International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 2529-2534

Received: 16-09-2019
Accepted: 18-10-2019

Lalit Prasad Singh Rajput
Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India

Keerti Tantwai
Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India

Sajjan Kumar Pooniya
Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India

Koji Tsuji
Faculty of Education, Chiba University, Yayoi, Inage, Chiba, Japan

Corresponding Author:
Keerti Tantwai
Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India

Genetic diversity among the landraces of kodo millet (*Paspalum scrobiculatum* L.) using ISSR markers

Lalit Prasad Singh Rajput, Keerti Tantwai, Sajjan Kumar Pooniya and Koji Tsuji

Abstract
Kodo millet (*Paspalum scrobiculatum* L.) is having high nutritional value, dietary fiber, antioxidant activity as well as drought tolerance characteristics. Present study was conducted for assessing genetic diversity using ISSR (Inter Simple Sequence Repeat) markers among a total of 42 landraces of kodo millet collected from five districts of Madhya Pradesh (India) viz. Rewa, Betul, Chhindwara, Dindori, and Jabalpur. Ten ISSR markers amplified a total of 63 loci while 56 loci showed 88.88% polymorphism. Average number of bands per primer was found to be 6.3 whereas, average number of polymorphic bands per primer was 5.6. Maximum number of alleles (09) was scored by the primer UBC-886 followed by 08 by UBC-807 whereas; minimum number of alleles (04) was scored by the primers UBC-812 and UBC-816. Cluster analysis was done and a dendrogram was generated using UPGMA (Weighted Pair Group Method with Arithmetic Mean). The highest PIC (Polymorphism Information Content) value of 0.58 was observed by primer UBC-884 revealing 07 alleles among 42 accessions. Percentage of the number of polymorphic loci within population among the three regions, the highest frequency of polymorphism was found in the Dindori region (69.84 ± 22.22). The highest genetic diversity was observed in Dindori region three other in region.

Keywords: Kodo millet, *Paspalum scrobiculatum*, landraces, genetic diversity, genetic resources, upgma, phylogenetic tree, molecular marker, ISSR, polymorphism

Introduction
Kodo millet' (*Paspalum scrobiculatum* L.) is a tropical millets crops which is primeval to India (de Wet *et al*. 1983) [5]. Kodo millet is widely distributed in damp habitats across the tropics and subtropics of the world and referred to as black rice or bird's grass in Africa. It was domesticated roughly 3000 years ago in India the only country today where kodo grain is harvested in significant quantities, mainly on the Deccan plateau. The grain contains a diverse range of high-quality protein (Geervani & Eggum 1989, Kulkarni & Naik 2000) [6, 12] and has high anti-oxidant activity (anti-cancer) even when compared to other millets (Hegde & Chandra 2005, Hegde *et al*. 2005, Chandrasekara & Shahidi 2011) [7, 8, 4]. Kodo millet like other millets is rich in macro and micro nutrient contents. It has higher protein content (8.3/100 g grain) as compared to rice, finger millet, barnyard millet and little millet. Its riboflavin content (0.10 mg/100 g grain) is also higher than rice and barnyard millet. It is also rich in magnesium (166 mg/100 g) and is considered as one of the nutritious millets (Muthamilarasan *et al*. 2014). Kodo mill *et al*so has considerable production potential in marginal, low fertility soils and chronic moisture deficient areas of the country and plays an important role for the food security of the people inhabiting dry and marginal lands. It is drought tolerant and can be grown in a variety of poor soil types from gravelly to clay (de Wet *et al*. 1983, M'Ribu & Hilu 1996) [5, 17]. Kodo millet has been reported to have higher free radical quenching potential when compared to other millets (Hegde & Chandra 2005) [7]. It provides low priced protein, minerals and vitamins in the form of sustainable food (Yadava & Jain 2006) [23]. Genetic diversity is a tool for effective plant breeding in a crop because the selection procedure of parental genotype in any plant breeding programme fundamentally depends on the nature and extent of genetic variability present in the gene pool. Genetic diversity in a crop gene pool can be estimated with morphological, biochemical, or DNA markers, among which DNA based molecular markers, are most preferred owing to their rapid and authentic results.

DNA based molecular markers utilized in characterizing diversity and phylogeny in kodo millet are few, like RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeat), binding with One Finger), SCoT (Start Codon Targeted Polymorphism) and SRAP (Sequence-Related Amplified Polymorphism) (M'Ribu & Hilu 1996, Kayande 2014, Kushwaha *et al*. 2015, Yadav *et al*. 2016, Singh 2016) [17, 11, 14, 22]. ISSR markers provide a powerful, rapid, simple, reproducible and inexpensive means to assess genetic diversity among closely related cultivars, characterization of accessions and identification of cultivar and varieties (Kumar *et al*. 2006) [13]. Inter-simple sequence repeats (ISSR) marker is found the best choice as it requires no information about the species. ISSRs are arbitrary multi loci markers produced by PCR amplification with a microsatellite primer. Further, such amplification does not require genome sequence information and leads to multi-locus and highly polymorphous pattern. ISSR markers amplify regions between adjacent and inversely oriented microsatellites using di-, tri-, tetra- and penta-nucleotide repeats. ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Mei *et al*. 2015) [16]. In present study, the genetic diversity among landraces of kodo millet was assessed using ISSR markers. The information generated from the present work will provide an estimate of the range of overall genetic variation of kodo millet existing in Indian state of Madhya Pradesh for designing suitable strategies for collection and conservation of germplasm for its enhancement and utilization in breeding programmes.

Materials and Methods

Plant materials - Forty two landraces of kodo millet were collected from five different geographical regions of Madhya Pradesh. Plants were grown in poly-house and fresh young leaf samples were collected for isolation of genomic DNA (Table-1).

DNA Extraction

Genomic DNA was isolated from young leaves of kodo millet using CTAB Protocol (Saghai-Maroof *et al*. 1984) [20] with some modifications. Chemical used for the extraction of DNA were 100mM Tris-HCl (pH 8.0), 20mM EDTA (pH 8.0), 0.5M NaCl, 2% CTAB (Cetyl Trimethyl-Ammonium Bromide), 0.2% β-mercaptoethanol, 2.5% PVP (Polyvinylpyrrolidone), 24:1 Chloroform-isoamyl alcohol (IAA), 3M sodium acetate (pH 4.8), Isopropanol (-20ºC), 70% ethanol, 5M NaCl. DNA quality was tested by agarose gel (0.8%) electrophoresis and visualized under UV light.

PCR Analysis

PCR reaction was prepared as described in Table-2. PCR amplification reactions carried out in volume of 20µl consisting 2µl of PCR buffer 1X, 2.4µl of MgCl2 2.5mM, 0.2µl of Taq Polymerase 5 U/µl, 0.5µl of dNTPs10mM, 2µl of Primer 10pM, 2µl of genomic DNA 50ng and nuclease free water was used to make up the total volume to 20µl. Amplifications were performed using "BIORAD T100 and Agilent Technologies Sure Cycler 8800" programmable thermal cycler with the cycling parameters that was programmed for ISSR with an initial denaturation step at 94 °C for 4 min followed by 45 cycles of denaturation at 94 °C for 45 second, annealing at 50 °C for 1 min and elongation at 72 °C for 2 min. In the final cycle, the elongation step at 72 °C was extended by 5 min.

Statistical Data Analysis

PCR product using ISSR primers were scored on the agarose gel as presence (1) or absence (0) of bands of molecular weight size in the form of binary matrix. The frequency of a null allele at a given locus was estimated by taking the square root of the frequency of null homozygosity (the absence of a band), which assumes that there are two alleles at a locus under Hardy-Weinberg equilibrium. Based on the estimated frequency of a null allele, frequency of heterozygosity (*H*) within population (*Hs*) and all individuals *(Ht*) were calculated. The genetic differentiation among population *(Gst)* was calculate as *(Ht- average Hs)/Ht* (Nei 1973). Polymorphism information content (PIC) values were calculated for each ISSR primer according to the formula: PIC = 1 - R (P_{ij}) 2, (Botstrin *et al*. 1980) [3]. Phylogenetic tree and PCA was constructed using Darwin software (Dissimilarly Analysis and Representation Window).

Results

In present study a total of 10 ISSR primers were amplified successfully and were selected on the basis of sharp and clear banding pattern for final ISSR analysis. The sequences of these ISSR primers are represented in (Table-3). Using 10 ISSR markers, a total of 63 loci were amplified (Table-4). Maximum numbers of allele (09) were scored by the primers UBC-886 followed by 08 number of alleles using UBC-807. Whereas minimum numbers of allele (04) were scored by the primers UBC-812 and UBC-816. Out of 63 amplified alleles, 56 were found to be polymorphic (88.88%). Average number of band per primer was 6.3 whereas the average number of polymorphic band per primer was 5.6. During present investigation, Polymorphism Information Content (PIC) was estimated for each of the 10 ISSR markers. Higher value of PIC score indicated higher polymorphism of the ISSR markers and therefore helped in selecting the best ISSR marker in phylogenetic analysis. The Highest PIC value (0.58) was observed for UBC-884 which has 07 alleles among the 42 accessions. Markers UBC807, UBC-825 and UBC-841 also had high PIC scores with high number of alleles. Lowest PIC value (0.41) was obtained from UBC-816. Percentage of the number of polymorphic loci within population among the three regions, the highest frequency of polymorphism was found in the Dindori region (69.84 ± 22.22), followed by the Chhindwara region (31.74 ± 26.98) and the lowest were in the Rewa region (26.98 ± 23.80) and Betul region (23.80 ± 15.87) (Table-6). Polymorphism was also detected within each region. The results also showed that the Dindori region had the highest *Hs* among the four regions (0.29), while the *Hs* of the Rewa region was 0.20 and the Betul region was 0.16 and Chhindwara region was 0.26. Ht was 0.30 and *Gst* on these four geographic regions was 0.23. Percentage of the number of polymorphic loci within region was the highest in the Dindori region (87.30%, n=28), second was in the Chhindwara region (53.96%, n=4), and the third was Rewa region (52.38%, n=6), lowest was in Betul region (36.50%, n=4) (Table-5). A dendrogram was generated using Unweighted Pair Group Analysis (UPGMA) in constructed using Darwin software (Dissimilarly Analysis and Representation Window) programme (Fig-1). Phylogenetic relationships the Dindori region formed a genetically distinct group based on their genetic distance from the individuals in

the Chhindwara, Betul, and Rewa region. The highest genetic diversity was observed in Dindori region.

Discussion

ISSR technique is a PCR based method which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. ISSR-PCR gives multi locus patterns which are very reproducible, abundant and polymorphic in plant genomes (Bornet & Branchard 2004) [2]. The major advantage of this method is its universality and ease of development (no need of sequence data) in addition to the reproducibility (Agostini *et al*. 2008, Jabbarzadeh *et al*. 2010) [1, 10] and low cost of the technique (Weising *et al*. 2005, Li *et al*. 2011) [21, 15]. 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 (Jabbarzadeh *et al*. 2010) [10]. Frequency of heterozygosity within region (*Hs*) was the highest found in Dindori region (0.29), and followed by Chhindwara region (0.26) lower value of *Hs* in the Betul region (0.16) and further lower value in Rewa region (0.20). The frequencies of each marker allele with greater numbers of alleles tend to have higher PIC values and thus are more informative (Hildebrand *et al*. 1994). The percentage of polymorphic loci within region was higher in the Dindori region (87.30, n=28) and lower in Betul (36.50, n=4). The *Hs* value of population higher in 0.29 and lower in Betul 0.16. This study also observed that some population of kodo millets were collected from the different geographical location. The results found that multiplicity of factors including the geographical location were responsible for the selection of diverse plant population. Phylogenetic relationship among population Dindori population were genetically distinct all other population Rewa, Chhindwara, and Betul. This study suggested Dindori region was different geographical and climate condition other region. However, in contrast to the expectations, all the landraces from one particular region do not grouped together. Similar observation was reported by Yadav *et al*. (2016) [22] that "Structure based analysis placed all the accessions into four sub-groups not strictly according to their geographical locations". Genetic diversity is very important for designing conservation strategies for threatened and endangered species and planning for crop improvement programmes. Higher level of genetic diversity observed among kodo millet using ISSR markers in the present investigation is supported by the results of Yadav *et al*. (2016) [22] who observed that the Neutral-ISSR markers were showing higher values as compared to functional markers (SCoT and SRAP) for the genetic relationship divers cultivar.

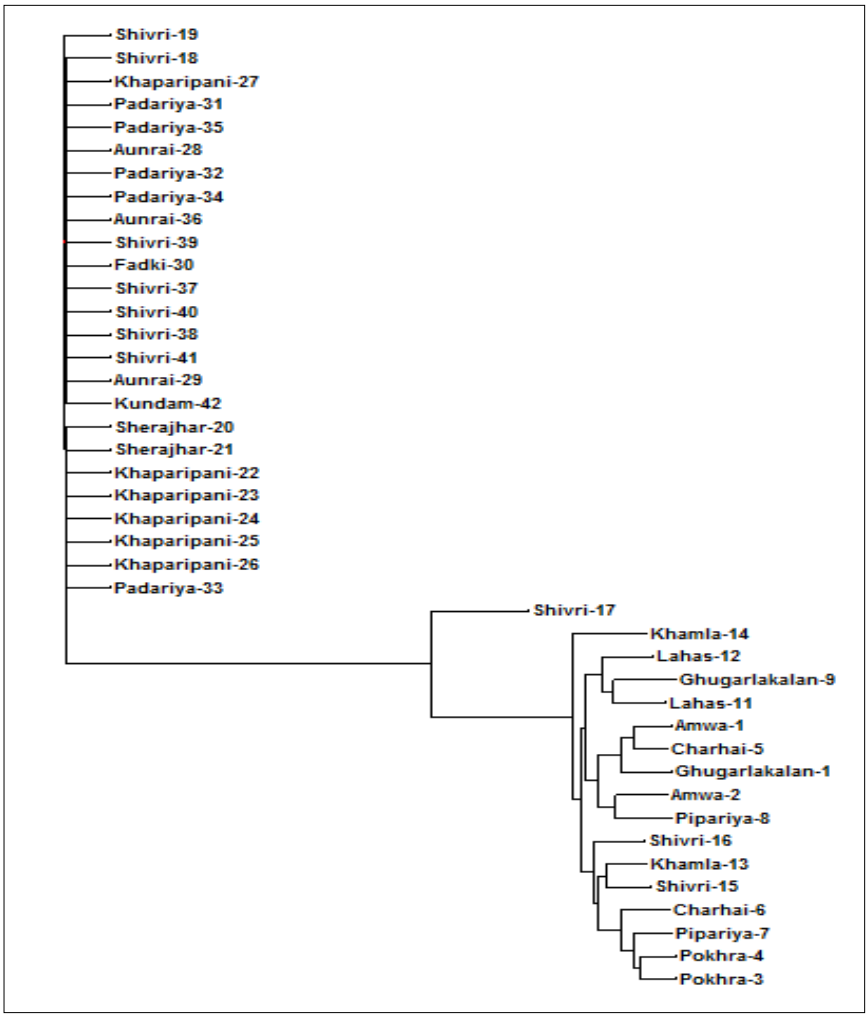

Fig 1: Phylogenetic tree construction using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis among 42 landraces of kodo millet.

Table 1: List of collected landraces of kodo millet

S. N.	Name of accessions	Collection site	Geographical location		District
			Latitude	Longitude	
1	REWKOD20171125-3	Amwa-1	N 34, 48, 19	E 82, 21, 54	Rewa
2	REWKOD20171125-8	Amwa-2			
3	REWKOD20171126-1	Pokhra-3	N 34, 48, 19	E 82, 21, 54	
4	REWKOD20171126-3	Pokhra -4			
5	REWKOD20171126-4	Charhai -5	N 34, 48, 19	E 82, 21, 54	
6	REWKOD20171126-6	Charhai -6			
7	CHHKOD20171127-1	Pipariya -7	N 22, 3, 26	E 78, 56, 17	Chhindwara
8	CHHKOD20171127-3	Pipariya -8			
9	CHHKOD20171127-8	Ghugarlakalan-9	N 22, 3, 26	E 78, 56, 17	
10	CHHKOD20171127-9	Ghugarlakalan-10			
11	BETKOD20171128-1	Lahas-11	N 21, 54, 4	E 77, 53, 45	Betul
12	BETKOD20171128-3	Lahas-12			
13	BETKOD20171128-7	Khamla-13	N 21, 54, 4	E 77, 53, 45	
14	BETKOD20171128-8	Khamla-14			
15	DINKOD20160830-1	Shivri -15	N 22, 50, 35	E 81, 14, 57	Dindori
16	DINKOD20160830-2	Shivri -16			
17	DINKOD20160830-3	Shivri -17			
18	DINKOD20160830-4	Shivri -18			
19	DINKOD20160830-5	Shivri -19			
20	DINKOD20160830-6	Sherajhar-20	N 22, 35, 17	E 81, 19, 16	Dindori
21	DINKOD20160830-7	Sherajhar-21			
22	DINKOD20160830-8	Khaparipani-22	N 22, 39, 20	E81, 16, 41	Dindori
23	DINKOD20160830-9	Khaparipani-23			
24	DINKOD20160830-10	Khaparipani-24			
25	DINKOD20160830-11	Khaparipani-25			
26	DINKOD20160830-12	Khaparipani-26			
27	DINKOD20160830-13	Khaparipani-27			
28	DINKOD20180219-1	Aunrai-28	N 22, 56, 36	E 81, 4, 37	Dindori
29	DINKOD20180219-2	Aunrai-29			
30	DINKOD20180219-3	Fadki-30	N 22, 59, 59	E 80, 57, 28	Dindori
31	DINKOD20180219-4	Padariya-31	N 22, 55, 17	E 80, 54, 4	Dindori
32	DINKOD20180219-5	Padariya-32			
33	DINKOD20180219-6	Padariya-33			
34	DINKOD20180219-7	Padariya-34			
35	DINKOD20180219-8	Padariya-35			
36	DINKOD20180315-1	Aunrai-36	N 22, 56, 36	E 81, 4, 37	Dindori
37	DINKOD20180315-2	Shivri-37	N 22, 50, 35	E 81, 14, 57	Dindori
38	DINKOD20180315-3	Shivri-38			
39	DINKOD20180315-5	Shivri-39			
40	DINKOD20180315-6	Shivri-40			
41	DINKOD20180315-7	Shivri-41			
42	JABKOD20180315-9	Kundam-42	N 23, 13, 7	E 80, 21, 3	Jabalpur

Table 2: PCR components with their concentrations used for PCR reaction

Sl. No.	Components	Concentrations	Volume
1.	10X PCR buffer	1X	2.0µl
2.	25mM $MgCl_2$	2.5mM	2.4µl
3.	10mM dNTPs	200µM	0.5µl
4.	Primer	10pM	2.0µl
5.	*Tag* Polymerase (5 Unit/µl)	1 unit	0.2µl
6.	Nuclease free H_2O	For makeup the volume	10.9µl
7.	DNA	50ng	2.0µl

Table 3: List of ISSR primers and their sequences

SN	Primer Code	Primer Sequence 5'-3'
1	UBC-834	5'-AGAGAGAGAGAGAGAGYT-3'
2	UBC-807	5'-AGAGAGAGAGAGAGAGT-3'
3	UBC-841	5'-GAGAGAGAGAGAGAGAC-3'
4	UBC- 853	5'-TCT CTC TCT CTC TCT CRT-3'
5	UBC-845	5'-CTC TCT CTC TCT CTC TRG-3'
6	UBC-812	5'-GAG AGA GAG AGA GAG AA-3'
7	UBC-816	5'-CAC ACA CAC ACA CAC AT-3'
8	UBC-825	5'-ACA CAC ACA CAC ACA CT-3'
9	UBC-884	5'-HBH AGA GAG AGA GAG AG-3'
10	UBC-886	5'-VDV CTC TCT CTC TCT CT-3'

Table 4: Populations used in this study and polymorphic loci within population.

SN	Region	Province	latitude	longitude	No. of individuals	No. of polymorphic loci within population	% of polymorphic loci within population
1	Rewa	Amwa	N 34, 48, 19	E 82, 21, 54	2	15	23.80
2	Rewa	Pokhra	N 34, 48, 19	E 82, 21, 54	2	17	26.98
3	Rewa	Charhai	N 34, 48, 19	E 82, 21, 54	2	11	17.46
4	Chhindwara	Pipariya	N 22, 3, 26	E 78, 56, 17	2	17	26.98
5	Chhindwara	Ghugarlakalan	N 22, 3, 26	E 78, 56, 17	2	20	31.74
6	Betul	Lahas	N 21, 54, 4	E 77, 53, 45	2	15	23.80
7	Betul	Khamla	N 21, 54, 4	E 77, 53, 45	2	10	15.87
8	Dindori	Shivri	N 22, 50, 35	E 81, 14, 57	5	33	52.38
9	Dindori	Sherajhar	N 22, 35, 17	E 81, 19, 16	2	14	22.22
10	Dindori	Khaparipani-	N 22, 39, 20	E81, 16, 41	6	28	44.44
11	Dindori	Aunrai	N 22, 56, 36	E 81, 4, 37	8	44	69.84
12	Dindori	Shivri	N 22, 50, 35	E 81, 14, 57	7	34	53.96

Table 5: Genetic diversity within each region

SN	Region	No. of population	Number of individuals	Number of polymorphic loci within region	% of polymorphic loci within region	*Hs* or *Ht*	*Gst*
1	Rewa	3	6	33	52.38	0.20	0.23
2	Chhindwara	2	4	34	53.96	0.26	
3	Betul	2	4	23	36.50	0.16	
4	Dindori	5	28	55	87.30	0.29	
5	All	12	42	56	88.88	0.30	

Table 6: Primer combinations and number of detected loci using ISSR markers among 42 landraces of kodo millet

SN	Primer	No. of total loci	No. of Polymorphic loci	% of polymorphic loci	Polymorphism Information Content (PIC)
1	UBC-834	6	4	66.66	0.46
2	UBC-807	8	7	87.50	0.50
3	UBC-841	6	5	83.33	0.53
4	UBC-853	7	6	85.71	0.49
5	UBC-872	6	6	100	0.51
6	UBC-812	4	3	75	0.39
7	UBC-816	4	3	75	0.41
8	UBC-825	6	6	100	0.55
9	UBC-884	7	7	100	0.58
10	UBC-886	9	9	100	0.52

Conclusion

The genetic relationship among 42 landraces of kodo millet was analysed using ISSR markers which proved to be effective and reliable tool for analysis. Genetically diverse landraces identified in present research. These findings not only highlighted the capacity of the ISSR technique for genetic diversity assessment but also help in the selection of diverse kodo millet landraces and further genetic improvement by tagging the desirable traits or for broadening the genetic base of Indian kodo millet.

Acknowledgement

This work was supported by JSPS KAKENHI Grant Number JP16K00907

References

1. Agostini G, Echeverrigaray S, Souza-Chies TT. Genetic relationships among South American species of Cunila D. Royen ex L. based on ISSR. Plant Systematics and Evolution. 2008; 274(34):135-141.
2. Bornet B, Branchard M. Use of ISSR fingerprints to detect microsatellites and genetic diversity in several related *Brassica taxa* and *Arabidopsis thaliana*. Hereditas. 2004; 140(3):245-247.
3. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment Length Polymorphisms. American Journal of Human Genetics. 1980; 32(3):314-331.
4. Chandrasekara A, Shahidi F. Determination of antioxidant activity in free and hydrolysed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. Journal of Functional Foods. 2011; 3(3):144-158.
5. De Wet JM, Brink DE, Rao KP, Mengesha MH. Diversity in kodo millet, *Paspalum scrobiculatum*. Economic Botany. 1983; 37(2):159-163.
6. Geervani P, Eggum BO. Nutrient composition and protein quality of minor millets. Plant Foods for Human Nutrition. 1989; 39(2):201-208.
7. Hegde PS, Chandra TS. ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. Food Chemistry. 2005; 92(1):177-182.
8. Hegde PS, Rajasekaran NS, Chandra TS. Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. Nutrition Research. 2005; 25(12):1109-1120.
9. Hildebrand CE, David C, Torney C, Wagner P. Informativeness of polymorphic DNA markers. Los Alamos Science. 1994; 20:100-102.
10. Jabbarzadeh Z, Khosh-Khui M, Salehi H, Saberivand A. Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. African Journal of Biotechnology. 2010; 9(37):6091-6095.

11. Kayande KD. Studies on Genetic diversity of kodo millet (*Paspalum scrobiculatum* L.) using Random Amplified Polymorphic DNA markers. M.Sc. Thesis JNKVV Jabalpur, 2014, 34.
12. Kulkarni LR, Naik RK. Nutritive value, protein quality and organoleptic quality of kodo millet (*Paspalum scrobiculatum*). Karnataka Journal of Agricultural Sciences. 2000; 13(1):125-129.
13. Kumar A, Arya L, Kumar V, Sharma S. Inter simple sequence repeat (ISSR) analysis of cytoplasmic male sterile, male fertile lines and hybrids of pearlmillet [*Pennisetum glaucum* (L.) R. Br.]. Indian Journal of Crop Science. 2006; 1(1, 2):117-119.
14. Kushwaha H, Jillo KW, Singh VK, Kumar A, Yadav D. Assessment of genetic diversity among cereals and millets based on PCR amplification using Dof (DNA binding with One Finger) transcription factor gene-specific primers. Plant Systematics and Evolution. 2015; 301(2):833-840.
15. Li S, Li J, Yang XL, Cheng Z, Zhang WJ. Genetic diversity and differentiation of cultivated ginseng (*Panax ginseng* CA Meyer) populations in North-east China revealed by inter-simple sequence repeat (ISSR) markers. Genetic Resources and Crop Evolution. 2011; 58(6):815-824.
16. Mei Z, Zhang C, Khan A, Zhu Y, Tania M, Luo P, Fu J. Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in *Angelica sinensis* (Oliv.) Diels varieties of China. Electronic Journal of Biotechnology. 2015; 18(2):96-102.
17. M'Ribu HK, Hilu KW. Application of random amplified polymorphic DNA to study genetic diversity in *Paspalum scrobiculatum* L. (Kodo millet, Poaceae). Genetic Resources and Crop Evolution. 1996; 43(3):203-210.
18. Muthamilarasan M, Venkata Suresh B, Pandey G, Kumari K, Parida SK, Prasad M. Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in foxtail millet. DNA research. 2013; 21(1):41-52.
19. Rohlf FJ. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0 user guide. Exeter Publication, Setauket, New York, 1998.
20. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences. 1984; 81(24):8014-8018.
21. Weising K, Nybom H, Pfenninger M, Wolff K, Kahl G. Applications of DNA fingerprinting in plants: principles, methods, and applications. CRC Press Boca Raton, London, New York, Singapore, 2005, 472.
22. Yadav Y, Lavanya GR, Pandey S, Verma M, Ram C, Arya L. Neutral and functional marker based genetic diversity in kodo millet (*Paspalum scrobiculatum* L.). Acta physiologiae plantarum. 2016; 38(3):75.
23. Yadava HS, Jain AK. Advances in kodo millet research. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi, 2006.