IJPSR (2021), Volume 12, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 April 2020; received in revised form, 19 March 2021; accepted, 22 March 2021; published 01 April 2021

EVALUATION OF THE GENETIC DIVERGENCE OF VARIOUS ACCESSIONS OF THE SPECIES *CARISSA CARANDAS* USING INTER SIMPLE SEQUENCE REPEAT PRIMERS

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Keywords:

Carissa carandas Linn., Genetic diversity, ISSR primers, Polymorphism

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ABSTRACT: *Carissa carandas* Linn. belonging to the family Apocynaceae, is an underutilized fruit plant. *C. carandas* has been used in many ethno medicines 6, 40, 30. Genetic diversity is a ubiquitous feature of all species in nature. ISSR markers are useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping, and evolutionary biology in a wide range of plant species 4, 12. In this investigation, 9 ISSR primers were used for fingerprinting of 9 different accessions of *C. carandas* grown in a different location. Of these 9 primers, the amplification of only 8 primers (4, 5, 6 and 7, HB10, HB12, HB13 and HB15) were satisfactory and reproducible. Results of the ISSR fingerprint showed 473 amplified fragments; 134 of them were polymorphic (28.33%). The sizes of the amplified fragments were ranged between 200 and 1700 bp in length. The primer HB-10 showed the highest polymorphism (46.67%), while primer 6 showed the lowest with only (8.70%). The results obtained in this study validate that ISSR is useful markers in genetic diversity studies and offer a promising perspective as a molecular tool for varietal identification and breeding program applications due to the polymorphism level detected by the primers. Tandem repeats flanking microsatellites are highly polymorphic, even among closely related cultivars due to mutations. The results showed a low level of polymorphism (28.33%) among *C. carandas* accessions growing in different locations by ISSR markers and revealed that low genetic diversity among the accessions analyzed, indicating its narrow genetic base and could be used for plantation and making value-added products.

INTRODUCTION: *C. carandas* (Karanda) was used in the Indian system of medicine, particularly Ayurvedic, Unani, and Homoeopathy medicine formulations since antiquity [24, 11] reported that the tribal healers of Western Ghat region of Karnataka, India used this species as a hepatoprotective and antihyperglycemic agent [29] have also reported that these species alleviate many diseases like malaria, epilepsy, nerve disorders, pain, *etc*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(4).2306-16

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(4).2306-16

But no scientific data was available at that time to validate this folkloric claim. From the reports published in recent times and scientific validity of the pharmacological importance of Carissa genus it is being evident now days.

The Scientific Classification of the Plant *Carissa carandas Linn.* is:

Kingdom	**:**	Plantae
Class	**:**	Angiospermae
Order	**:**	Gentianales
Family	**:**	Apocynaceae
Subfamily	**:**	Rauvolfiodeae
Genus	**:**	*Carissa*
Species	**:**	*carandas*

Scientific Name: *Carissa carandas* Linn. the plant *C. carandas* is an evergreen shrub or small crooked tree, diffuse, rank-growing, straggly, woody with short stem, paired thorns, usually growing to 10 or 15 ft (3-5 m) high; and rich in white, gummy latex. The stem is fresh green, cylindrical in shape, and the branches are set with sharp, simple or forked thorns in pairs [18.] Leaves are simple, evergreen, opposite and coriaceous with fragrant white flowers in lax cymes. The flowers are regular, bisexual [14]. The fruit clusters of 3 to 10 smooth, glossy; enclosing very acid to fairly sweet, often bitter, juicy, red or pink, juicy pulp [25].

Various parts of *C. carandas* have been used in many ethno medicines. The whole plant is usually used as an anthelmintic, astringent, appetizer, antipyretic, antidiarrheal, anti-cancerous, in biliary, stomach disorders like acidity, flatulence, poor digestion, rheumatism, brain disease, and wound healing [19]. Earlier studies in the traditional system of medicine reported that the extract of the plant has shown to possesses cardiotonic, antipyretic, and antiviral activity [6, 40, 30].

It is also used as a slimming diet. Stem used to strengthen tendons; obstinate skin diseases. The white wood or yellow wood of *C. carandas* is sometimes burned as fuel. A leaf decoction of Karonda is used against fever, diarrhoea, oral inflammation, syphilitic pain, earache and also has anti-pyretic activities. It is pounded with horse urine, lime-juice and camphor as a remedy for the itch and leaves boiled in water which acts as an appetite stimulant [20, 2, 27].

The karaunda fruits are rich in minerals, fiber, vitamins, iron and a fair amount of vitamin C. It is very useful for cure of anaemia. Its unripe fruit is sour, bitter in taste and the ripe fruit is sweet, cooling, acid, thermogenic, astringent, aphrodisiac, appetizer, antipyretic, antiscorbutic; lessen-thirst, biliousness *i.e* bad digestion, stomach pain and constipation, analgesic, anti-inflammatory, anti-microbial, antidiabetic , anticancerous agents and lipase activity [25, 11, 34] useful in diseases of the brain, skin, hyperdipsia, diarrhoea, anorexia, intermittent fevers, mouth ulcer, skin disorders, sore throat, cardiotonic, anticonvulsant, anthelmintic, antiviral and nematicidal activity; makes one lethargic; diminishes sexual power.

The fruit of *Carissa carandas* pickled with ginger hastens digestion and acts as a mild laxative. Its fruits and seed latex are used for treating rheumatoid arthritis, anorexia, indigestion, colic, hepatomegaly, splenomegaly, piles, cardiac diseases, oedema, amenorrhoea, fever, and nervine disorder [28]. The intake of an aqueous boiled root extract cleans the uterus after childbirth. Root paste is used for diabetic, ulcer, stomach disorders, vermifuge, intestinal worms, scabies, purtuitis, and urinary disorders [35].

Alcoholic extract of roots exhibit hypotensive activity *i.e.* reduces the blood pressure [4,] and aqueous extract of roots exhibited various pharmacological activities including histamine-releasing, antidiarrheal, antihelmintic, stomachic, sapsmolytic, anticonvulsions, epilepsy, cardiotonic, analgesic, anti-inflammatory, remedy for itches and lipase activities [5, 15, 44, 42].

The fruits of *C. carandas L.* have been used as tanning and dyeing agents. Leaves served as fodder for the tussar silkworm. Pounded roots served as a fly repellent. The white or yellow wood of *C. carandas*is hard, smooth, and useful for fashioning spoons, combs, household utensils, and miscall-aneous products of turnery. The DNA sequences flanking microsatellites are generally conserved within individuals of the same species. ISSR repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of tandem repeating units.

In addition, when the genetic basis of the species or genus to be analyzed is not well-known, rapid and robust results are necessary. To date, no report is available on applications of molecular markers (ISSR) in studies on the genetic diversity of *Carissa carandas*. In the case where gene flow between neighboring populations is not limited, populations of longer geographical distances will generally show greater diversification [26].

Different accessions of *Carissa carandas* Linn. collected from different locations exhibited morphological similarities (leaf and length, flower morphology and fruit morphology, *etc*.), but their ISSR fingerprinting may differ markedly. Based on this knowledge, one can suggest appropriate strategies and policies for the crop breeding

program and biodiversity conservation strategies through the definition of appropriate units of management in order to prevent depletion of biodiversity due to man-made efforts.

An understanding of the genetic diversity responsible for individual species adaptations and responses to their environment under natural conditions (intraspecific diversity) is a foundation for understanding almost all ecological and evolutionary processes.

Therefore, dendrogram could be partially explained genetic relationships among the plant species of *C. carandas* L. grown in different locations of NE Region and could be used for plantation and making value-added products.

It is important to point out that the genetic variation that a population of organisms possesses is the fuel that allows them to be able to change or evolve in response to changing environmental conditions. This distribution pattern of genetic variation of *C. carandas* L. populations will provide important baseline data for conservation and collection strategies for this species.

Therefore, analysis of ISSR data could be useful to detect the genetic difference between accessions of *C. carandas* L. grown in different locations. However, a detailed study is desirable to understand all the aspects related to genetic variations.

Hence, further information is required on gene flow patterns within and between accessions to protect and select the superior variety of the species. To generate information about the potential health-enhancing as well as health-protective activity of *Carissa carandas* Linn. and to introduce underutilized fruit of Karanda (*Carissa carandas L.*) for its commercial exploitation, the present investigation is a part of such efforts on *Carissa carandas Linn.* of Assam, North East India.

By taking into account, their strong antioxidant ability to target multiple ROS and antibacterial activities, genetic divergence within cultivars was studied to develop herbal formulations for human health as an antioxidant and as antimicrobial agent with less cytotoxicities.

To date, no report is available on applications of molecular markers (ISSR) in studies on the genetic diversity of different accessions of *C. carandas*. The present investigation is an attempt to explore the extent of genetic diversity based on ISSR polymorphism in *C. carandas* for selection and crop improvement.

Objectives of the Study: Evaluation of the genetic divergence of various accessions of the species *Carissa carandas Linn.* using molecular markers (ISSR). Cluster analysis by Dendrogram.

MATERIALS AND METHODS:

Study Area: For the Genetic divergence assay of *Carissa carandas* Linn., samples were collected from 9 different areas of Assam, NE Region in the month of August 2016 **Table 1**, **Fig. 1** & **2**.

The plant materials were transferred to plastic bags for transport from the field to the laboratory. Permanent storage was at -20 °C. The voucher specimens were identified by the Department of Botany, Gauhati University, Assam.

FIG. 1: *CARISSA CARANDAS* PLANT BEARING FRUITS AND FLOWERS

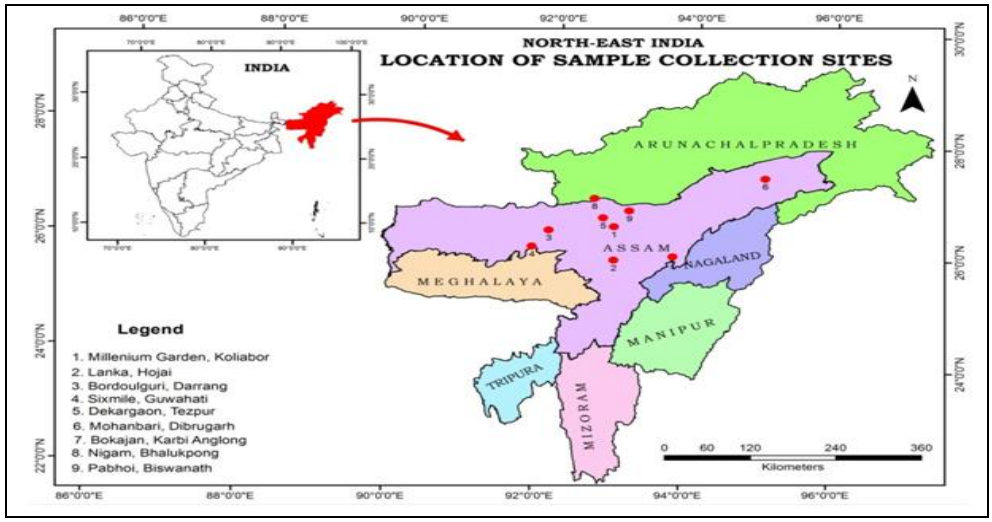


FIG. 2: MAP SHOWING *CARISSA CARANDAS* LINN. GROWING AREAS AND STUDYING SITES AT ASSAM

TABLE 1: VARIOUS ACCESSIONS OF *CARISSA CARANDAS* LINN

S. no.	ID	Accessions
1	K	Kaliabor, Nagaon
2	L	Lanka, Hojai
3	D	Dekargaon, Tezpur
4	M	Mohanbari, Dibrugarh
5	B	Bokajan,Karbi Anglong
6	S	Six mile, Guwahati
7	P	Pabhoi,Biswanath Chariali
8	BM	Bordoulguri,Mangaldoi
9	N	Nigam, Bhalukpong

Chemicals: All the chemicals were of analytical grade obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai.

DNA Isolation: In the case of *Carissa carandas*, genomic DNA was isolated by using liquid nitrogen due to the presence of high contents of secondary metabolites. Total genomic DNA was extracted from fresh leaves of *C. carandas* by using HipurA^{TM} Plant DNA Isolation Kit (CTAB Method) with minor modifications. First of all the plant material was washed, dried, and weighed (0.6g) and then extracted in a pre-chilled mortar and pastel. The plants were crushed in 2% Cetyl trimethyl ammonium bromide (CTAB), and then homogenized material was transferred to Eppendorf tubes. The samples were incubated at 65ºC for 30 minutes with occasional mixing. An equal volume of phenol was added to an aqueous DNA sample in Eppendorf tube and incubated at 25ºC overnight period. After overnight incubation, the samples were centrifuged at 2500g for five minutes. The supernatant was taken out and treated with 500µl chloroform iso-amyl alcohol. The tubes were inverted gently and then centrifuged at 2500g for 5 min. The chloroform: iso-amyl treatment was repeated again. The supernatant was treated with chilled iso-propanol and left for 3 h at 4 ºC for precipitation of DNA. Then the supernatant was removed, and the pellet was washed with 70% ethanol and allowed to be dried. Finally, the pellet was incubated at 37 ºC in 40µl TE (Tris EDTA) buffer and RNase A to get rid of RNA.

Quantification of Genomic DNA by Spectrophotometer and Quality Checking by Electrophoresis: The concentration of genomic DNA was estimated by comparing the size and intensity of each sample band with those of sizing standard, 100 bp ladder (Himedia). DNA was quantified with UV/VIS spectrophotometer by measuring OD 260/280, and purity was checked by running it on 0.8% agarose gel prepared in 1× TAE buffer (Tris Acetate Ethylene Diamine Tetra Acetic Acid), stained with Ethidium Bromide (10 mg/mL). The DNA is pure enough (OD260/280 =1.68)[33], for ISSR- PCR analysis. The quantified DNA samples were diluted in TE buffer to make a final concentration of 25 ng/µL for PCR reactions and stored at -20 °C for further use.

ISSR Analysis: For ISSR analysis, 9 selected inter simple sequence repeats (Bangalore Genei Pvt. Ltd., Bangalore, India) were used in **Table 2** for PCR amplification. DNA amplifications were performed in Bio-Rad thermocycler, under the following conditions: These were carried out in a final volume of 25 µL reactions containing 12.5 µl PCR master mix (kit Himedia), 7.5 µl dH_2O, 3.0 µL template DNA and 2.0 µl primer. DNA amplifications were performed in Bio-Rad thermo cycler, under the following conditions: These were carried out in a final volume of 25 µL reactions containing 12.5 µl PCR master mix (kit HiMEDIA), 7.5 µl dH_2O, 3.0 µL template DNA, and 2.0 µl primer **Table 3.** PCR was set at an initial denaturation for 5 min at 95 °C, followed by 35 cycles of 94 °C for 30 sec, 37 °C for 30 sec for primer annealing, 72 °C for 30 sec. for primer extension, and 72 °C for 12 min for completion of primer extension, using the ISSR primers. The soaking temperature was 4 °C **Table 4**. After completion of the PCR, 2.5 mL of 6X loading dye (MBI Fermentas, Lithuania) was added to the amplified product and deep-frozen at –20 °C for future use.

TABLE 2: LIST OF ISSR PRIMER [36]

S. no.	ISSR Primer Name	Sequence (5'-3')
1	4	AGG AGG AGG AGG
2	5	GAG AGA TGA GA GA T
3	6	GACA GACA GACA GACA
4	7	T GA GA GA T GA GA GA
5	HB-09	GTG TGT GTG TGT GC
6	HB-10	CTC TCT CTC TCT CTC TTG
7	HB-12	CAC CAC CAC GC
8	HB-13	GAG GAG GAG GC
9	HB-15	GTG GTG GTG GC

TABLE 3: THE REAGENTS USED TO AMPLIFY THE GENOMIC DNA

S. no.	Constituent	Quantity
1	2X PCR TaqMixture, 2X	12.5 µl
2	Upstream Primer, 10 µM	2.0 µl
3	Template DNA	3.0 µl
5	Molecular Biology Grade Water	7.5 µl
	Total	25.0 µl

TABLE 4: THE ISSR-PCR REACTION STEPS

S. no.	Activity	Temperature	Time	Cycles
1	Initial denaturation	95°C	5 min	1
2	Denaturation	94°C	30 sec	
3	Annealing	37°C	30 sec	35
4	Extension	72°C	30 sec	
5	Final extension	72°C	12min	1
6	Storage	4°C	Forever	

Agarose Gel Electrophoresis: Electrophoresis was performed at a constant voltage at 60 V in TAE 1X buffer for 3 h. The amplicons were separated in 2 % agarose gel for ISSR. After electrophoresis, the ISSR patterns were visualized with a UV transilluminator and photographed with gel documentation system by Gel Doc 2000 (Bio-Rad, USA) to detect polymorphism between different accessions of *C. carandas* grown in different locations under study and the DNA bands generated by each primer were counted.

The amplicon size was determined by comparison with the ladder (Range 100-1500 bp) (Gene Ruler 100 bp ladder plus). In order to evaluate the reproducibility of the DNA profile, DNA isolation and PCR reactions were carried out 3 times, and only well-defined and reproducible bands were scored.

Data Scoring: The data were scored as '1' for band presence and '0' for absence for each primer genotype combination for ISSR analysis. All bands (monomorphic and polymorphic) were considered to avoid under/overestimation of the genetic similarity [8].

The data were integrated together to form a data matrix for the estimation of genetic distance between the samples collected from different areas. Cluster analysis of *C. carandas* genotypes was performed based on the similarity coefficient between samples and based on morphological, molecular data using Numerical Taxonomy and Multivariate Analysis System (NTSYS) pc version 2.01[31, 38] to determine genetic diversity and similarity among genotypes.

RESULTS: Northeast India abounds with a large variety of major and minor fruits usually mature during summer (May-July) and is available in the market. A few fruits are available throughout the year, and others are season-specific.

Carissa carandas is important as a source of antioxidants. In this investigation, 9 ISSR primers were used for fingerprinting of 9 samples of *Carissa carandas*. of these 9 primers, the amplification of only 8 primers (4, 5, 6, 7, HB10, HB12, HB13, HB15) was satisfactory and reproducible. The rich diversity in the morphological and chemical characteristics of the

fruits of different accessions of *C. carandas* validates the existence of genetic diversity. A low level of polymorphism (28.33%) among *C. carandas* accessions by ISSR markers revealed low genetic diversity among the accessions analyzed, indicating its narrow genetic base.

ISSR-PCR Fingerprinting: In this study, the banding patterns of ISSR fragments had done using the nine arbitrary primers with the nine *C. carandas* of the same cultivars grown in different location **Table 1**. Out of 9 ISSR primers, 8 have shown amplifications and revealed some degree of polymorphism among selected species.

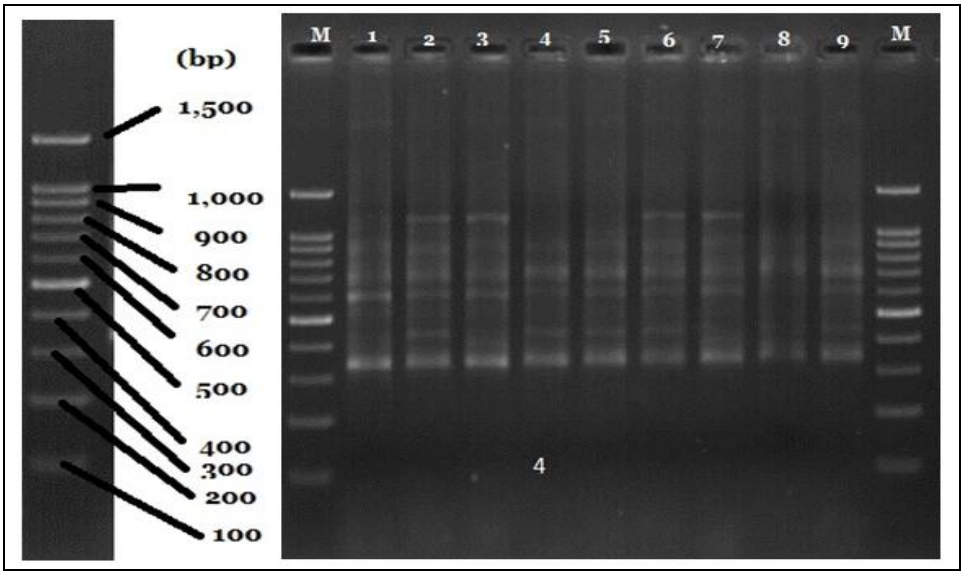
Results of the ISSR fingerprint showed 473 amplified fragments; 134 of them were polymorphic (28.33%). The total number of amplified and polymorphic fragments obtained with each primer presented in **Table 6**. On average, 40 bands were produced by each primer. The highest band number was produced with primer HB-12 (81 bands), while the lowest was produced with primer HB-15 (41 bands), respectively. The molecular size of the amplified fragments was ranged between 200 and 1700 bp in length. The primer HB-10 showed the highest polymorphism (46.67%), while primer 6 showed the lowest with only (8.70%), **Table 5**.

The ISSR banding pattern obtained with primers are depicted in **Fig. 3.** The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others, and 'monomorphic' if present in all the individuals.

Among the bands obtained using ISSR primers, 28.33% were polymorphic, and only 11.11% were considered monomorphic. This ISSR data was further utilized for the construction of dendrogram. A degree of polymorphism was observed with ISSR primers suggesting that they could be used as suitable molecular markers for diversity analysis.

TABLE 5: TOTAL NUMBER OF AMPLIFIED DNA FRAGMENTS AND POLYMORPHISM GENERATED IN *C. CARANDAS*GENOTYPES USING 8 ISSR MARKERS

Molecular type	Primer code	Range of amplicons (bp)	Polymorphic bands	Total bands	Polymorphic Percentage %
ISSR	4	350-1200	15	45	33.33
	5	320-950	12	69	17.39
	6	300-1100	6	69	8.70
	7	200-1400	26	74	35.14
	HB-09	0	0	0	0
	HB-10	490-1600	21	45	46.67
	HB-12	350-1600	36	81	44.44
	HB-13	300-1700	10	49	20.41
	HB-15	400-920	8	41	19.51
	Total = 9 primers	200-1700	134	473	28.33



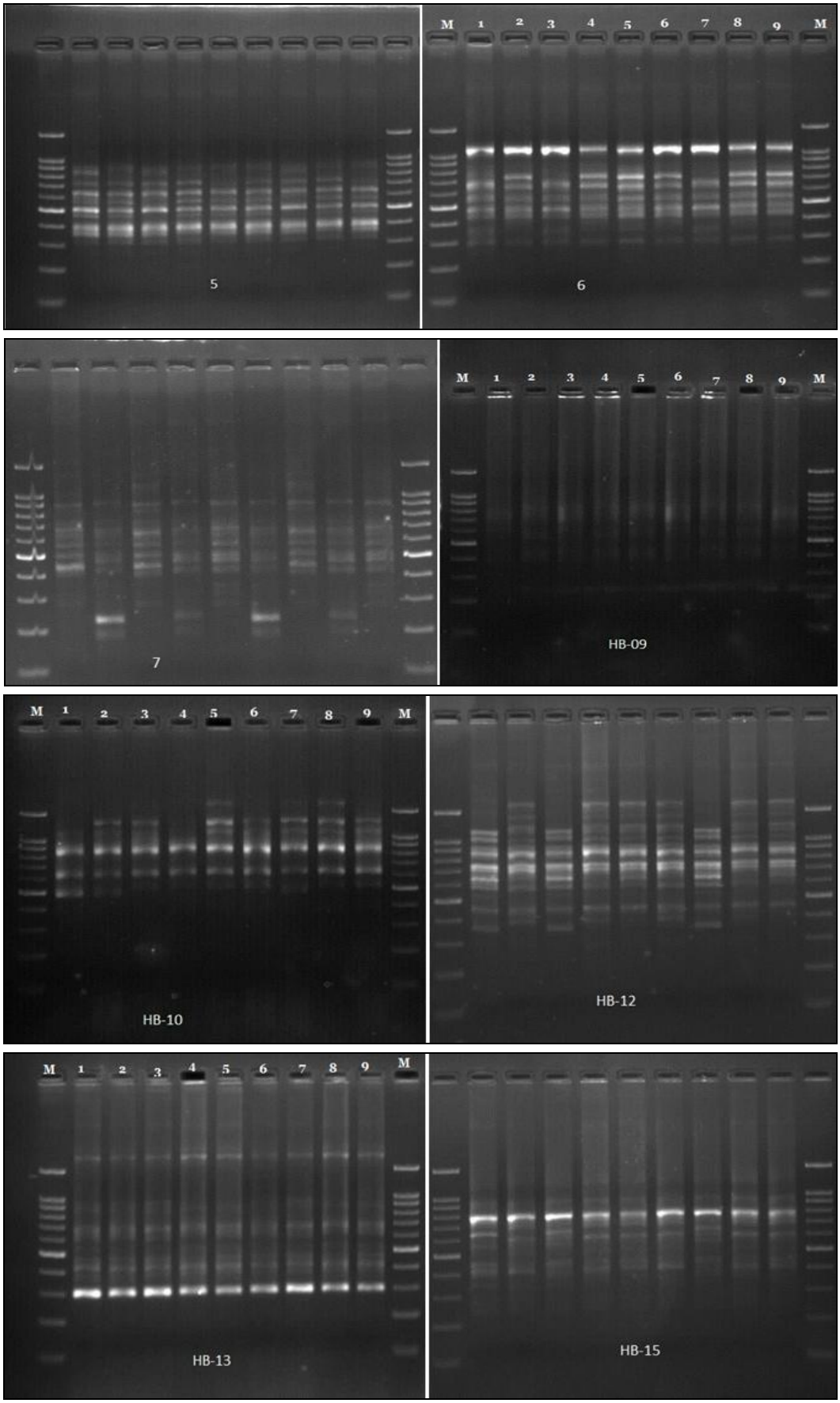


FIG. 3: ISSR BANDING PATTERNS OF 9 ACCESSIONS OF CARISSA CARANDAS L. (M= MARKER 4, 5, 6, 7, HB9, HB10, HB12, HB13, HB15) FIG. B-J Fig. a= Ladder specifications**.** Lane M= Molecular weight marker Himedia 100bp**.** Lane 1-9= DNA from plants of nine different accessions

TABLE 6: TOTAL NUMBER OF AMPLIFIED DNA FRAGMENTS AND POLYMORPHISM GENERATED IN *C. CARANDAS* GENOTYPES USING 8 ISSR MARKERS

Molecular type	Primer code	Range of amplicons (bp)	Polymorphic bands	Total bands	Polymorphic Percentage %
ISSR	4	350-1200	15	45	33.33
	5	320-950	12	69	17.39
	6	300-1100	6	69	8.70
	7	200-1400	26	74	35.14
	HB-09	0	0	0	0
	HB-10	490-1600	21	45	46.67
	HB-12	350-1600	36	81	44.44
	HB-13	300-1700	10	49	20.41
	HB-15	400-920	8	41	19.51
	Total= 9 primers	200-1700	134	473	28.33

Data Scoring: The data were scored as '1' for band presence and '0' for absence for each primer genotype combination for ISSR analysis.

Cluster Analysis: The clustering results of different accessions suggested that *C .carandas* L. undergoes a major part of genetic variation by environmental factors. Genetic diversity refers to the variation at the level of individual genes (polymorphisms), and provides a mechanism for populations to adapt to their ever-changing environment. Genetic diversity is a resource for the species' own survival and future evolution, it also promotes selective breeding. The global pool of genetic diversity represents all the information pertinent to all biological structures, functions and processes on this planet [16].

Apart from genetic drift, inbreeding depression may also be one of the factors, which may lead to genetic variation[37]. An understanding of these genetic processes is required in order to fully evaluate the consequences of fragmentation and its relationship to genetic variation. Assessment of genetic diversity is essential for germplasm characterization to identify potential parents, management, and utilization of genetic resources. Molecular markers (ISSR) are valuable tools for the analysis of genetic relatedness, the identification, and selection of desirable genotypes for crosses as well as for germplasm conservation in gene banks. Moreover, the polymorphism determined by these markers is one of the valuable parameters for the study of populations and understanding of their genetic difference [45, 1]. Each amplification fragment was considered as a dominant allele for a given locus. The ISSR amplification products obtained with different ISSR primers were scored as present (1) and absent (0) of clear, visible, and reproducible bands for each primer accession combination in each species. Cluster analysis was performed based on the Jaccard's similarity coefficient [13] between samples by un-weighted pair group method using arithmetic averages UPGMA method to show the phenetic representation of genetic relationships as revealed by the similarity matrix, and the corresponding dendrogram was constructed with the Numerical Taxonomy and Multivariate Analysis System (NTSYS) pc version 2.01 [31] by using an unweighted pair group method of arithmetic mean (UPGMA).

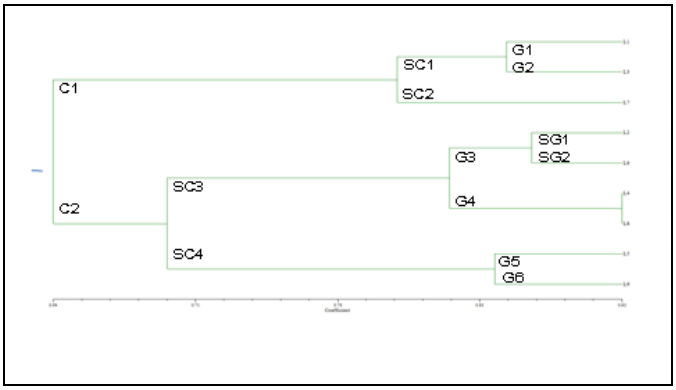


FIG. 4: UPGMA CLUSTER ANALYSIS SHOWING DIVERSITY AMONG THE SAMPLES OF *C. CARANDAS* L.

According to the dendrogram, 9 different accessions of *C. carandas* species mainly split into two clusters and shared the same node at a similarity level of 0.64. Cluster 1 (C 1) consisted of L1,L3 and L7while Cluster 2 (C 2) was represented by L2,L4,L5,L6,L8,L9. Cluster 1diverged to form two sub-clusters *i.e.* sub-cluster SC 1, SC 2, SC 3, SC 4 **Fig. 5.** Sub-cluster 1 consisted ofL1 and L3, while sub-cluster 2 contained only L7.

Subcluster 2 further, divided into two groups *i.e.* group 1 (G1) and group 2 (G2). Both groups exhibited 0.92 similarities. Group 1 contained L1, whereas group 2 containedL3 which formed a separate group. Group 3 further diverged to form sub group 1 (SG 1) and subgroup 2 (SG 2). Subgroup 1 consisted L2; Subgroup 2 consisted L6. Both were 0.92 similar and formed separate lines. G4 consisted of L4 and L8 and both the members ofG4 showed 0.92 similarities and formed same line. There is close similarity in between L4 and L8.Sub cluster 4 is further divided into two groups *i.e.* group 5 (G 5) and group 6 (G 6). Both were 0.92 similar and formed separate lines. Cluster analyses were implemented by NTSYS PC software.

DISCUSSION: Genetic diversity is a ubiquitous feature of all species in nature. Genetic divergence among the genotypes plays an important role in the selection of wider variability parents for different characters. Molecular markers have been the development of genetic and physical maps of genomes. ISSRs are randomly distributed throughout the genome. ISSR involves PCR-based amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite directions. ISSR markers usually show high polymorphism [21], although the level of polymorphism has been shown to vary with the detection method used [26] done RAPD analysis among different accessions of *C. carandas* [7] reported a reproducibility level of more than 99% after performing repeatability tests for ISSR markers by using DNA samples of the same cultivar grown in different locations. The use of molecular markers has become a common practice in studies of population structure, genetic diversity for pre-breeding and breeding germplasm and in distinguishing one individual genotype to preserve the property of breeding rights [22].

In addition, ISSR markers are useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping, and evolutionary biology in a wide range of plant species [4, 12]. DNA-based markers have their applications in fingerprinting genotypes, seed purity determination and in phylogenetic analysis by which plant conservation can be made easy. The innovation of Polymerase Chain Reaction (PCR) made the development of DNA-based markers easier. DNA-based molecular markers have wide application in drug analysis and are greatly used for the characterization of medicinally important plant species [41]. In the present study, the Inter Simple Sequence Repeat (ISSR) technique was employed for characterizing the selected species of *Carissa carandas Linn.* Applying molecular markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibilities for evaluating genetic diversity and determining of inter and intraspecific relationships between genotypes.

For population and evolutionary genetic studies, characterization of genetic diversity and measurement had always been a primary concern. The main emphasis of the present study was to analyze the genetic diversity at intraspecific level and differentiation of species at a molecular level. ISSR molecular markers used in this particular study is one of the most popular DNA marker system and highly suitable for analysis of genetic relationships and quick fingerprinting. Meghwal *et al.,* 2014 [26] observed that out of 18 decamer random primers, seven primers detected intra-specific variation generating scorable amplicons reproducible patterns and generated 60 marker bands in the range of 250 bp to 2.5 kb. Among these, 15 markers were polymorphic, amounting to 25 % polymorphism, and exhibited 11.1 to 57.1% polymorphism in the banding pattern. However, a low level of polymorphism amounting to only 25% among *C. carandas* accessions by RAPD markers revealed low genetic diversity among the accessions analyzed, indicating its narrow genetic base in the available germplasm.

In this investigation, 9 ISSR primers were used for fingerprinting of 9 samples of *Carissa carandas*. Of these 9 primers, the amplification of only 8 primers were satisfactory and reproducible. Each amplification fragment was considered as a dominant allele for a given locus. The presence or absence of the band was scored as 1 or 0, respectively, obtaining the molecular identification profile for each individual. Cluster analyses were implemented by the UPGMA method, and the corresponding dendrogram was constructed by NTSYS PC software. The results could be used for the identification of ideal genotypes for the

extraction of drugs by correlating the molecular fingerprints with desirable morphological and biochemical features. It may also be helpful in devising strategies to protect the genetic diversity of this species. For efficient conservation and management of such threatened species, the genetic composition of the species in different geographic locations needs to be assessed. Yet, there has been no previous report on the use of these methods to characterize the genetic diversity of *Carissa carandas.* The objective of this study was to determine the genetic variation between species of *Carissa carandas* grown in different locations of the NE Region by using ISSR markers.

CONCLUSION: Medicinal plants are sources of important therapeutic aids for alleviating human ailments [10]. Due to lack of awareness, several medicinal plant species are on the verge of extinction, and many more fruits remained underutilized. Moreover, the use of these underutilized fruits can be a substitute to make balanced diets for humans. Molecular characterization using ISSR primers revealed variation among *C. carandas* populations growing in different locations. The results obtained in this study validate that ISSR is a useful marker in genetic diversity studies and offers a promising perspective as a molecular tool for varietal identification and breeding program applications due to the polymorphism level detected by the primers. Genetic improvement of any crop species depends upon the existence, nature, and extent of genetic variability available for manipulation[43].

In this study, the banding patterns of ISSR fragments observed using the nine arbitrary primers with the nine *C. carandas* of the same cultivars growing in a different location. Genetic divergence among the genotypes plays an important role in the selection of wider variability parents. The DNA sequences flanking microsatellites causing variation in the number of repeating units are generally conserved within individuals of the same species, variation in the number of tandem repeats, n, results in different PCR product lengths. These repeats are highly polymorphic, even among closely related cultivars due to mutations. To date, no report is available on applications of molecular markers (ISSR) in studies on the genetic diversity of *Carissa carandas*.

In this investigation, 9 ISSR primers were used for fingerprinting of 9 samples of Carissa *carandas*. Of these 9 primers, the amplification of only 8 primers (4, 5, 6, 7, HB10, HB12, HB13, and HB15) was clear and reproducible. Results of the ISSR fingerprint showed 473 amplified fragments; 134 of them were polymorphic (28.33%). The results showed that low polymorphism levels were detected by the ISSR primers for C.carandas allowing it to adapt environmental variations and narrow genetic baseline data for conservation and collection strategies for this species. The variability of different characters among different accessions of *C. carandas* in different locations could be used for plantation and making value-added products. Therefore, dendrogram could be partially explained genetic relationships among the plant species of *C. carandas L.*

ACKNOWLEDGEMENT: Gauhati University: Department of Biotechnology, Government of India, is duly acknowledged for financial assistance and for supporting the work.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Alvarez A, JL Fuentes, Puldon V, Gomez PZ, Mora L, Duque MC, Gallego G and Tohme JM: Genetic diversity analysis of Cuban traditional rice (Oryza sativa L.) varieties, based on microsatellite markers. Genetics Newsletter 2017; 30(4): 1109-17.
2. Anonymous: Wealth of India: Raw materials, Vol III, New Delhi, Council of Scientific and Industrial Research 2018; 294-96.
3. Bhaskar VH and Balakrishnan N: Analgesic, anti-inflamatory and antipyretic activities of *Pergularia deamiaand Carissa carandas.* DARU Journal of Pharmaceutical Science 2019; 17(3): 168-74.
4. Christopoulos MV, Rouskas D, Tsantili E and Bebeli PJ: Germplasm diversity and genetic relationships among walnut (*Juglan sregia* L.) cultivars and Greek local selections revealed by Inter-Simple Sequence Repeat (ISSR) markers. Scientia Horticulturae 2017; 125(4): 584-92.
5. Devmurari V, Shivanand P, Goyani MB, Vaghani S and Jivani NP: A review: Carissa Congesta: Phytochemical constituents, traditional use and pharmacological properties. Pharmacognosy Reviews 2019; 3(6): 375-77.
6. Dhawan BN and Patnaik GK: Investigation on some new cardio-active glycosides. Indian Drugs 2017; 22(6): 285-90.
7. Fang DQ, Roose ML, Krueger RR and Federici CT: Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs and inter-simple sequence repeat markers. Theor Appl Genet 2017; 95: 211-19.

8. Gherardi M, Mangin B, Goffinet B, Bonnet D and Huguet T: A method to measure genetic distance between allogamous populations of alfalfa (*M. sativa*) using RAPD molecular marker. Thr Applied Genetics 2017; 98: 406-12.
9. Glew, RS, Dorothy, J, Chuang, LT, Huang, YS, Millson, M and Glew RH: Nutrient content of four edible wild plants from West Africa. Plant Foods for Human Nutrition 2018; 60: 187-93.
10. Ingale AG: *In-vitro* regeneration of Aloevera (*A. barbadensis*). Bioinfolet 2018; 3: 93-94.
11. Itankar PR, Lokhande SJ, Verma PR, Arora SK, Sahu RA and Patil AT: Antidiabetic potential of unripe *Carissa carandas* Linn. fruit extract. Journal of Ethnopharmacology 2011; 135(2): 430-33.
12. Jabbarzadeh Z, Khosh-khui M, Salehi H and Saberivand A: Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. African Journal of Biotechnology 2012; 9(37): 6091-95.
13. Jaccard P: Nouvelles Recherchessur la distribution florale. Bulletin Soc Vaud Sci Nat 2017; 44: 223-70.
14. Jayaweera DMA: Medical Plants used in Ceylon, Colombo. The Nat Science Council of Sril 2018; 42-49.
15. Joglekar SN and Gaitonde BB: Histamine releasing activity of *Carissa carandasroots* (Apocynaceae). Japanese of J. of Pharmacol 2017; 20: 367.
16. Britto JDA and Sujin RM: A study on genetic variability among five cotton cultivars. Indian Journal of Botanical Research 2018; 4(1): 29-32.
17. Hegde K, Thakker SP, Joshi AB, Shastry CS and Chandrasekhar KS: Anticonvulsant activity of Carissa carandasLinn. root extract in experimental mice. Tropical Journal of Pharmaceutical Research 2019; 8(2): 117-25.
18. Kirtikar KR and Basu BD: Indian Medicinal Plants. Edition 2nd Allahabad 2012; 775-77.
19. Kirtikar KR and Basu BD: Indian Medicinal Plants. Allahabad 2013; 1546-49.
20. Kirtikar KR and Basu BD: Indian Medicinal Plant, Vol I, 2nd Ed, Dehradun. Inter Book Distributors 2015; 1546-48.
21. Kojima T, Nagaoka T, Noda N and Ogihara Y: Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theor Appl Genet 2020; 96: 37-45.
22. Langridge P and Chalmers KJ: The principle: Identification and application of molecular markers. In: Lörz, H and Wenzel, G eds. Biotechnology in Agriculture and Forestry, 55. Molecular Marker Systems in Plant Breeding and Crop Improvement 2014; 1: 13-22.
23. Leung WTW: Food composition table for use in Africa. FAO and US Department of Health. Education and Welfare Rome and Washington 1968.
24. Meena AK, Bansal P and Kumar S: Plants-herbal wealth as apotential source of ayurvedic drugs and herbal wealth as apotential source of ayurvedic drugs. Asian J Tradit Med 2019; 4: 4.
25. Balakrishnan N and Bhaskar VH: Karaunda (*Carissa carandas* Linn.)- As a phytomedicine: A review. Kongposh Publications 2019; 95.
26. Meghwal PR, Singh SK, Singh A and Pathak R: Characterization of Karonda (*Carissa carandas*) accessions under arid region. Journal of Applied Horticulture 2014; 16(2): 157-60.
27. Pakrashi SC, Datta S and Ghosh PP: Indian medicinal plants XVII. Phytochemical examination of Carissa SPP. Phytochemistry 2012; 7(3): 495-6.
28. Pushpangadan P: Karanda: Rural India's rich fruit. Down to earth; Thoms Press India. New Delhi 2017; 12: 52-53.
29. Rahmatullah M, Noman A, Hossan MS, Harun-Or-Rashid M, Rahman T, Chowdhury MH and Jahan RA: Survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. Am Eur J Sustain Agric 2019; 3: 862-76.
30. Rajasekaran A, Jeyasudh V, Kalpana B and Jayakar B: Preliminary Phytochemical and antipyretic evaluation of Carissa carandas L. Ind J of Nat Prod 2019; 15(1): 27-29.
31. Rohlf FJ: NTSYS pc: Numerical Taxonomy and Multivariate analysis system Version 2.02e. Exeter software, New York 1998.
32. Jain SK: Ethno-botany in India: some thoughts on future work. Ethno-botany, 2010; 22: 01 -04.
33. Sambrook and Russell: Molecular Cloning: A Laboratory Manual. Third Edi. CSHL Press 2012; 78-85.
34. Sharma A, Reddy GD, Kaushik A and Shanker K: Natural Product Sciences 2017; 13(1): 6-10.
35. Sharma A, Tiwari RK, Kaushik A and Tyagi LK: Continental J of Pharmaceutical Sciences 2017; 1: 9-14.
36. Shaw RK, Acharya L and Mukherjee AK: Assessment of genetic diversity in highly valuable medicinal plant *Catharanthus roseus* using molecular markers. Crop Breed Appl Biotech 2019; 9: 52-59.
37. Sherwin WB and Moritz C: Managing and monitoring genetic erosion. In: Youn AG. Clarke GM. (Eds.). Genetics. Deomography and viability of fragmented populations. Cambridge University Press. Cambridge 2020; 9-34.
38. Sneath PHA and Sokal RR: Numerical Taxonomy. The Principles and Practice of Numerical Classification. WF Freeman & Co., San Francisco, 1973; 573.
39. Sundriyal M and Sundriyal RC: Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. Economic Botany 2012; 55: 377-90.
40. Taylor RSL, Hudson JB, Manandhar NP and Tower GHN: Antiviral Activities of Medicinal Plant of Southern Nepal. Journal of Ethno pharmacolog 2016; 53(2): 97-104.
41. Tharachand C, Selvaraj IC and Mythili MN: Molecular markers in characterization of medicinal plants: An overview. Research in Plant Biology 2019; 12.
42. Vohra MM and De NN: Comparative cardiotonic activity of *Carissa carandas* L. and *Carissa spinarum* A. Indian J Med Res 1963; 51: 937-40.
43. Waugh R and Powell W: Using RAPD markers for crop improvement. Tibtech 2019; 10: 186-01.
44. Zaki A, El- Tohamy S and El-Fattah S: Study of Lipid content and volatile oil of the different organs of *Carissa carnadas* Linn. And *Carissa grandiflora* Dc. growing in Egypt. Egyptian J of Pharma Sci 2020; 22(1-4): 127-41.
45. Zietkiewicz E, Rafalski A and Labuda D: Genome fingerprinting by simple sequence repeat (SSR)-anchored polymorphism chain reaction amplification. Genomics 2014; 20(2): 176-83.

How to cite this article:
Saikia D and Handique PJ: Evaluation of the genetic divergence of various accessions of the species *Carissa carandas* using inter simple sequence repeat primers. Int J Pharm Sci & Res 2021; 12(1): 2306-16. doi: 10.13040/IJPSR.0975-8232.12(1).2306-16.