujarat is 534 (000' ha), 935 (000't) and 1751 kg/ha respectively (Anon., 2019). Gujarat, Rajasthan and Andra Pradesh are the major castor seed producing states in our country. Mehsana and Banaskantha are the largest castor producing districts in Gujarat (Anon., 2019). Castor has the ability to grow in low rainfall and low fertility area so the crop is suitable for the dry land farming.

Morphological markers play a key role in distinguishing among different genotypes and assessing genetic relationships. Traits such as plant height, days to flowering, leaf shape, seed weight, and seed colour are commonly used descriptors. These characteristics are generally governed by single or a few genes (Levi *et al*., 2001). Morphological descriptors are traditionally important as they are easily observable in the field without requiring specialized equipment. However, biochemical and molecular markers offer greater precision and faster results in genotype identification.

Establishing clear criteria for distinctness is essential for varietal registration and seed quality assurance. Genetic purity, defined as the "trueness to type," ensures that cultivars conform to the characteristics described by the breeder. Maintaining genetic purity is a critical component of seed quality control programs, as genetically impure seeds can adversely affect yield, uniformity, and overall product quality. In this context, the present study focuses on assessing the genetic purity of castor genotypes using morphological, biochemical, and molecular markers.

Material and Methods

The experimental material comprised sixteen diverse castor genotypes, namely GCH 4, GCH 7, GCH 8, GCH 9, SKP 84, SKI 215, JP 96, DCS 89, DCS 94, GC 2, GC 3, GAC 11, GNCH 1, PCS 124, VP 1(M), and 48-1 to assess the genetic purity of these castor (*Ricinus communis* L.) genotypes using morphological, biochemical, and molecular markers. Morphological evaluation included seed characters, plant traits, and seed quality parameters. Biochemical analysis involved tests such as potassium hydroxide (KOH) bleach test (Mckee, 1973), sodium hydroxide (NaOH) bleach test (Agarwal, 1998), phenol-based grain coloration (Jaiswal and Agrawal, 1995), peroxidase test (Agrawal and Pawar, 1990), and copper sulphate-ammonia test (Agrawal and Pawar, 1990).

Molecular characterization was carried out using SSR markers *viz.*, Castor_SSR_231, Castor_SSR_229, Castor_SSR_194, Castor_SSR_270, Castor_SSR_102, Castor_SSR_277, Castor_SSR_123, Castor_SSR_74, Castor_SSR_112 and Castor_SSR_253 to obtain precise genetic information. The study was conducted during late *kharif* 2020–21 at the Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, following a Randomized Block Design (RBD). Observations on fruit yield and its component traits were recorded for each genotype across different treatments and replications.

Qualitative and quantitative analysis of genomic DNA

The isolation of good quality DNA is the prerequisite for molecular research. Genomic DNA from sixteen different castor genotypes was isolated following CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle 1987) with slight modifications and quantified by UV spectrophotometer. The quality of DNA was determined at A260/A280 ratio.

Construction of dendrogram and protein data analysis

The dendrogram was constructed based on UPGMA (un weighted pair-group method with arithmetic mean) by using Jaccard's similarity coefficient through NTSYSpc (version 2.0) software to analyse similarity index and hierarchial clustering pattern. Also for protein data analysis, sixteen genotypes were tested in SDS PAGE. The bands were scored for their presence (+) or absence (-) in each genotype.

Results and Discussion

Morphological characterization

Seed Characters

Based on seed caruncle, the genotypes were grouped into small in ten genotypes (GCH 9, SKP 84, DCS 89, DCS 94, GC 3, GAC 11, GNCH 1, PCS 124, VP 1(M) and 48-1) and big in six genotypes (GCH 4, GCH 7, GCH 8, SKI 215, JP 96 and GC 2). Based on seed mottling, the genotypes were grouped into high in thirteen genotypes (GCH 4, GCH 7, GCH 8, GCH 9,

SKP 84, SKI 215, JP 96, DCS 89, GC 2, GC 3, PCS 124, VP 1(M) and 48-1) and low in three genotypes (DCS 94, GAC 11 and GNCH 1) Table 1. Similar findings based on seed characters were also reported by Varier *et al.* (1999), Shaheen (2002), Gupta and Aggarwal (2012), Salamatbakhsh *et al.* (2012), Ahmed *et al.* (2014), Rao *et al.* (2014), Vasco-Leal *et al.* (2018), Chaudhari *et al.* (2019) and Drumond *et al.* (2019).

Plant characters

Based on the variations in anthocyanin colour on hypocotyl, the genotypes were grouped as present in seven genotypes (GCH 9, DCS 89, GC 2, GC 3, GAC 11, PCS 124 and 48-1) and absent in nine genotypes (GCH 4, GCH 7, GCH 8, SKP 84, SKI 215, JP 96, DCS 94, GNCH 1 and VP 1(M)). Based on the variations in leaf: anthocyanin pigmentation of young emerging leaves, the genotypes were grouped as present in eleven genotypes (GCH 4, GCH 7, GCH 9, SKP 84, SKI 215, DCS 89, GC 2, GC 3, GAC 11, GNCH 1 and 48-1) and absent in five genotypes (GCH 8, JP 96, DCS 94, PCS 124 and VP 1(M)). Based on the variations in types of bloom, the genotypes were grouped as double bloom in five genotypes (SKI 215, DCS 89, GAC 11, PCS 124 and 48-1) and triple bloom in eleven genotypes (GCH 4, GCH 7, GCH 8, GCH 9, SKP 84, JP 96, DCS 94, GC 2, GC 3, GNCH 1 and VP 1(M)) (Figure 1). Based on the variations in stem colour, the genotypes were grouped as mahogany in nine genotypes (GCH 4, GCH 7, GCH 8, SKP 84, SKI 215, DCS 89, GC 2, GAC 11 and GNCH 1), red colour in three genotypes (GCH 9, GC 3 and 48-1)) and green stem colour in four genotypes (JP 96, DCS 94, PCS 124 and VP 1(M)) (Figure 2). Based on the variations in types of internodes, the genotypes were grouped as elongated in fourteen genotypes (GCH 4, GCH 7, GCH 8, GCH 9, SKI 215, JP 96, DCS 89, DCS 94, GC 2, GC 3, GAC 11, GNCH 1, PCS 124 and 48-1) and condensed in two genotypes (SKP 84 and VP 1(M)) (Figure 3). The length of 4th leaf from top varied significantly among the genotypes. The length of 4th leaf from top ranged from 22.37 (PCS 124) to 36.90 cm (48-1) with a mean of 31.17 cm. Based on the variations in leaf lascination, the genotypes were grouped as shallow in all sixteen genotypes (Figure 4). The number of lobes on leaf varied significantly among the genotypes. The number of lobes on leaf ranged from 8.0 (SKI 215) to 11.33 (GCH 9) with a mean of 9.60. The petiole length varied significantly among the genotypes. The petiole length ranged from 25.13 (GCH 9) to 37.83 cm (DCS 89) with a mean of 32.26 cm.

Double bloom: SKI 215 Triple bloom: GCH 4

Fig. 1 : Types of bloom castor genotypes

Green: JP 96 Mahogany: GCH 7 Red: GCH 9

Fig. 3 : Types of internodes of castor

Condensed: SKP 84 Elongated: JP 96 Shallow: GCH 4

Fig. 4 : Leaf lascination

Absent: DCS 89 Sparse: GCH 4 Dense: GCH 9

Fig. 5 : Capsule spininess of castor genotypes

Seed placed for germination on paper Germinated Seedling

Fig. 6 : Germination test of castor genotypes

Based on the variations in petiole surface, the genotypes were grouped as smooth in all sixteen genotypes. Based on the variations in capsule spininess, the genotypes were grouped as absent in three genotypes SKI 215, DCS 89 and 48-1, sparse in three genotypes (GCH 4, GCH 7 and GCH 8) and dense in ten genotypes (GCH 9, SKP 84, JP 96, DCS 94, GC 2, GC 3, GAC 11, GNCH 1, PCS 124 and VP 1(M)) (Figure 5). The capsule length varied significantly among the genotypes. The capsule length ranged from 1.70 (DCS 94) to 2.8 cm (GCH 9 94) to with a mean of 2.17 cm. Based on the variations in location of branches, the genotypes were grouped as basal in twelve genotypes (GCH 4, GCH 7, GCH 8, GCH 9, SKP 84, SKI 215, JP 96, DCS 94, GC 3, GAC 11, GNCH 1 and VP 1(M)) and top in four genotypes (DCS 89, GC 2, PCS 124, and 48-1) (Table 1 and 2). Similar findings based on plant characters were also reported by Shaheen (2002), Sakure *et al.* (2010), Gupta and Aggarwal (2012), Rao *et al.* (2014), Meena *et al.* (2015), Hu *et al.* (2016), Chaudhari *et al.* (2019) and Drumond *et al.* (2019).

Seed quality parameters

The germination percentage varied among different castor genotypes (Table 2, Figure 6). The mean seed germination percentage of the genotypes was 88.65 per cent. Among 16 genotypes, the highest

seed germination percentage was observed in GCH 9 (95.33 %) followed by five genotypes (SKP 84, DCS 89, SKI 215 and 48-1) and the lowest was observed in PCS 124 (67.33 %). The seedling fresh weight varied among different castor genotypes (Table 2). The mean seedling fresh weight of the genotypes was 8.34 g. Among 16 genotypes significantly the highest seedling fresh weight was observed in the genotype GCH 8 (11.90 g) followed by DCS 89 (11.89 g), SKI 215 (11.76 g), SKP 84 (9.49 g) and JP 96 (9.03 g) and the lowest seedling fresh weight was observed in the genotype VP 1(M) (3.75 g). The seedling dry weight varied among different castor genotypes (Table 2). The mean seedling dry weight of the genotypes was 0.95 g. Among 16 genotypes significantly the highest seedling dry weight was observed in the genotype GCH 8 (1.40 g) followed by GCH 9 (1.19 g), SKI 215 (1.15 g), GCH 7 (1.07 g) and DCS 89 (1.07 g) and the lowest seedling dry weight was observed in the genotype PCS 124 (0.58 g). The root length ranged from GCH 9 (7.77 cm) to SKI 215 (17.53 cm) with a mean root length of 12.85 cm. The shoot length ranged from GCH 9 (6.53 cm) to GCH 4 (13.57 cm) with a mean shoot length of 9.75 cm (Table 2). Similar findings based on plant characters were also reported by Salamatbakhsh *et al.* (2012), David *et al.* (2013), Ahmed *et al.* (2014), Ribeiro *et al.* (2014) and Drumond *et al.* (2019).

Fig. 6 : Germination test of castor genotypes

Chemical tests

On the basis of chemical tests as shown in table 3, genotypes were classified into different groups. Based on potassium hydroxide (KOH) bleach test, the genotypes were grouped as orange colour in ten genotypes, dark orange red colour in two genotypes and no colour in four genotypes. Based on sodium hydroxide (NaOH) bleach test, the genotypes were grouped as orange colour in thirteen genotypes, dark orange red colour in two genotypes and no colour in one genotype. Based on grain colouration with phenol, the genotypes were grouped as light brown colour in six genotypes, brown colour in seven genotypes, dark brown in two genotypes and no colour in one genotype. Based on peroxidase test (Figure 7), the genotypes were grouped as light brown colour in six genotypes, brown colour in seven genotypes, dark brown in two genotypes and no colour in one genotype. Based on copper sulphate ammonia test, the genotypes were grouped as dark brown colour in six genotypes, black colour in three genotypes and no colour in seven genotypes.

Similar results and grouping based on chemical test were also made by Reddy et al. (2008) in cotton, Chavan (2010) in soybean, Mansing (2010) in wheat, Sathisha *et al.* (2012), Kallihal *et al.* (2013) and Prasad *et al.* (2013) in sunflower, Rao *et al.* (2013) in peanut and Chandusingh *et al.* (2017) in rice.

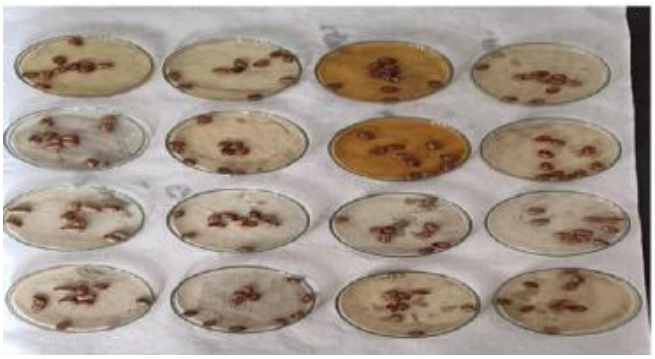


Fig. 7 : Peroxidase test of castor genotypes

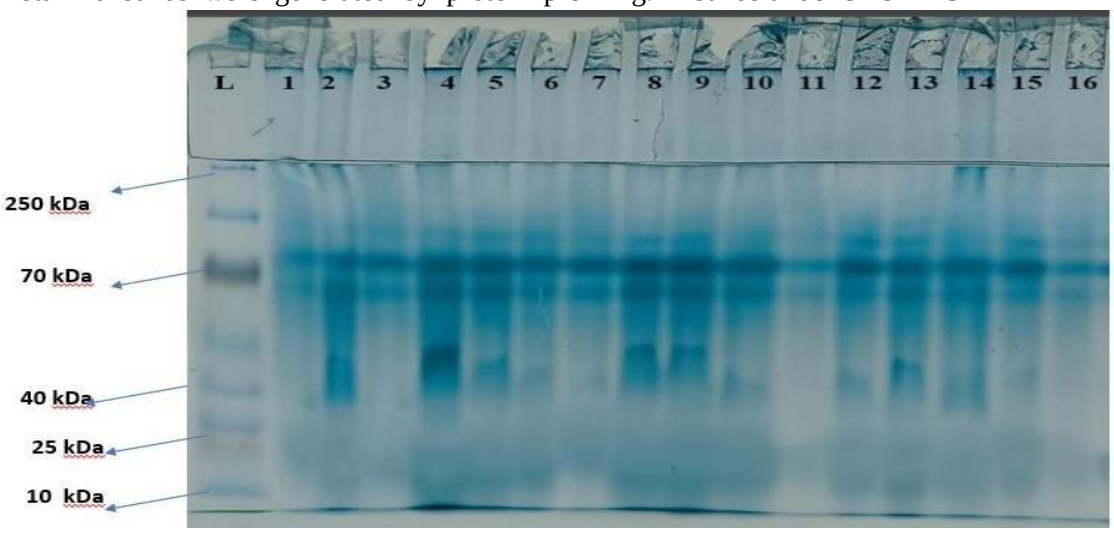
Proximate analysis

The moisture content ranged from JP 96 (3.90 per cent) to SKP 84 (4.91 per cent) with a mean seed germination percentage of 4.43 per cent. The ash content ranged from JP 96 (3.65 per cent) to 48-1 (5.33 per cent) with a mean seed germination percentage of 4.62 per cent. The oil content ranged from SKI 215 (45.00 per cent) to DCS 89 (48.67 per cent) with a mean seed germination percentage of 47.25 per cent. The total soluble sugar ranged from GC 2 (0.95 per cent) to SKP 84and GC 3 (2.18 per cent) with a mean seed germination percentage of 1.48 per cent. The total protein ranged from SKI 215 (17.08 per cent) to SKP 84 (24.69 per cent) with a mean seed germination percentage of 20.37 per cent. The mineral content varied among different castor genotypes. Maximum Zn content was observed in SKP 84 (0.14 mg/L), Fe content was observed maximum in SKI 215 (0.75 mg/L), K, S and P content was observed maximum in GCH 4 (11.30, 0.36 and 0.188 mg/L) respectively as shown in table 4. Similar results based on proximate analysis were also reported by Vasco-Leal *et al*. (2018) and Chaudhari *et al*. (2019).

Genetic purity assessment using biochemical marker (SDS PAGE)

Protein data analysis

All sixteen genotypes were tested in SDS PAGE. Total 16 bands were generated by protein profiling. The bands were scored for their presence (+) or absence (-) in each genotype. Data entry was done into a binary data matrix as discrete variables. On the basis of total protein there was no variation observed in the bands under SDS PAGE



1. GCH 4	5. SKP 84	9. DCS 94	13.GNCH 1	L = 10 kDA
2.GCH 7	6. SKI 215	10. GC 2	14.PCS 124	Protein
3. GCH 8	7. JP 96	11. GC 3	15.VP 1(M)	ladder
4. GCH 9	8. DCS 89	12. GAC 11	16. 48-1	

Plate 1: SDS- PAGE protein profiling of castor genotypes

Genetic purity assessment using molecular marker (SSR markers)

Qualitative and quantitative analysis of genomic DNA

In present investigation, the average concentration of DNA was 368.66 ng/µl, quantified by UV spectrophotometer. The genotype SKP 84 showed the highest concentration of 563.4 ng/µl and genotype 48-1 showed lowest concentration of 196.0 ng/µl. The average ratio was found to be 1.89. The genotype VP 1(M) showed highest ratio of 1.94 and lowest 1.73 was found to be of genotype SKP 84. This could be due to the interference of various compounds in plant tissue during the procedure. Quality of DNA was also confirmed on agarose gel electrophoresis. It showed compact band without RNA contamination.

Simple Sequence Repeats (SSR) analysis

In present investigation, a purified DNA extracted from castor leaves was obtained by CTAB method screened on agarose gel electrophoresis indicated the further use of DNA for amplification. Genotype GCH 9 did not show polymorphism with any primer, hence the source of genotype GCH 9 was true to type. SSR analysis of ten primers detected eighteen alleles among the genotypes. Eight primers Castor_SSR_231, Castor_SSR_229, Castor_SSR_194, Castor_SSR_270, Castor_SSR_277 and Castor_SSR_123 produced two alleles and Castor_SSR_102 produced three alleles and Castor_SSR_74, Castor_SSR_112 and Castor_SSR _253 produce one allele (monomorphic) with an average of 1.8 alleles per loci. The amplified fragmented ranged from 115 bp (with primer Castor_SSR_102) to 635 bp (with primer Castor_SSR_123) suggested presence of sufficient genetic diversity in the number of repeats between the different alleles. Similar observations were also recorded by Gajera *et al.* (2010), Gautam *et al.* (2013) and Thatikunta *et al.* (2016).

The details of amplification product size, total number of alleles and PIC of each primer are given in Table 5. The gel images for the primers run on 3% agarose gel are presented in plates 20 to 29. The Polymorphism Information Content (PIC) value of microsatellite locus, a measure of the allelic diversity of the genotypes. The PIC values of these SSR markers varied from 0.22 (primer Castor_SSR_270) to 0.57 (primer Castor_SSR_102) with a mean of 0.27 (Table 5). Similar findings were also observed by Lakhani *et al.* (2015) and Kachhadiya *et al.* (2019)

1. 48-1	5. GCH 7	9. JP 96	13. GC 2	L = 100 bp DNA ladder
2. DCS 89	6. GAC 11	10.VP 1(M)	14.DCS 124	
3. GNCH 1	7. GCH 8	11.DCS 94	15. GC 3	
4. GCH 4	8. GCH 9	12. SKP 84	16. SKI 215	

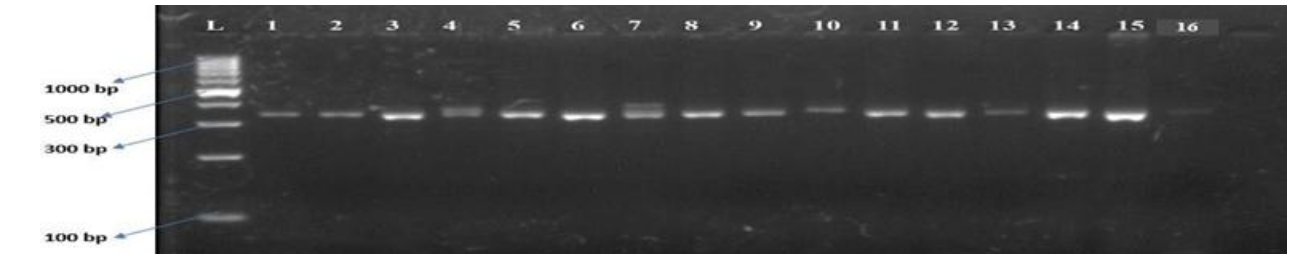


Plate 2: SSR profiling of Castor_SSR_231 in 16 genotypes of castor

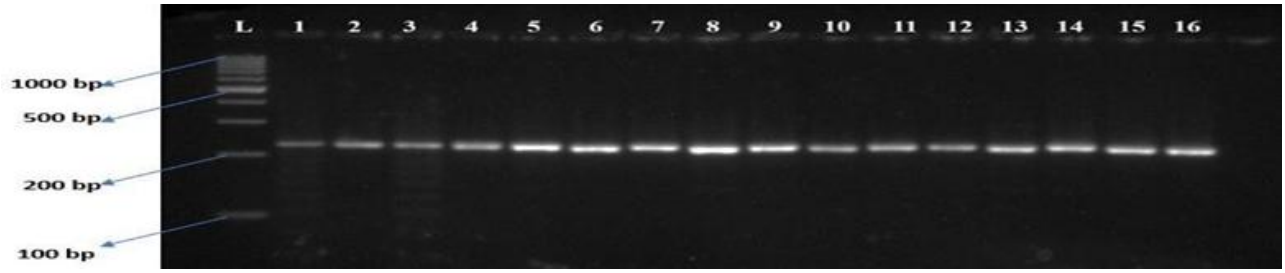


Plate 3: SSR profiling of Castor_SSR_229 in 16 genotypes of castor

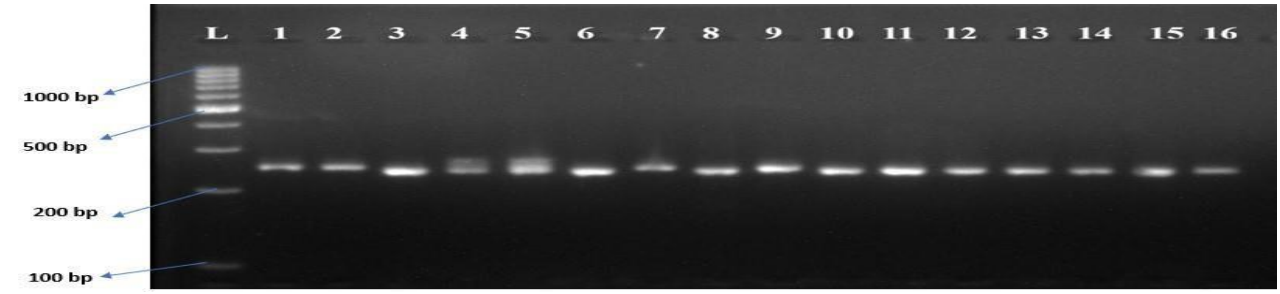


Plate 4: SSR profiling of Castor_SSR_194 in 16 genotypes of castor

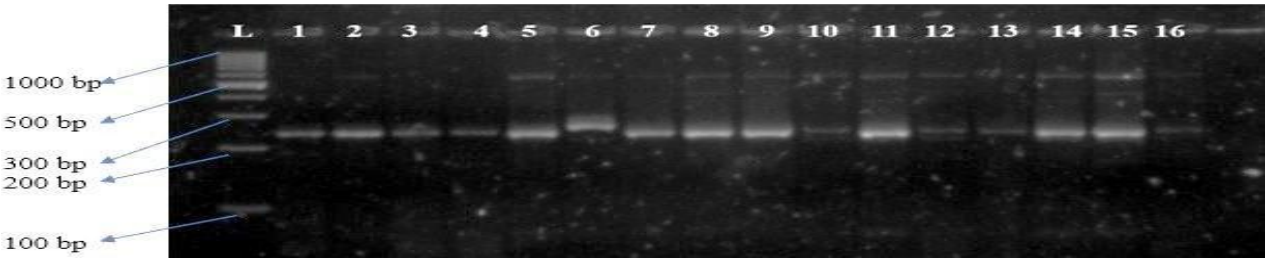


Plate 5: SSR profiling of Castor_SSR_270 in 16 genotypes of castor

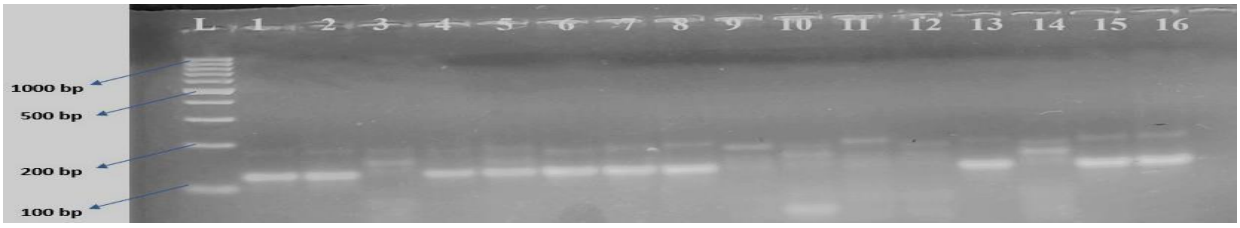


Plate 6: SSR profiling of Castor_SSR_102 in 16 genotypes of castor

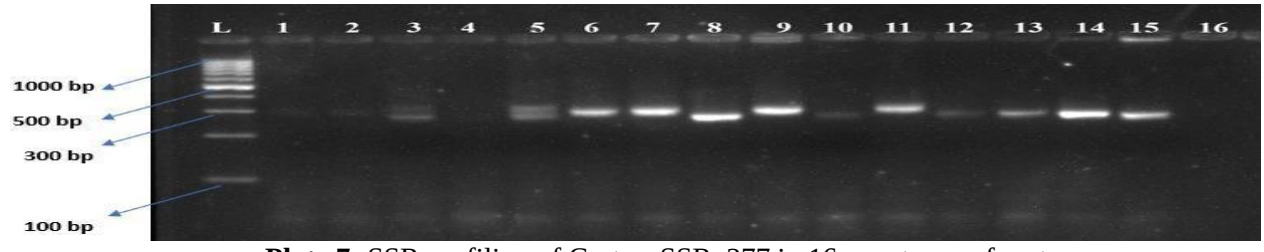


Plate 7: SSR profiling of Castor_SSR_277 in 16 genotypes of castor

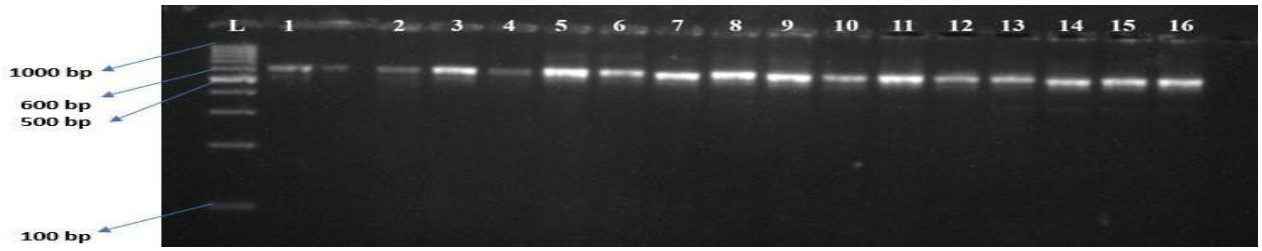


Plate 8: SSR profiling of Castor_SSR_123 in 16 genotypes of castor

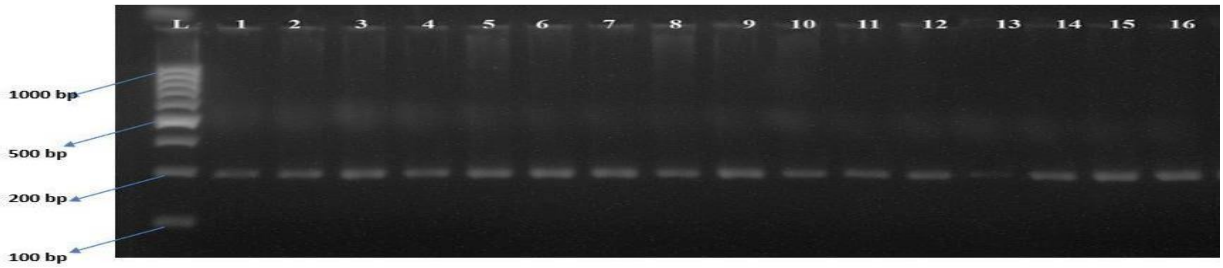


Plate 9: SSR profiling of Castor_SSR_74 in 16 genotypes of castor

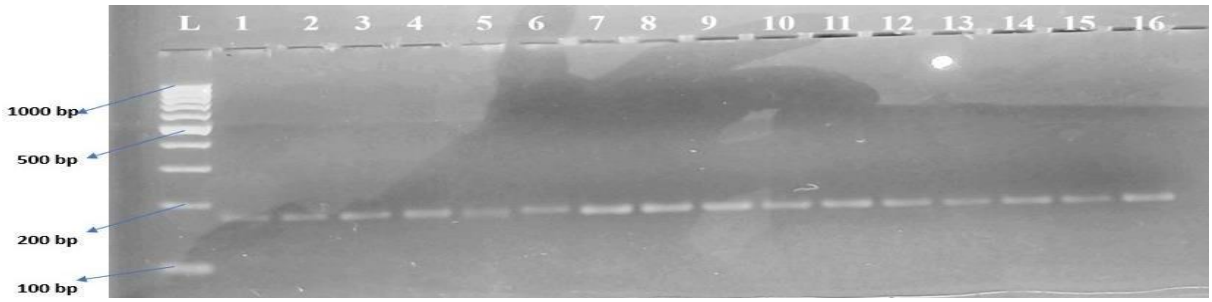


Plate 10: SSR profiling of Castor_SSR_112 in 16 genotypes of castor

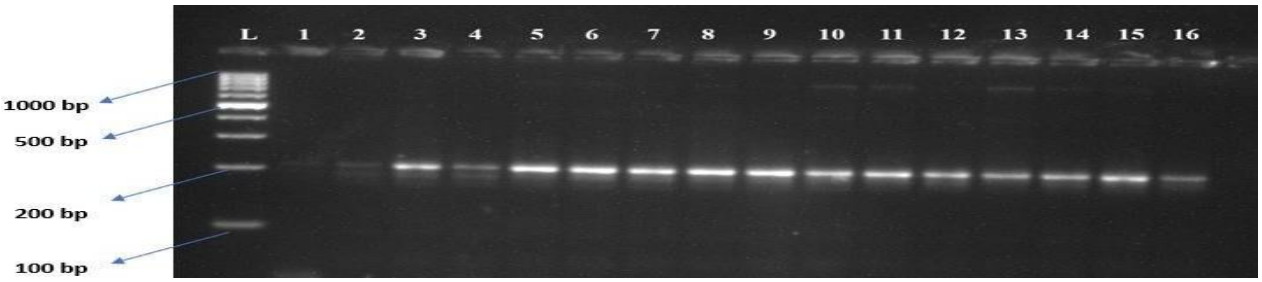


Plate 11: SSR profiling of Castor_SSR_253 in 16 genotypes of castor

Table 5: Results of SSR analysis of sixteen castor genotypes

Sr. No.	Primer name	Product size (bp)	Number of Alleles	PIC	Polymorphis m
1.	Castor_SSR_231	305-385	2	0.44	Yes
2	Castor_SSR_229	204-237	2	0.32	Yes
3	Castor_SSR_194	245-273	2	0.41	Yes
4.	Castor_SSR_270	225-285	2	0.22	Yes
5.	Castor_SSR_102	115-195	3	0.57	Yes
6.	Castor_SSR_277	254-312	2	0.50	Yes
7.	Castor_SSR_123	605-635	2	0.32	Yes
8.	Castor_SSR_74	203	1	0.00	No
9.	Castor_SSR_112	189	1	0.00	No
10.	Castor_SSR_253	207	1	0.00	No
	Total		18	2.78	
	Mean		1.8	0.27	

Similarity index and Hierarchial clustering pattern

Based on the simple sequence repeat data, cluster analysis was performed by using genetic similarity values and a dendrogram (Fig.8) was generated showing the genetic relationships among sixteen genotypes of castor. The dendrogram was constructed based on UPGMA (un weighted pair-group method with arithmetic mean) by using Jaccard's similarity coefficient through NTSYSpc (version 2.0) software. Similarity indices were estimated on the basis of eleven primers ranged from 0.35 to 1.0. The maximum similarity value (1.0) was observed between 48-1 and DCS 89, followed by 0.92 observed between SKP 84 and GC 3. The minimum similarity value (0.35) was observed between DCS-124 and SKI 215, followed by 0.38 observed between GNCH 1 and SKI 215 and JP 96 and SKI 215 indicating the presence of genetic diversity among the castor genotypes. Jaccards's similarity coefficient values for sixteen genotypes ranged from 0.35 to 1.0 indicating moderate level of genetic variation existing among the castor genotypes studied (Appendix B). Similar results were also observed by Senthil *et al*. (2009) and Wang *et al*. (2017).

Clustering pattern of dendrogram generated by pooled SSR data of sixteen genotypes by using eleven primer pairs constructed using UPGMA showed two major clusters A and B formed at a similarity coefficient of 0.61 (Fig: 8). The similarity coefficient was ranged from 0.61-1.00 which is an indicative of presence of moderate variation among genotypes. Cluster A was sub-divided into two sub- clusters A1 and A2. Thirteen genotypes were grouped in one major cluster A. Sub-cluster Al included castor genotypes *viz.*, 48-1, DCS 89, GCH 4, SKI 215, VP 1(M), SKP 84, GC 3, DCG-89, GC 2 and GCH 7. Sub-cluster A2 comprised of genotypes GNCH 1, GAC 11 and GCH 9. Cluster B included GCH 8, JP 96 and DCS-124 genotypes. Similar results were also observed by Thatikunta *et al.* (2016) and Wang *et al*. (2017).

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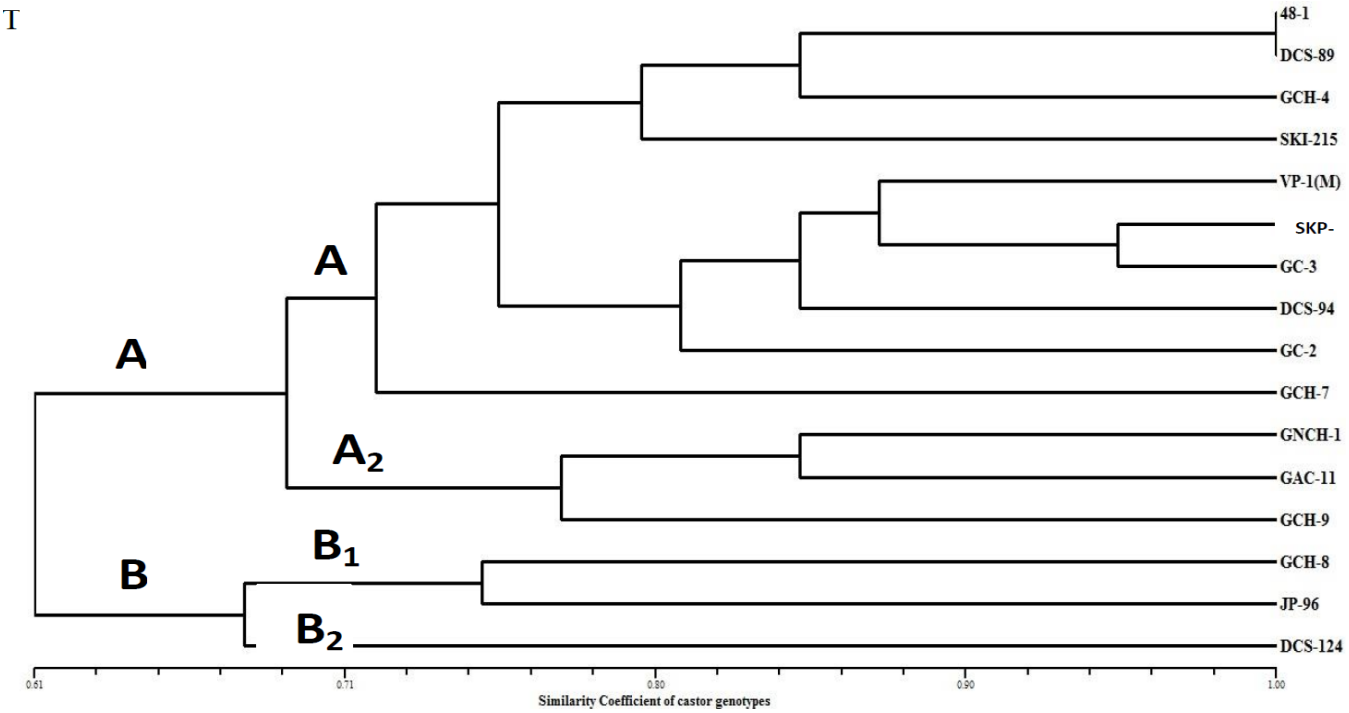

Fig. 8 : Dendrogram showing clustering of sixteen castor genotypes constructed using UPGMA based on Jaccard's similarity co-efficient obtained from SSR based PCR analysis

Conclusions

The investigation demonstrated that the combined use of morphological, biochemical, and molecular markers is effective for assessing genetic purity in castor (*Ricinus communis* L.) genotypes. Morphological characterization revealed considerable variation among the sixteen genotypes in traits such as seed characteristics, pigmentation, stem colour, internode type, and yield-related parameters, enabling clear phenotypic grouping. Biochemical tests further supported genotype differentiation through distinct colour reactions and seed quality parameters, although limited variation was observed in protein profiling using SDS-PAGE. Molecular analysis using SSR markers confirmed the presence of adequate genetic variability among the genotypes, as evidenced by polymorphic alleles, moderate PIC values, and a wide range of similarity coefficients.

Future thrusts

Incorporation of advanced molecular tools like SNP markers, genotyping-by-sequencing (GBS), focusing on gene expression and functional markers associated with key traits (like yield and oil content) with multi-location validation can strengthen genotype characterization. Also, the identified diverse genotypes can be utilized in breeding programmes to develop high-yielding, genetically stable, and uniform varieties suited for commercial cultivation and industrial applications.

Table 1 : Identification of castor genotypes based on seed caruncle, seed mottling, anthocyanin colour on hypocotyl, leaf: anthocyanin pigmentation of young emerging leaves, types of bloom, stem colour, types of internodes, leaf lascination, petiole surface, capsule spininess and plant: location of branches

Genotypes	Seed caruncle	Seed mottling	Anthocyanin colour on hypocotyl	Leaf: anthocyanin pigmentation of young emerging leaves	Types of bloom	Stem colour	Types of internodes	Leaf lascination	Petiole surface	Capsule spininess	Plant: Location of branches
GCH 4	Big	High	Absent	Present	Triple	Mahogany	Elongated	Shallow	Smooth	Sparse	Basal
GCH 7	Big	High	Absent	Present	Triple	Mahogany	Elongated	Shallow	Smooth	Sparse	Basal
GCH 8	Big	High	Absent	Absent	Triple	Mahogany	Elongated	Shallow	Smooth	Sparse	Basal
GCH 9	Small	High	Present	Present	Triple	Red	Elongated	Shallow	Smooth	Dense	Basal
SKP 84	Small	High	Absent	Present	Triple	Mahogany	Condensed	Shallow	Smooth	Dense	Basal
SKI 215	Big	High	Absent	Present	Double	Mahogany	Elongated	Shallow	Smooth	Absent	Basal
JP 96	Big	High	Absent	Absent	Triple	Green	Elongated	Shallow	Smooth	Dense	Basal
DCS 89	Small	High	Present	Present	Double	Mahogany	Elongated	Shallow	Smooth	Absent	Top
DCS 94	Small	Low	Absent	Absent	Triple	Green	Elongated	Shallow	Smooth	Dense	Basal
GC 2	Big	High	Present	Present	Triple	Mahogany	Elongated	Shallow	Smooth	Dense	Top
GC 3	Small	High	Present	Present	Triple	Red	Elongated	Shallow	Smooth	Dense	Basal
GAC 11	Small	Low	Present	Present	Double	Mahogany	Elongated	Shallow	Smooth	Dense	Basal
GNCH 1	Small	Low	Absent	Present	Triple	Mahogany	Elongated	Shallow	Smooth	Dense	Basal
PCS 124	Small	High	Present	Absent	Double	Green	Elongated	Shallow	Smooth	Dense	Top
VP 1(M)	Small	High	Absent	Absent	Triple	Green	Condensed	Shallow	Smooth	Dense	Basal
48-1	Small	High	Present	Present	Double	Red	Elongated	Shallow	Smooth	Absent	Top

Table 2 : Identification of castor genotypes based on leaf number of lobes, petiole length (cm), capsule length (cm), number of spikes per plant, number of capsules per spike, number of seeds per capsule and seed yield per plant (g),

Genotype	Leaf number of lobes	Length of 4th leaf from top	Petiole length (cm)	Capsule length (cm)	Number of spikes per plant	Number of capsules per spike	Number of seeds per capsule	Seed yield per plant (g)	Germination percentage	Seedling fresh weight (g)	Seedling dry weight (g)	Root length (cm)	Shoot length (cm)
GCH 4	9.00	28.57	31.37	2.13	7.00	65.33	3	382.51	92.67	7.99	0.79	9.47	13.57
GCH 7	10.67	32.50	31.73	2.23	7.00	47.33	3	372.38	85.33	7.93	1.07	10.03	11.90
GCH 8	10.67	34.24	29.43	2.80	6.00	47.00	3	396.93	93.67	11.90	1.40	12.19	10.53
GCH 9	11.33	35.42	25.13	2.20	7.43	49.00	3	367.67	95.33	8.61	1.19	7.77	6.53
SKP 84	8.67	33.81	28.00	1.80	6.33	40.67	3	288.65	95.00	9.49	0.79	11.88	9.23
SKI 215	8.00	34.55	30.40	2.20	5.67	46.67	3	365.37	94.67	11.76	1.15	17.53	10.93
JP 96	8.67	31.52	35.80	2.20	5.33	48.67	3	380.61	92.33	9.03	0.96	13.90	8.60
DCS 89	11.33	30.83	37.83	2.20	7.00	46.00	3	371.20	95.00	11.89	1.07	17.23	11.67
DCS 94	8.67	27.11	35.87	1.70	6.67	37.00	3	359.00	91.33	6.53	0.87	12.80	10.33
GC 2	10.33	28.20	36.13	2.03	6.33	49.67	3	382.06	76.00	7.91	0.76	12.57	7.10
GC 3	9.67	28.73	33.54	2.10	5.33	38.67	3	264.32	89.33	6.77	0.89	13.03	8.23
GAC 11	9.67	29.16	36.10	1.97	6.67	37.00	3	393.39	90.67	6.75	1.00	9.63	11.90
GNCH 1	9.67	30.27	33.13	2.40	6.00	40.33	3	359.11	93.33	7.91	0.96	15.70	7.80
PCS 124	10.00	22.37	33.53	2.10	7.00	47.33	3	381.55	67.33	7.73	0.58	16.47	7.20
VP 1(M)	9.00	34.64	27.77	2.30	6.67	48.00	3	364.32	71.00	3.75	1.00	13.80	8.95
48-1	8.33	36.90	30.50	2.40	6.41	50.84	3	354.69	94.67	7.57	0.84	11.70	11.60
Mean	9.60	31.17	32.26	2.17	6.42	46.21	3	361.48	88.65	8.34	0.95	12.85	9.75
S.Em ±	0.39	1.37	1.87	0.08	0.37	3.70	0.0	23.82	1.68	0.22	0.01	0.27	0.20
C.D. at 5 %	1.14	3.97	5.39	0.22	1.06	10.69	0.0	68.81	4.86	0.65	0.05	0.80	0.60
CV %	7.10	7.63	10.02	6.12	9.84	13.50	0.0	11.31	3.30	4.72	3.51	3.75	3.71

Table 3 : Identification of castor genotypes based on potassium hydroxide (KOH) bleach test, sodium hydroxide (NaOH) test, grain colouration with phenol, peroxidase test and copper sulphate ammonia test.

Genotypes	Potassium hydroxide (KOH) bleach test	Sodium hydroxide (NaOH) test	Grain colouration with phenol	Peroxidase test	Copper sulphate ammonia test
GCH 4	Orange	Orange	Brown	Brown	Dark brown
GCH 7	Orange	Orange	Brown	Brown	Dark brown
GCH 8	Dark orange red	Dark orange red	Dark brown	Dark brown	Black colour
GCH 9	Orange	Orange	Brown	Brown	Dark brown
SKP 84	No colour	No colour	No colour	No colour	No colour
SKI 215	Orange	Orange	Brown	Brown	Dark brown
JP 96	Dark orange red	Dark orange red	Dark brown	Dark brown	Black colour
DCS 89	Orange	Orange	Brown	Brown	Dark brown
DCS 94	No colour	Orange	Light brown	Light brown	No colour
GC 2	No colour	Orange	Light brown	Light brown	No colour
GC 3	No colour	Orange	Light brown	Light brown	No colour
GAC 11	Orange	Orange	Light brown	Light brown	No colour
GNCH 1	Orange	Orange	Light brown	Light brown	No colour
PCS 124	Orange	Orange	Light brown	Light brown	No colour
VP 1(M)	Orange	Orange	Brown	Brown	Black colour
48-1	Orange	Orange	Brown	Brown	Dark brown

Table 4: Identification of castor genotypes based on moisture content (%), ash content (%), oil content (%), total soluble sugar (%) and total protein (%)

Genotypes	Moisture content (%)	Ash content (%),	Oil content (%)	Total soluble sugar (%)	Total protein (%)	Zinc (Zn) content (mg/L)	Iron (Fe) content (mg/L)	Potassium (K) content (mg/L)	Sulphur (S) content (mg/L)	Phosphorous (P) content (mg/L)
GCH 4	4.32	4.61	48.33	1.32	19.04	0.11	0.74	11.30	0.36	0.188
GCH 7	4.19	4.62	47.67	1.26	20.39	0.09	0.67	8.67	0.26	0.149
GCH 8	4.08	4.74	47.67	1.22	22.17	0.08	0.36	8.38	0.22	0.147
GCH 9	4.10	4.85	47.67	1.50	22.84	0.13	0.37	8.75	0.19	0.149
SKP 84	4.91	4.79	46.17	2.18	24.69	0.14	0.64	10.94	0.19	0.153
SKI 215	4.84	3.96	45.00	1.45	17.08	0.10	0.75	9.96	0.19	0.151
JP 96	3.90	3.65	47.33	1.44	18.13	0.07	0.25	6.41	0.19	0.153
DCS 89	4.60	4.09	48.67	1.39	19.46	0.08	0.45	8.58	0.19	0.152
DCS 94	4.63	4.27	46.00	1.55	22.30	0.03	0.46	9.34	0.34	0.153
GC 2	4.04	5.03	47.33	0.95	20.62	0.05	0.33	9.64	0.18	0.153
GC 3	4.41	4.82	47.67	2.18	21.80	0.02	0.48	9.13	0.20	0.145
GAC 11	4.54	4.02	46.67	1.56	17.54	0.11	0.39	7.15	0.19	0.143
GNCH 1	4.30	5.20	47.17	1.48	18.18	0.13	0.39	11.05	0.18	0.152
PCS 124	4.05	5.28	48.33	1.38	21.25	0.06	0.51	8.54	0.20	0.147
VP 1(M)	4.21	4.82	46.33	1.85	21.26	0.03	0.30	7.90	0.27	0.147
48-1	4.38	5.33	48.00	0.97	19.19	0.04	0.26	10.38	0.20	0.148
Mean	4.34	4.62	47.25	1.48	20.37					
S.Em ±	0.08	0.06	0.28	0.03	0.43					
C.D. at 5 %	0.23	0.19	0.81	0.10	1.26					
CV %	3.24	2.53	1.03	4.23	3.72					

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