International Journal of Technical Research and Applications e-ISSN: 2320-8163,
www.ijtra.com Volume 3, Issue 2 (Mar-Apr 2015), PP. 56-61

MORPHO-PHYSIOLOGICAL AND GENETIC VARIATION OF THE ALGAE COLLECTED FROM DIFFERENT WATER BODIES OF VARANASI AND CHANDAULI REGION USING ISSR MOLECULAR MARKER

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Abstracts: In general algae shows ability to survive in wide range of environmental conditions. Under natural condition, they usually grow in the mixed community. Many algae have a special adaptibility to form net in water bodies and on surfaces were water falls continuously. This communication deals with the dynamics of the net forming and non net forming algal species from different water bodies. Total 16 samples of algae collected from Varanasi and Chandauli district, and their species varification done, of them 14 species were declared, as two similar species were sampled. Out of them 6 were mat forming and 8 non mat forming species. Sampling done from two different types of water habitat i.e., stagnant and running. pH of the water bodies varied from 7-10. ISSR marker based diversity studied for 11 algal species which include 6 mat forming and 5 non mat forming. Total 97 bands amplified from 12 ISSR primers of them 77 bands were polymorphic. Number of amplified band varies from 6 to 11 with an average 8.08 band from each primer. PIC ranged from 0.407 to 0.654 and there mean is 0.536. Jaccord's similarity coefficient varied from 0.60 to 0.88 with mean value 0.74. Algal species grouped in two major groupes. This study provides information for future exploration and collection of different algal species in Uttar Pradesh, India, and needs more research to find out industrial application of mat forming algal species.

Key words: Diversity, ISSR markers, Algae, Mat formation

I. INTRODUCTION

Algae are one of the most useful natural resources that can be used to produce different bioactive compounds such as vitamins, proteins, unsaturated fatty acids, antioxidants and carotenoids etc. [9]. Algae most commonly occur in fresh water, marine or brackish, they can also be found in almost every other environment on earth, from algae growing in the snow to those living as lichens on bare rocks, to desert soils, to those in hot springs. Some algae are predominantly terrestrial occurring in soil. Most of the algae in streams and rivers are those that are able to attach themselves to a stable bottom substrate. The algae that are common in smaller streams and rivers are attached diatoms, blue green algae and green algae (particularly Spirogyra and Cladophora) [19]. Algae grow in different shapes like long strands or chains of cells called as filamentous. Some forms of filamentous algae grow in a submerged mat over the pond bottom, especially in shallow areas. Filamentous algae mats are more common in ponds during the spring, but they can persist throughout the year. There are a number of species of mat-forming algae, and some types are more easily controlled than others. Algal filaments may feel slimy, cottony or like steel wool, depending on the species. Dark-colored mats can result from a type of algae called Lyngbya. Mat-forming algae are major constituents of the aquatic weed flora. Excessive growths of these macrophytic algae, which occur alone or in association with vascular plants, can limit recreational activities, restrict culture and harvest activities in fish culture ponds, reduce flow and clog intakes in water conveyance systems, and reduce habitat diversity [11] Common, mat-forming green algae are Hydrodictyon, Oedogonium, Pithophora, Rhizoclonium and Spirogyra. Most of the algal species are not harmful except those produced toxins. Morphological traits observed through the light microscope have been traditionally used to determine the species and the diversity of algae, many species have complex life cycle with different morphological stages affected by environmental conditions. The morphology alone is not able to recognize strains which have various shapes in diverse environmental conditions and the cryptic species (due to recent speciation) with similar morphological traits however they are different genetically [6]. Molecular and genetic characters are affected less than the morphological characters by environmental conditions, hence they are more stable [13]. In addition to the necessity of the morphological study, there is a need to the molecular study of organisms in order to differentiate them geographically [4]. The combination of molecular and morphology provide a robust way to determine organisms with lower mistakes. Most of the molecular marker tools are valuable methods to investigate population genetic and diversity which were developed quickly over the three past decades [2]. ISSR markers are reliable, highly polymorphic, low cost and less laborious, need only a small amount of DNA and are very fast when compared to most other molecular markers [20]. Although the reproducibility of RAPD technique is low and is dominant [3]. ISSR do not require DNA sequence data and in terms of reproducibility, ISSR is comparable to SSR [5]. Therefore, this study was conducted for the collection and identification of different mat forming and non mat forming algae and their correlation with habitat along with molecular diversity among mat forming algae and between mat forming and non mat forming algae.

II. MATERIALS AND METHODS

A. Field Sampling

Total 16 samples were collected from different locations of Varanasi and Chandauli district of Uttar Pradesh, India. The samples were brought to the laboratory, washed under running tap water and preserved in 4% formaldehyde solution. Mounts of these samples were prepared microscopic observation done to identify the algal species, following the keys given by [14]. From the 16 samples total 14 different algae species identified as two similar species collected from two different location (Table. 1).

A. ph Determination

pH of the water from where algae sample collected measured by using pH meter (EUTECH instruments OAKTON), 5 samples from each site were taken into 50 ml tube to measure the pH.

B. Dna Isolation

Genomic DNA of eleven algae sample which include 6 mat forming and 5 non mat forming algae was extracted by CTAB method [7] with minor modifications (Table. 2). Quality and quantity of each DNA sample were confirmed by agarose gel electrophoresis and through spectrophotometer respectively. Concentration of each DNA sample was adjusted to 40 ng/µl through dilution.

C. Pcr Amplification

A total of 12 ISSR markers were selected, synthesized and used for DNA amplification from 11 DNA samples of different algae species. The PCR reaction mixture (25 µl) consisted of 2.5 µl 10 × buffer, 0.75 µl MgCl2 (25 mM), 0.5 µl dNTP (25 mM), Taq DNA polymerase 0.5 µl (3 U/µl), 18.75 µl ddH2O, 1 µl primers (10 pm) and 1 µl (40 ng) genomic DNA. All the reagents were procured from MBI Fermentas, USA. The amplification was carried out in a thermal cycler (Dyad, Bio-rad, USA) at initial denaturing step at 94℃ for 4 min. followed by 36 cycles of 94℃ for 1 min., 55–60℃ for 1 min. and 72℃ for 2 min. In the last cycle, primer extension was performed at 72℃ for 05 min. and storage at 4℃ till electrophoresis. The amplification products were separated in 2.5% agarose gel, and after electrophoresis the products were visualized in a gel documentation system (Alfa Imager 2200, Alfa Innotech Corporation, California). The 1000-bp DNA ladder (Fermentas) was used as molecular size marker (Fig. 1).

D. Analysis

The genomic DNA fragments from ISSRs generated clear and unambiguous bands of various molecular weight sizes were scored for the presence (1) and absence (0) of the corresponding band among the samples in the form of binary matrix and the data matrix was subjected to further analysis using NTSYSpc version 2.11W [17]. The SIMQUAL program was used to calculate the Jaccard's coefficient [10]. The similarity matrix was computed using ISSR markers based on Jaccard's coefficient following the UPGMA methods using SHAN programme of NTYSYSpc to estimate similarity indices and genetic relatedness among and within net forming and non mat forming algae. The similarity index (SI) values were computed as a ratio of number of similar bands to the total number of bands in pair wise comparison of the accessions. A dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) cluster algorithm. Polymorphic information content (PIC) of each ISSR markers was calculated as per the formula: $PICi = 1 - \sum Pij2$. Where, PICi is the polymorphic information content of a marker i; Pij is the frequency of the jth pattern for marker i and the summation extends over n patterns.

III. RESULTS

A. Relation Between Algal Mats And Ph

Algal presence increases the alkalinity of the water. The increase in pH could be due to either increase of carbonates and increased photosynthetic activities of producers. Increase in acidity of water initially causes general increase in filamentous algae. However, high level of water acidity due to pollution by acid forming chemicals results in decrease algal growth in water body. Most algae disappear completely in water below pH 5.8. In our collection most of the algae belong to stagnant water, while in industrial areas algae species was low in number. Variation of pH with mat formation shows that pH of the water sample was increases constantly as the volume of mat increases. This was observed at Chakia agricultural field where at same place, 3 types of mat were present. It was observed that in agricultural areas where water was highly alkaline and pH ranged between 9-10.5, thick mats of Hydrodictyon and Spirogyra were observed. At one place where mat was in les amount the pH of water was in the range of 7-7.5. In the middle, net formation was comparatively more than the previous one and pH was about 8.5. Whereas in areas like Ram Nagar Industrial areas, the algae were present in very scarce amount, pH was neutral to less basic, which showed that less amount of algae could not alter the pH of water much. The analysis of the composition and growth pattern of algal flora in a water body can be used to identify the type and the level of water pollution.

B. Species Composition And Nature Of Habitat

Habitat of the collected 14 species observed, of them most found in stagnant water while few in running water, one species Pithophora were found on running water on wall. Out of 14 species collected 6 found as mat forming algae while remaining were not able to form mat. As listed in (Table 1), sample collection covers varied location which include agricultural farm, Ghats of Ganges, drainage of Varanasi city falling into Ganges, cement and ply factories, Lakhania dari and Madhuban BHU. Appearance of each algal sample is as listed in (Table 1). Although it is clear from our observation algal species showed varied type of living habitat which makes them adaptive to their surrounding environments.

C. Characterization Of Issr Loci

A total of 97 amplification products were obtained from 12 ISSR primers, out of this 77 (79.4%) amplification products were polymorphic while remaining 20 amplification product was monomorphic. The mean number of alleles per primer was 8.08, and the size of amplified products ranged from 300bp to 2400bp. The average PIC value was estimated to be 0.536, and in terms of PIC values, UBC-809 (0.654), UBC-811 (0.599) and UBC-850 (0.587) were recorded to be most informative. Primer UBC-841 recorded the least PIC value of 0.407 (Table 3). Pair-wise

comparison was performed among all the accessions. Out of 12 ISSR primers two primers UBC-808 and UBC-809 which gives 6 and 7 amplification products respectively, shows 100% polymorphic bands, while lowest polymorphic amplification products obtained from UBC-836 with polymorphic amplification products 62.50%, average 80.17% polymorphic amplification products were obtained from each ISSR primers.

D. Cluster Analysis Based On Issr Markers

Jaccord's similarity coefficients calculated from ISSR data varied from 0.60 to 0.88 with a mean value of 0.74. The highest similarity coefficient (0.88) was observed between species Chaotophora and species Chlorella, whereas, it was the lowest between species Xanthidium and species Pithophora. The dendrogram based on Jaccord's similarity coefficient revealed that 11 species were grouped into two major clusters consisting of 08 and 03 species, respectively (Fig 2). Cluster I could be further sub-divided into three sub-clusters; IA (6 species), IB (1 species) and IC (1 species). The species Xanthidium and species Pithophora were separated singly in sub group IB and IC at similarity coefficient 0.63 and 0.69, respectively. Major group 2 consists of 3 algal species. The dendrogram showed a close similarity among species Chaotophora and species Chlorella. The species Spirogyra were separated from species Ulotherix as they fall either side of the dendrogram, indicating a high genetic diversity among these two species. Subgroup 1A consists of 6 algal species which include Spirogyra, Hydrodictyon, Zygnema, Scenedesmus, Scytonema and Cladophora, algal species included in this subgroup are all mat forming except Cladophora. Subgroup 1B include species Xanthidium and subgroup 1C having species Pithophora which is the second species of major group 1 which is non mat forming.

IV. DISCUSSION

Banaras (also known as Kashi) is one of the most ancient, religious and visited cities in Uttar Pradesh. The river Ganges is a major river of Uttar Pradesh catering to the needs of agriculture and human consumption. Although very few report on collection of different algal species from water bodies. In our experiment we have collected 14 algal species from different location of Varanasi and Chandauli district of Uttar Pradesh, it has been observed that the different water bodies shows availability of different types of algal species, this may be due to better adaptability of particular algal species in individual areas. It was observed in Ram Nagar Industrial area that the effluents discharged from a polythene factory had very little algal growth. Species of Chlorella were present on the dead leaves. As chlorella is an indicator of water pollution, therefore presence of Chlorella in the drains throws light upon the being polluted. In the front of cement industry, the cement kiln was being discharged into the sewage. The cement kiln has got many toxic materials which do not allow algae to grow abundantly, Xanthidium species was found and that to in patches. In industrial areas, the patchy presence of algae may be due to high CO2 content, it becomes a limiting factor in photosynthesis which limits the growth of algae. The frequency of occurrence of mats in agricultural areas was more than those found in industrial areas. Spirogyra was the most common mat forming algae. Agricultural farms were dominated by species like Hydrodictyon, Anabaena, Aulosira, Nostoc, Zygnema etc. Aulosira was present as flakes.

In eutrophicated lakes, the number of algae is more but they do not form mat. In eutrophicated lakes the BOD level increases, eutrophicated lakes also have high NO3 and phosphate content which discourages algal mat formation. Mat formation helps in the survival of Spirogyra. Spirogyra wherever presents forms thick mats. At waterfalls where water is falling continuously on rocks, Spirogyra was found attached with rocks forming thick mats. In industrial areas, places where normal water was falling on the wall, Spirogyra formed thick mats despite the prevailing stress conditions. At Assi Ghats Spirogyra was present in thick mats where water was falling continuously on damp walls. This shows that even little amount of moisture also favors algal growth. The water bodies where thick mats are present, water flow slows due to restriction created by algal mats. Algal presence increases the alkalinity of the water. The increase in pH could be due to either increase of carbonates and increased photosynthetic activities of producers.

The polymorphic information content of the markers ranged from 0.407 (UBC-841) to 0.654 (UBC-809) with an average of 0.536. Overall, this study revealed high genetic diversity (8.08 alleles per primer), which ranges from 6 to 11 alleles. Possibly this is due to species variation of algae. The previous reports of [16] reported an average of 8 markers per primer in Cajanus cajan. Maciel et al. Such a high variation in the number of fragments produced by these primers may be attributed to the differences in the binding site throughout the genome of the algal species. Ajibade et al. [1], reported on the generation of the ISSR fragments ranging from 4 to 12 markers in Vigna unguiculata and 8 markers in Phaseolus vulgaris [8]. This indicates that the ISSR marker is applicable in assessing molecular relativity among and within non mat forming and mat forming algal species.

The dendrogram constructed using the UPGMA method based on Jacard's similarity matrix (Fig 1), showed the genetic variation among and within mat forming and non mat forming algal species studied, this is in accordance to an earlier report in which Alexandrium minutum species showed high genetic diversity using the RAPD technique, even between strains from same bloom [12]. ISSR-PCR is a quick, produce sufficient polymorphism and reliable fingerprint method to distinguish potato cultivars [15]. The regression and association between the genetic diversity and geographical locations were visible in many cases [18].

In this experiment it is clear that the grouping of mat forming and non mat forming species is moreover distinct as in major group 1 which include 8 algal species, 6 species are mat forming. Out of them 6 species grouped in subgroup 1A, five species are mat forming except one. This indicated molecular dissimilarity between mat forming and non mat forming algal species based on ISSR markers. Only two species i.e. Xanthidium and Cladophora which is in major group 1 is non mat forming. The major group 2 doesn't have any subgroup includes 3 algal species, all of them are non mat forming algal species, in this group two non mat forming species Chaotophora and Chlorella shows very close similarity as they separates together in the dendrogram (Fig 1).

V. CONCLUDING REMARKS

Species living in a common area or near to each other have genetic relationships and, which explains the UPGMA results illustrated by the dendrogram clusters of species according to their geographical location. The massive genetic diversity of the species provides scientists with good opportunities to find new bioactive compounds. Although morphological characteristics are useful for the detection of species and genetic diversity, they depend on environmental conditions and vary under diverse conditions and they are not precise enough to detect the strains and populations, hence the use of the ISSR have proven to be more powerful methods to distinguish mat forming and non mat forming algal species. So we can states that the ISSR markers used in this experiment may be useful in identification of mat forming and non mat forming algal species.

VI. ACKNOWLEDGEMENTS

WAA is thankful to UGC for providing financial support in the form of MANF-JRF.

REFERENCES

[1] Ajibade SR, Weeden NF, Michite S (2000) Inter simple sequence repeat analysis of genetic relationships in the genus Vigna. Euphytica 111: 47–55

[2] Arif I, Bakir M, Khan H, Al-Farhan A, Al Homaidan A, Bahkali A, Al Sadoon M, Shobrak MA (2010) Brief Review of Molecular Techniques to Assess Plant Diversity. Int J Mol Sci 11: 2079–2096

[3] Bardakci F (2001) Random Amplified Polymorphic DNA (RAPD) Markers. Turk J Bio l25: 185–196

[4] Bittencourt-Oliveira MC, Massola JNS, Hernandez-Marine M, Romo S, Moura AN (2007) Taxonomic investigation using DNA fingerprinting in Geitlerinema species (Oscillatoriales, Cyanobacteria). Phycol Res 55:214–221

[5] Bornet B, Branchard M (2001) Non anchored Inter Simple Sequence Repeat (ISSR) Markers: Reproducible and Specific Tools for Genome Fingerprinting. Plant Mol Biol Rep 19:209–215

[6] Cianciola E, Popolizio T, Schneider C, Lane C (2010) Using Molecular-Assisted Alpha Taxonomy to Better Understand Red Algal Biodiversity in Bermuda. Diversity 2:946–958

[7] Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

[8] Galvan MZ, Bornet B, Balatti PA, Branchard M (2003) Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (Phaseolus vulgaris L.). Euphytica 132:297–301

[9] Hughes DA (1999) Effects of dietary antioxidants on the immune function of middle-aged adults. Proc Nutr Soc 58:79–84

[10] Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44:223--270

[11] Lembi CASW, Neal O, Spencer DF, (1988) Algae as weeds: economic impact, ecology, and management alternatives. In: Lembi, C.A., and J. R. Waaland, J.R. (eds). Algae and Human Affairs. Cambridge University Press, 455-481

[12] Martinez R, Anibarro C, Fernandez S (2006) Genetic variability among Alexandrium tamarense and Alexandrium minutum strains studied by RAPD banding pattern analysis. Harmful Algae 5:599–607

[13] Park Y, Lee JK, Kim N (2009) Simple Sequence Repeat Polymorphisms (SSRPs) for Evaluation of Molecular Diversity and Germplasm Classification of Minor Crops. Molecules 14:4546–4569

[14] Prescott GW (1951) Algae of the Western great lakes area. WMC Brown Publishers, Dubuque, Iowa

[15] Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98:107–112

[16] Ratnaparkhe MB, Gupta VS, Venmurthy MR, Ranjekar PK (1995) Genetic fingerprinting of pigeon pea (Cajanus cajan (L.) Millsp.) and its wild relatives using random amplified polymorphic DNA markers. Theor Appl Gen 91:893–898

[17] Rohlf FJ (1998) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.02. Exeter Software, New York, USA

[18] Singh R, Zaki NM, Ting N, Rosli R, Soon-Guan T, Leslie LE, Ithnin M, Cheah S (2008) Exploiting an oil palm EST database for the development of gene-derived SSR markers and their exploitation for assessment of genetic diversity. Biologia 63:227–235

[19] Thiamdao S (2011) Diversity of Edible Freshwater Macroalgae. Thailand, (Ph.D. Dissertation. Chiang Mai University)

[20] Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genetics 20:176–183.

Figure Captions:

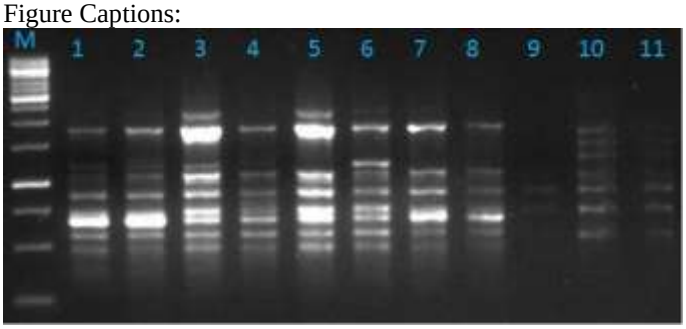

Fig. 1: Amplification with UBC-811 marker, M indicates 1 kb ladder, 1-11 is algal sample as per listed in Table 2.

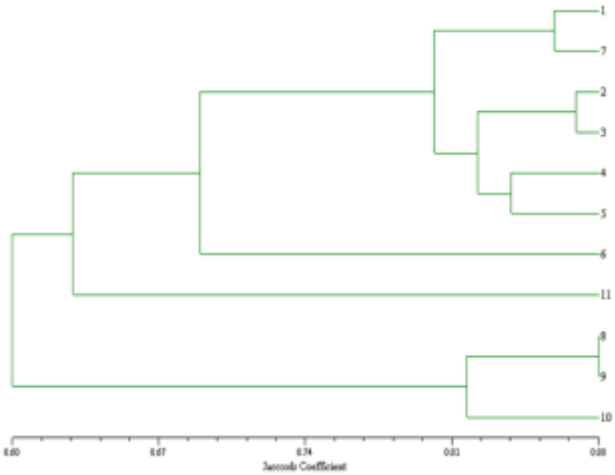

Fig. 2: UPGMA dendrogram of 11 species of mat forming and non mat forming algae species based on the 12 ISSR primers. Groups IB and IC each contains a single species. Numbers are species as per listed in Table 2.

S.No	Name of Algae	Location	Water	Mat formation
1	*Spirogyra*	Agricultural farm, Varanasi, and Lakhaniya Dari, Chandauli	Stagnant water	Yes
2	*Anabaena*	Agricultural farm , Varanasi	Stagnant water	No
3	*Aulosira*	Agricultural farm , Varanasi	Stagnant water	No
4	*Nostoc*	Agricultural farm, Varanasi	Stagnant water	No
5	*Hydrodictyon*	Agricultural area, Chakia (Chandauli) and Ravidas Ghat, Varanasi	Stagnant water	Yes
6	*Zygnema*	Agricultural area, outer Chakia (Chandauli)	Stagnant water	Yes
7	*Chaotophora*	Ravidas Ghat, Varanasi	Floating water, contaminated	No
8	*Chlorella*	Ram Nagar ply factory, Varanasi	Stagnant water, contaminated	No
9	*Xanthidium*	Ram Nagar cement factory, Varanasi	Stagnant water, contaminated	No
10	*Scenedesmus*	Pipeline of Ravidas Ghat, Varanasi	Continuously falling fresh water	Yes
11	*Scytonema*	Ravidas Park drain, Varanasi	Stagnant water	Yes
12	*Ulotherix*	Assi Ghat, Varanasi	Running water, contaminated	No
13	*Cladophora*	Madhuban BHU, Varanasi	Stagnant water	No
14	*Pithophora*	Ram Nagar plastic factory, Varanasi	Falling water on wall.	Yes

Table 1: Name of algae species, location of their collection, nature of their water habitat and mat forming nature, of 14 algal species collected from 16 different locations of Varanasi and Chandauli district of Uttar Pradesh, India.

	Name of Algae	Mat formation
1	*Spirogyra*	Yes
2	*Hydrodictyon*	Yes
3	*Zygnema*	Yes
4	*Scenedesmus*	Yes
5	*Scytonema*	Yes
6	*Xanthidium*	No
7	*Cladophora*	No
8	*Chaotophora*	No
9	*Chlorella*	No
10	*Ulotherix*	No
11	*Pithophora*	Yes

Table 2: List of algae species which has been taken for DNA isolation and ISSR based PCR Amplification for molecular diversity study of mat forming and non mat forming algae species.

S. No.	ISSR Primer	Nucleotide sequence (5'……3')	Tm (ºC)	PIC	Alleles	% polymorphic alleles
1	UBC-807	AGAGAGAGAGAGAGAGT	55.0	0.495	6	66.66
2	UBC-808	AGAGAGAGAGAGAGAGC	56.8	0.551	6	100.00
3	UBC-809	AGAGAGAGAGAGAGAGG	58.0	0.654	7	100.00
4	UBC-810	GAGAGAGAGAGAGAGAT	55.0	0.476	7	85.71
5	UBC-811	GAGAGAGAGAGAGAGAC	52.0	0.599	10	80.00
6	UBC-812	GAGAGAGAGAGAGAGAA	50.9	0.594	11	72.72
7	UBC-836	AGAGAGAGAGAGAGACYA	55.0	0.418	8	62.50
8	UBC-840	GAGAGAGAGAGAGAGAY	58.0	0.531	9	77.78
9	UBC-841	GAGAGAGAGAGAGAGACTC	58.0	0.407	9	66.67
10	UBC-842	GAGAGAGAGAGAGAGAYG	59.1	0.540	9	77.78
11	UBC-850	GTGTGTGTGTGTGTGTCTC	60.0	0.587	6	83.33
12	UBC-853	TCTCTCTCTCTCTCTCRT	55.0	0.584	9	88.89
			Mean	0.536	8.08	80.17

Table 3: ISSR primers ID, sequence information, annealing temperature, polymorphic information content (PIC), number of alleles and % of polymorphic alleles, in this study are presented.