The Journal of Agricultural Science

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Molecular profiling of foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region for genetic variability and nutritional quality

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Crops and Soils Research Paper

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Cite this article: Trivedi AK, Arya L, Verma SK, Tyagi RK, Hemantaranjan A, Verma M, Sharma VP, Saha D (2018). Molecular profiling of foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region for genetic variability and nutritional quality. *The Journal of Agricultural Science* **156**, 333–341. https://doi.org/10.1017/S0021859618000382

Received: 21 October 2017
Revised: 3 March 2018
Accepted: 27 April 2018
First published online: 28 May 2018

Key words:
Amino acid; carbohydrate; dietary fibre; foxtail millet; SSR marker

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Abstract

Agriculture in the Central Himalayan Region depends on the availability of suitable germplasm as well as natural conditions. Due to extreme weather conditions, food and nutrition security is a major issue for communities inhabiting these remote and inaccessible areas. Millets are common crops grown in these areas. Foxtail millet (*Setaria italica* (L.) P. Beauv) is an important crop and forms a considerable part of the diet in this region. The aim of the present study was to explore, collect, conserve and evaluate the untapped genetic diversity of foxtail millet at the molecular level and discover variability in their nutritional traits. A total of 30 accessions having unique traits of agronomic importance were collected and molecular profiling was performed. A total of 63 alleles were generated with an average of 2.52 alleles per locus and average expected heterozygosity of 0.37 ± 0.231. Significant genetic variability was revealed through the genetic differentiation (Fst) and gene flow (Nm) values. Structure-based analysis divided whole germplasm into three sub-groups. Rich variability was found in nutritional traits such as dietary fibre in husked grains, carbohydrate, protein, lysine and thiamine content. The collected germplasm may be useful for developing nutritionally rich and agronomically beneficial varieties of foxtail millet and also designing strategies for utilization of unexploited genetic diversity for food and nutrition security in this and other similar agro-ecological regions.

Introduction

Since pre-historic times, food plants have been domesticated, selected, exchanged and improved by farmers in traditional ways within traditional production systems. This process has also been utilized for scientific crop improvement, which led to the Green Revolution and a significant rise in crop yields. Globally, approximately half of the increase in food production can be attributed to genetic improvement. Millions of lives depend upon the extent to which crop genetic improvement can keep pace with the growing global population, changing the climate and shrinking environmental resources (Ronald, 2011). Plant geneticists, as well as plant breeders, consider molecular marker-assisted selection (MAS) a useful additional tool in crop improvement/breeding programmes to optimize selection efficiency (Dwivedi *et al.*, 2007; Xu and Crouch, 2008). Recently, the amount of molecular genetic markers for relevant plant breeding traits has increased (Lammerts van Bueren *et al.*, 2010). Without the knowledge of linkage to genes for specific traits of interest (for the plant breeder), molecular markers can still be used to determine the genetic relatedness between two different individual plants or the genetic diversity within a gene pool (Lammerts van Bueren *et al.*, 2010). Although increasing, use of molecular markers is still modest in plant breeding. A major reason for this is the lack of appropriate markers with high selective value for many traits of interest to breeders (Tuvesson *et al.*, 2009). Particularly, those quantitative traits which depend largely on environmental factors, GEI (genotype–environment interactions), are integrated into QTL (quantitative trait loci) analysis, resulting in markers reflecting the amount and direction of the reaction of the plant to an environmental input. Such markers are valuable over a larger range of environments (Backes and Østergard, 2008). Several genes contributing to one trait can be pyramided in one genotype using linked markers. Hence, molecular profiling would be useful particularly in the utilization of untapped genetic diversity. Crop diversity is gradually shrinking in most parts of the world, with most of mankind living off a few plant species and the human diet composed mainly of a few major crops such as wheat, rice, maize and

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potatoes. However, crop diversity with known traits can provide the basis for offering higher yielding and more reliable plants, which can support low-income farmers and consumers. It can also deliver security to the poor by making harvests more resilient to environmental changes.

Millet, which is one of the oldest foods known to man and possibly the first cereal grain used for domestic purposes, is grown in the Central Himalayan Region (CHR). Foxtail millet (*Setaria italica* (L.) P. Beauv) is an important annual crop of the genus *Setaria*, family Gramineae. It is a self-pollinated crop (Leonard and Martin, 1963) where cross-pollination averages about 0.04 (Li *et al.*, 1935). Foxtail millet is not just a cereal of the Old World, it is also used widely in Africa, the Americas, Australia and Eurasia (Lin *et al.*, 2012). It is a good source of dietary fibre and certain amino acids such as lysine and thiamine, which are otherwise low/deficient in cereal food. Dietary fibres found in carbohydrates aid digestion by moving food quickly through the intestines. A fibre-rich diet helps to decrease constipation and other digestive problems, lower blood cholesterol and reduce the risk of heart disease (Jones, 2001; Jones *et al.*, 2006). In human nutrition, lysine is an essential amino acid involved in the creation of collagen and absorption of calcium. It prevents cold sores (caused by the virus called herpes simplex labialis). A deficiency of lysine can lead to anaemia, bloodshot eyes and fatigue (Sahley and Birkner, 2000). Another amino acid analysed in seeds, thiamine, is an essential nutrient for normal body function (Lonsdale, 1990). Its deficiency may result in damage to the nervous system as well as to the heart and other muscles, which are the symptoms of beriberi disease (Lee, 1994). Thiamine is important due to its role as a coenzyme for reactions catalysed by enzymes; thiamine is required for mitochondrial oxidative decarboxylation (Sica, 2007), the pentose phosphate pathway and the citric acid cycle (Wooley, 2008). Due to a lack of stable and improved varieties, foxtail millet production is unstable and the market is chaotic. Therefore, there is an urgent need to develop varieties with stable and higher yield to stabilize the fluctuation of the production (Li *et al.*, 2014). In this context, evaluation of untapped genetic diversity of foxtail millet for nutritional traits is very important because this will enable the development of nutritionally rich varieties, particularly for resource-poor and malnourished regions, using the existing genetic diversity of crops.

With a relatively small genome (515 Mb), foxtail millet is a suitable plant for molecular and genetic research (Wang *et al.*, 1998). Foxtail millet (*S. italica*) exhibits numerous properties (for instance, C_4 photosynthesis) that make it an ideal model for functional genomic studies in the Panicoid grasses (Diao *et al.*, 2014). With a high-quality reference genome sequence (Bennetzen *et al.*, 2012) and a high-density haplotype map of genome variation (Jia *et al.*, 2013) and other genomic data (Kumari *et al.*, 2013; Pandey *et al.*, 2013; Suresh *et al.*, 2013; Muthamilarasan *et al.*, 2014; Zhang *et al.*, 2014; Yadav *et al.*, 2015), this species can now truly be considered as a novel model system for genetic and genomic studies in other cereal and millet crops (Doust *et al.*, 2009; Li and Brutnell, 2011; Lata *et al.*, 2013; Muthamilarasan and Prasad, 2015) to develop new varieties suitable for different regions.

Without irrigation and application of fertilizers, foxtail millet can survive and produce economic yield comparable with other crops of similar lifespan cultivated with optimal inputs. Thus, collection and nutritional evaluation of untapped genetic diversity might open some new avenues of research and breeding. Therefore, in the present study, an effort has been made to assess the extent of variability in 30 accessions of foxtail millet collected from the CHR using molecular markers and nutritional quality traits in order to identify specific germplasm for crop improvement.

Materials and methods

Plant materials and seed quality analysis

Plant exploration and germplasm collection expeditions were conducted in the CHR of India, i.e. Uttarakhand State, which is a large geographical area known for vagaries of weather. A total of 30 accessions (including four controls used for comparison) having unique traits of agronomic importance were collected from areas with different climatic conditions as well as different altitudes in this region and were evaluated for nutritional quality and genetic variability for three consecutive years (2011–2013) at one site located at 29°24′N, 79°30′E, 1480 m a.s.l. Four accessions, IC337300, IC355776, IC418394 and IC469880, which are preferred by the farming community across the region and occupy the largest area, were used as controls for comparison. Total dietary fibre content was determined using the AOAC method 2001.03 (AOAC 2005). Total carbohydrate content was estimated by the anthrone reagent method (Morris, 1948), while protein content was determined by the folin ciocalteau reagent method (Lowry *et al.*, 1951) and fat content by AOAC method 996.01 (AOAC 1998). Estimation of lysine and thiamine amino acid content was achieved following the methods of Hurrell and Carpenter (1981) and Chen *et al.* (1999), respectively. All data are given on a dry weight basis.

Simple sequence repeat markers for molecular evaluation

The DNA was extracted and purified from seeds using mini CTAB (cetyl trimethylammonium bromide) method (Saghai-Maroof *et al.*, 1984) and a working solution of 20 ng/µl was prepared for simple sequence repeat (SSR) amplification. These accessions were profiled with genome-wide SSR markers (Zhang *et al.*, 2014). Genomic SSR markers were selected based on their high polymorphism information content (PIC) value (0.7 or more) and represented different chromosomes. All of the 25 SSR loci (Table 1) were run at one touchdown cycle, whereas earlier different annealing temperatures were used for polymerase chain reaction (PCR) amplification (Zhang *et al.*, 2014). The PCR was carried out using 15 µl reaction mixture that included 5.68 µl H_2O, 1.5 µl of 10× buffer, 1.2 µl of 25 mM $MgCl_2$, 0.3 µl of 10 mM dNTPs mix, 0.6 µl of 10 mM forward and reverse primer each and 5 µl of 20 ng/µl DNA. All reagents were from MBI fermentas (St. Leon-Rot, Germany). The PCR was performed using touchdown cycle: initial denaturation at 94 °C for 3 min, then ten cycles of denaturation at 94 °C for 30 s, touchdown annealing starting at 62 °C for 30 s and decreasing 0.7 °C per cycle and extension at 72 °C for 1 min followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at temperature 55 °C for 30 s and primer extension at 72 °C for 1 min with a final extension step at 72 °C for 4 min, 3% metaphor agarose gel was used for separating the SSR amplification products and photographed using a SYNGENEG-Box Gel Documentation unit (Syngenta, Cambridge, UK).

Statistical analysis

Experiments were conducted for three consecutive years (2011–2013) in a randomized block design (RBD) with three replications. Data for each parameter were analysed for statistical

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

Table 1. Sequence of the simple sequence repeat (SSR) primer pairs used for molecular profiling of foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region

SSR loci	Forward sequence	Reverse sequence
SICAAS2017	F-GGCGCCATGGGTGGATGCGGAATTGTA	R-TCAGTTGTCTCCCTCGCCGCTTCAGGA
SICAAS3010	F-TGCGTGTGGCGATGTGAGATGGA	R-ACCATCGGTTTCGTGGCTTGGC
SICAAS3052	F-TGGAGGACTTGGACACCTCTCTCTCTC	R-GCAAACCTGCTTTCAGCTATCGACCCA
SICAAS8028	F-GTTGCAGTTTCCATGCAGTTAG	R-TCGAGAACATCTTGTGAGCATC
SICAAS9030	F-GCAAGCAACAGTCCATGGCCGA	R-GCTGCGAGCTTGCGTGACAGA
SICAAS7038	F-GCGTTAATGGGCTGGAATTATG	R-TCATGTACGTCGCTAATCGTGA
SICAAS5049	F-GGTTTCTGCTTCTCCTCCGCTCCA	R-AGGACACGGAGACCCTGCTGAACT
SICAAS5020	F-CCAACAAAACAATGCCCTGGGTAAGCA	R-CGTGAGCGCCCTTTAGAGGAGGGTA
SICAAS9054	F-GGGATTTGGGTGGGGACGGC	R-CGCGCCACGCCTCTGTAACT
SICAAS9067	F-GCTAGGCGGCTTCCCAAGGC	R-GCCGCCATCTCCTCCCCGTA
SICAAS7024	F-TGGTCGACGTTCTCCACTCTTA	R-CCTTGTGCGATTTTATCCCTTC
SICAAS4010	F-ACTCCTCCAGCCAACCAACCAACCAAG	R-TCCCTCCCCCTGTTCTTCCTTTCAGCG
SICAAS5013	F-GCTGGTGCAGCAGATGCTACGTTC	R-GGATTTCCTTTGTCACCCTGCCCGC
SICAAS4024	F-TATGGGCCTCGCTTATGGGCCCAACCA	R-ATACTGGGCCTCCGGCAGCATCGAA
SICAAS4037	F-TGATGGGCTTGATTGGGACGGCTCG	R-TCCCTTTTGTCGCACGCACGCTGA
SICAAS5081	F-ACCACAAATGCTGGCCTTAC	R-GGTTGTACCGATCCGAGAGA
SICAAS5034	F-GTGTCCTCGCTCTCCACCAAGACTC	R-ACGAGACGTCAAATCCTGCAAATGGC
SICAAS6101	F-CATGGTGCCTTGCATTTAGA	R-TGCAGTTCAGTGAGACATACAAAAC
SICAAS6035	F-AATACCACACAAGCATCAGGAG	R-GGCGATGGAGTGCATTTTATTA
SICAAS9095	F-GGCCCCTGGTAGTAACCTTTTA	R-AAGATGAACGATCCCAAGTACG
SICAAS3056	F-TGCGCAGGCAGCTTGTTGTTGT	R-AATCGGATTTCCCTCCGCCCGT
SICAAS4061	F-CTGTTATGTGATGCTGCCAAT	R-AATGACTCCAAAATGCACAGC
SICAAS4027	F-ACAACGCCACAGCCAAAGCCTCCCTCT	R-TGTGCGAGCCAGGCCAAACACAAGGCT
SICAAS5005	F-TCTGCCATCAGGCCAAGTCACAGT	R-CGGCACCCCCATTTTCTTGAGCCA
SICAAS6055	F-GGCCCACCCAATATAACAAAC	R-ACAAAGTGATGTGCTCCATGA

significance using two-way analysis of variance (ANOVA) to compare the means considering accession and trait as independent variables.

Alleles were scored and the presence or absence of alleles was converted to '1' and '0', respectively, for data analysis. The software program NTSYS-PC ver 2.1 (Rohlf, 2000) was used to calculate Jaccard's similarity coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis. Nei's gene diversity statistics were calculated using POPGENE version 1.32 (Yeh *et al.*, 1999). The PIC was calculated using the formula $1-\sum p_{ij}^2$ (Anderson *et al.*, 1993), where p_{ij} is the frequency of *j*th allele for *i*th SSR locus. Software STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) was run using a burn-in of 1 00 000, a run length of 1 00 000 (admixture model) and number of populations was inferred using Structure Harvester (Earl and von Holdt, 2012) keeping *K* values (1–10) with five iterations at each *K* value.

Results

Seed quality traits are the main criteria for selection of a food crop to be grown at large scale and commercialized in any region. From the present study, it is evident that substantial variability is available in the dietary fibre, carbohydrate and fat content of the seeds. The fibre content of seeds varied from 4.88% (in IC357343) to 5.95% (in IC338633) whereas carbohydrate content varied from 58.50% in IC337300 to 61.18% in IC406534 (Table 2). In addition, fat content ranged from 3.97% in IC337335 to 5.08% in IC337327. Protein content in seeds was found to vary from 10.03% in IC337300 to 12.29% in IC355800. Amino acid lysine in seeds ranged from 2.31 mg/g (in IC337300 and IC355794) to 2.78 mg/g (in IC383568), while thiamine content was found to vary from 5.46 µg/g (in IC337307) to 5.95 µg/g (in IC338633). Thousand grain weight were found to vary from 1.17 g (in IC338639) to 2.24 g (in IC337318) (Table 2). There is a statistically significant ($P \leqslant 0.05$) correlation between dietary fibre and thiamine content, indicating that accessions rich in dietary fibre will also be rich in thiamine content (Table 3), as well as carbohydrate and lysine content, indicating that accessions rich in carbohydrate will also be rich in lysine content.

A total of 25 genome-wide SSR loci were used for genotyping to investigate genetic variation (Fig. 1). The number of alleles ranged from two to four, with an average of 2.5 ± 0.77 alleles/SSR locus. Furthermore, statistical analysis using POPGENE version

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

Table 2. Variability in the nutritional traits and seed weight of the foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region

Sl. No.	IC No.	Dietary fibre (%)	Carbohydrates (%)	Fat (%)	Protein (%)	Lysine (mg/g)	Thiamine (µg/g)	1000 grain weight (g)
1	**IC337300**	**5.51**	**58.5**	**4.21**	**10.03**	**2.31**	**5.51**	**2.12**
2	**IC355776**	**5.87**	**60.93**	**4.89**	**11.47**	**2.72**	**5.87**	**2.11**
3	**IC418394**	**5.93**	**60.06**	**4.61**	**10.12**	**2.36**	**5.93**	**2.06**
4	**IC469880**	**5.91**	**59.38**	**4.15**	**11.98**	**2.32**	**5.91**	**2.23**
5	IC337303	5.33	58.69	4.79	11.03	2.56	5.53	2.21
6	IC337307	5.46	59.16	4.37	10.51	2.42	5.46	1.87
7	IC337311	5.61	60.2	4.81	11.12	2.36	5.61	2
8	IC337313	5.53	60.31	4.66	11.66	2.53	5.53	2.02
9	IC337318	5.55	60.45	4.08	11.89	2.38	5.55	2.24
10	IC337327	5.71	60.85	5.08	10.12	2.47	5.71	1.83
11	IC337335	5.89	61.11	3.97	10.72	2.62	5.89	1.89
12	IC337338	5.22	59.12	4.24	10.45	2.42	5.78	2.09
13	IC337340	5.81	59.65	4.52	10.95	2.35	5.81	1.45
14	IC338633	5.95	59.93	4.36	10.22	2.52	5.95	2.04
15	IC338639	5.88	59.32	4.68	10.8	2.48	5.88	1.17
16	IC338650	5.72	60.66	4.37	11.55	2.55	5.72	1.79
17	IC340855	5.66	58.77	4.05	12.13	2.39	5.66	1.39
18	IC340963	5.68	59.86	5.06	10.48	2.66	5.68	1.23
19	IC341376	5.82	60.72	4.77	11.14	2.65	5.82	1.96
20	IC341382	5.92	60.88	4.28	10.85	2.59	5.92	1.6
21	IC355794	5.74	60.65	4.73	12.21	2.31	5.74	1.97
22	IC355800	5.76	60.34	4.8	12.29	2.46	5.76	1.32
23	IC357343	4.88	60.08	4.46	10.83	2.56	5.69	1.78
24	IC383467	5.62	60.18	4.82	11.62	2.62	5.86	1.88
25	IC383568	5.94	60.68	4.16	11.78	2.78	5.94	1.69
26	IC383633	5.77	60.42	4.39	11.25	2.71	5.77	2.16
27	IC393056	5.72	60.12	4.57	10.43	2.69	5.72	1.73
28	IC406534	5.88	61.18	4.23	10.65	2.52	5.88	1.72
29	IC436955	5.85	59.23	4.52	10.4	2.61	5.85	1.57
30	IC469863	5.91	58.89	4.07	11.22	2.56	5.90	2.01
	Minimum	**4.88**	**58.50**	**3.97**	**10.03**	**2.31**	**5.46**	**1.17**
	Maximum	**5.95**	**61.18**	**5.08**	**12.29**	**2.78**	**5.95**	**2.24**
	Average	**5.70**	**60.01**	**4.49**	**11.06**	**2.51**	**5.76**	**1.84**
	STDEV	**0.24**	**0.77**	**0.31**	**0.67**	**0.13**	**0.14**	**0.30**
	CV%	**4.24**	**1.29**	**6.92**	**6.06**	**5.32**	**2.50**	**16.32**

1.32 software revealed an average effective number of alleles, Shannon's Information Index, expected heterozygosity, Nei's expected heterozygosity, average heterozygosity of 1.8 ± 0.61, 0.6 ± 0.32, 0.4 ± 0.23, 0.4 ± 0.22 and 0.2 ± 0.11, respectively (Table 4). The observed heterozygosity of the germplasm ranged from 0.000 to 0.414 with an average of 0.06 ± 0.104, while PIC values varied from 0.064 to 0.66 with an average of 0.366. Genetic differentiation (Fst) among germplasm populations was 0.42 and the gene flow (Nm) was 0.34 (Table 4). Allelic data were converted into a 0/1 binary matrix based on the absence or presence of alleles, respectively, and was subjected to UPGMA cluster analysis (based on Jaccard's similarity coefficient matrix (Supplementary Table 1)) to further elucidate the genetic relatedness among different accessions (Fig. 2). The most distant accessions were IC469880 and IC469863 and most similar were IC337313 and IC337318. The Bayesian approach was followed

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

Table 3. Correlation matrix of foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region based on nutritional traits

	Dietary fibre (%)	Carbohydrates (%)	Fat (%)	Protein (%)	Lysine (mg/g)	Thiamine (μg/g)	1000 grain weight (g)
Dietary fibre (%)	1						
Carbohydrates (%)	0.315						
Fat (%)	−0.076	0.181					
Protein (%)	0.073	0.17	−0.089				
Lysine (mg/g)	0.171	**0.381**	0.135	−0.043			
Thiamine (μg/g)	**0.664**	0.327	−0.145	−0.023	0.315		
1000 grain weight (g)	−0.18	0.008	−0.194	0.003	−0.13	−0.161	1

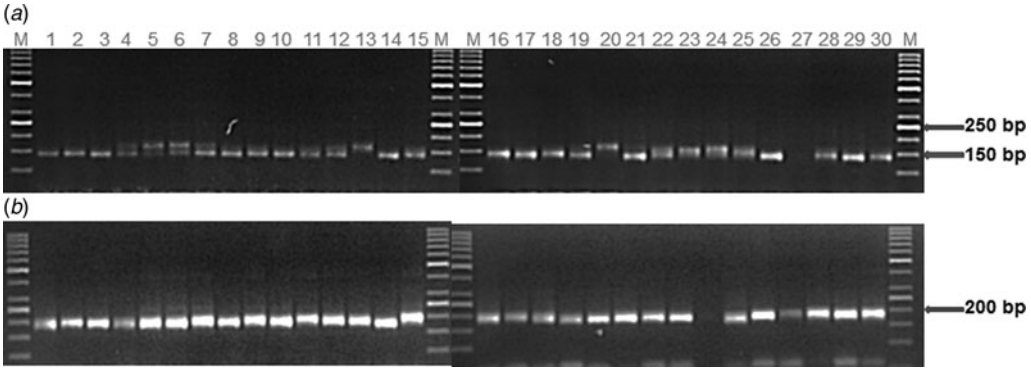

Fig. 1. Gel of foxtail millet accessions with SICAAS5049 (a) and SICAAS5020 (b) SSR loci,1: IC337300, 2: IC337303, 3: IC337307, 4: IC337311, 5: IC337313, 6: IC337318, 7: IC337327, 8: IC337335, 9: IC337338, 10: IC337340, 11: IC338633, 12: IC338639, 13: IC338650, 14: IC340855, 15: IC340963, 16: IC341376, 17: IC341382, 18: IC355776, 19: IC355794, 20: IC355800, 21: IC357343, 22: IC383467, 23: IC383568, 24: IC383633, 25: IC393056, 26: IC406534, 27: IC418394, 28: IC436955, 29: IC469863, 30: IC469880.

to infer population structure using STRUCTURE 2.3.4 software and delta *K* value was calculated using Structure Harvester. The highest delta *K* value was reported at $K = 3$, indicating that all 30 accessions could be grouped into three well-defined subgroups (Fig. 3).

Discussion

Foxtail millet germplasm from the CHR was found to have ample variability in nutritional traits. After achieving food security in different parts of the world, nutritional security is the next focus of global agricultural research. The rich variability in fibre content of millet germplasm found in the present study could be due to the difference in their genetic makeup. Accumulating evidence favours the view that increased intake of dietary fibre has beneficial effects including prevention or alleviation of maladies such as cardiovascular disease, diabetes, diverticulosis and colon cancer (Abdul-Hamid and Luan, 2000). Some of the collected accessions have higher fibre content than all four controls, i.e. widely cultivated accessions, used in the current study. Information on fibre is now the third most sought-after health information in supermarkets in countries such as India, Australia, Western Europe and North America (Mehta, 2005). Hence, accessions high in fibre content might be preferable for breeders and important for a foxtail improvement programme.

Millets are considered as carbohydrates and dietary carbohydrate is essential for gastrointestinal integrity and functioning (Flight, 2006). They supply the body with the energy required for its various activities (Eastwood, 2003) and help in transporting crucial micronutrients. In seeds of any species, carbohydrate content usually remains stable; however, considerable variability in this nutritional constituent makes the germplasm useful for breeding programmes.

Millets, particularly foxtail millet, are low in saturated fat; however, they are a good source of polyunsaturated fats (Akoh and Min, 2007), which help to lower low-density lipoprotein (LDL) cholesterol. In turn, low-LDL cholesterol reduces the risk of heart disease (Chow, 2008), so low-LDL foods such as foxtail millet become an important component of nutritional security (Diniz *et al.*, 2004).

In addition, considerable variability was found in the protein content of seeds. Although cereal grains contain relatively little protein compared with legume seeds, cereals are the most important food crops. They provide over 200 million tonnes of protein for the nutrition of humans and livestock, which is about three times the amount derived from the more protein-rich legume seeds (Shewry and Halford, 2002). In addition to their nutritional importance, cereal seed proteins also influence the utilization of the seed in food processing. The storage proteins of cereals are of immense importance in determining the quality and end-use properties of the grain (Shewry and Halford, 2002). Essential amino acids are crucial for nutritional security; hence, two amino acids (lysine and thiamine) were analysed in the germplasm and showed remarkable variability in their content. The food supply of developed countries is rich in lysine; however, in poor countries where cereals dominate the food supply, lysine is the most limiting amino acid in the food supply (Baker, 2007). It is a strictly indispensable amino acid in humans and animals

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

Table 4. Characteristics of simple sequence repeat (SSR) loci used for diversity analysis in foxtail millet (*Setaria italica* (L.) P. Beauv) from Central Himalayan Region

SSR loci	Chromosome (location, Kb)	na	ne	I	Exp_Het	Nei	Ave_Het	PIC	Fst	Nm
SICAAS2017	Chr.2 (41 333.1)	2	1.4	0.46	0.29	0.29	0.11	0.285	0.68	0.12
SICAAS3010	Chr.3 (4137.9)	3	2.59	1.02	0.63	0.61	0.22	0.615	0.65	0.13
SICAAS3052	Chr.3 (50 461.4)	3	2.92	1.08	0.67	0.66	0.48	0.657	0.24	0.8
SICAAS8028	Chr.8 (34 290.9)	4	1.38	0.56	0.28	0.27	0.25	0.302	0.26	0.72
SICAAS9030	Chr.9 (6681.4)	3	2.94	1.09	0.67	0.66	0.34	0.66	0.46	0.3
SICAAS7038	Chr.7 (29 176.6)	4	2.58	1.15	0.62	0.61	0.31	0.612	0.53	0.22
SICAAS5049	Chr.5 (23 544.2)	3	1.78	0.78	0.45	0.44	0.34	0.438	0.23	0.85
SICAAS5020	Chr.5 (28 017.3)	2	1.15	0.25	0.13	0.13	0.13	0.128	0.43	0.33
SICAAS9054	Chr.9 (23 440.3)	2	1.07	0.15	0.07	0.07	0.02	0.067	0.07	3.43
SICAAS9067	Chr.9 (58 046.7)	2	1.56	0.54	0.36	0.36	0.25	0.358	0.28	0.66
SICAAS7024	Chr.7 (20 228.2)	2	1.92	0.67	0.49	0.48	0.27	0.48	0.46	0.29
SICAAS4010	Chr.4 (40 053.8)	2	1.62	0.57	0.39	0.38	0.31	0.384	0.26	0.7
SICAAS5013	Chr.5 (12 297.9)	4	1.62	0.78	0.39	0.38	0.26	0.381	0.51	0.24
SICAAS4024	Chr.4 (1918.4)	4	2.78	1.14	0.65	0.64	0.34	0.64	0.43	0.33
SICAAS4037	Chr.4 (33 729.8)	2	1.15	0.26	0.14	0.13	0.06	0.133	0.8	0.06
SICAAS5081	Chr.5 (46 767.8)	2	1.23	0.33	0.19	0.19	0.14	0.186	0.33	0.52
SICAAS5034	Chr.5 (37 301.8)	2	2	0.69	0.51	0.5	0.23	0.499	0.55	0.21
SICAAS6101	Chr.6 (3493.8)	2	1.6	0.56	0.38	0.38	0.26	0.375	0.43	0.33
SICAAS6035	Chr.6 (35 880.9)	2	1.07	0.15	0.07	0.06	0.06	0.064	0.3	0.57
SICAAS9095	Chr.9 (2878.5)	2	1.07	0.15	0.07	0.07	0.06	0.066	0.47	0.29
SICAAS3056	Chr.3 (518.2)	2	1.43	0.48	0.31	0.3	0.21	0.302	0.3	0.59
SICAAS4061	Chr.4 (36 164.2)	2	1.47	0.5	0.33	0.32	0.25	0.32	0.36	0.44
SICAAS4027	Chr.4 (38 133.1)	2	2	0.69	0.51	0.5	0.2	0.499	0.5	0.25
SICAAS5005	Chr.5 (3492.1)	2	1.17	0.28	0.15	0.15	0.12	0.147	0.37	0.43
SICAAS6055	Chr.6 (7657.6)	3	2.28	0.9	0.57	0.56	0.36	0.561	0.37	0.43
Mean		2.52	1.75	0.61	0.37	0.37	0.22	0.366	0.42	0.34
St. Dev		0.77	0.61	0.32	0.2	0.2	0.11	0.198		

na, observed number of alleles; ne, effective number of allele; I, Shannon's information index; Exp_Het, expected heterozygosity; Nei, Nei's gene diversity, Ave_Het, average heterozygosity; PIC, polymorphism information content; Fst, genetic differentiation; Nm, gene flow.

(Tome and Bos, 2007); hence, untapped genetic diversity of foxtail millet having considerable variability in lysine content might be important for human nutrition as well as for feed.

Thiamine, also known as vitamin B_1, is essential for energy metabolism (Bettendorff *et al.*, 2014). Thiamine deficiency is uncommon in economically developed regions due to diversified diets and thiamine fortification of grains (Nathoo *et al.*, 2005; Dwyer *et al.*, 2015); however, severe thiamine deficiency leading to beriberi does occur in areas where dietary sources of thiamine are limited, such as Southeast Asia (Khounnorath *et al.*, 2011; Coats *et al.*, 2012). Ample variability in the thiamine content of foxtail millet germplasm signifies its potential relevance for breeding.

Foxtail millet is considered as a food suitable for diabetics, so its popularization and cultivation may help to minimize this global problem (Kam *et al.*, 2016). Unlike other common cereal foods such as rice and wheat, foxtail millet releases glucose steadily without affecting the body's metabolism (Jali *et al.*, 2012).

The available foxtail millet diversity has vast scope for supporting commercially grown crops by reducing pressure on their availability; it is also a cheap source of nutrients and can be raised at low management cost (Sankhala *et al.*, 2004).

Diversity based on phenotypic and morphological characters usually varies with environment. Molecular markers have been proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Unlike morphological traits, molecular markers are not affected by environment (Staub *et al.*, 1997). Collecting DNA marker data to determine whether phenotypically similar cultivars are genetically similar would, therefore, be of great interest in breeding for economically important traits (Duzyaman, 2005). In the present study, the number of alleles ranged from two to four, with an average of 2.5 ± 0.77 alleles/SSR locus, which is comparable with an earlier study by Jia *et al.* (2009), i.e. 2.5 alleles/SSR locus, and higher than other previous reports: 2.15 alleles/SSR locus (Pandey *et al.*, 2013), 2.2 alleles/SSR locus (Gupta *et al.*, 2012; Kumari

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

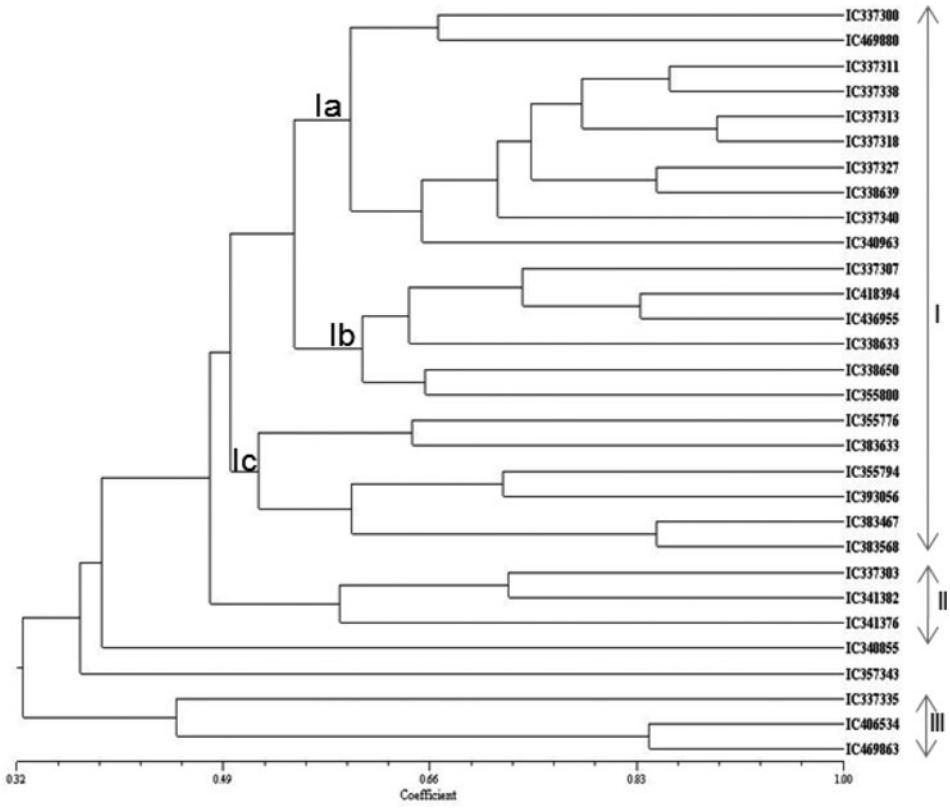

Fig. 2. UPGMA cluster of 30 foxtail millet accessions based on simple sequence repeat (SSR) markers.

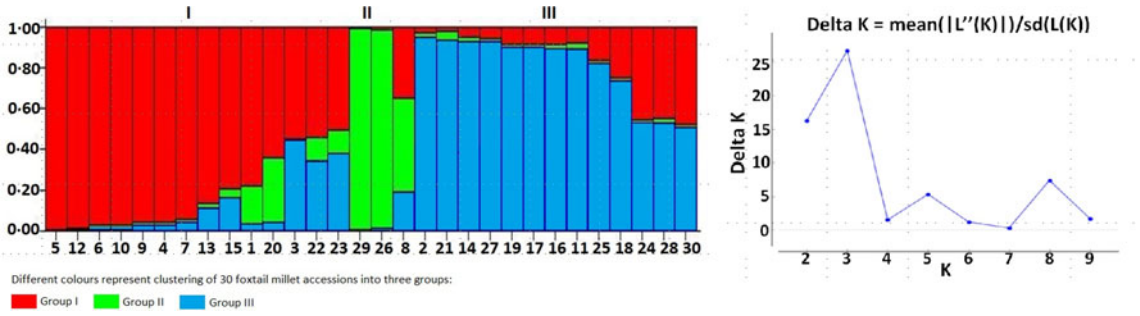

Fig. 3. Structure and ΔK statistics-based graphical representation of estimated number of clusters for K values 1 to 10. 1: IC337300, 2: IC337303, 3: IC337307, 4: IC337311, 5: IC337313, 6: IC337318, 7: IC337327, 8: IC337335, 9: IC337338, 10: IC337340, 11: IC338633, 12: IC338639, 13: IC338650, 14: IC340855, 15: IC340963, 16: IC341376, 17: IC341382, 18: IC355776, 19: IC355794, 20: IC355800, 21: IC357343, 22: IC383467, 23: IC383568, 24: IC383633, 25: IC393056, 26: IC406534, 27: IC418394, 28: IC436955, 29: IC469863, 30: IC469880. Colour online.

et al., 2013) and 2.4 alleles/SSR locus (Lin *et al.*, 2012). The observed heterozygosity of the germplasm is comparable with that obtained earlier by Gupta *et al.* (2012). The PIC values are also comparable with the earlier report by Gupta *et al.* (2012), i.e. 0.45, and lower than values of 0.69 and 0.45 reported by Jia *et al.* (2009) and Liu *et al.* (2011), respectively. Jia *et al.* (2009) and Liu *et al.* (2011) found higher diversity in Chinese germplasm as compared with the Indian germplasm used in the present study. This may be because different SSR markers were used in these studies; secondly, China is the centre of origin of foxtail millet whereas region-specific (i.e. CHR of India) germplasm was used in the present study for evaluation of nutritional quality. The Shannon's Information Index, Nei's expected heterozygosity and PIC values obtained in the present study also suggest considerable variability in the *S. italica* germplasm analysed. Based on the present analysis, the most informative SSR markers identified for *S. italica* are SICAAS3052, SICAAS9030, SICAAS4024, SICAAS3010 and SICAAS7038. According to Wright's (1931) rule, if the value of Nm is <1, populations will diverge. A low Nm value is generally believed to occur in self-pollinating systems such as foxtail millet. In the present study, the obtained Nm value was 0.34, indicating significant genetic differentiation. It has been observed that the accessions IC338633, IC406534, IC337327, IC355800, IC383568, IC338633 and IC337318 showed the highest values of dietary fibre, carbohydrate, fat, protein, lysine, thiamine and 1000 seed weight, respectively, which are higher than all four controls included in the present study, hence may be utilized in foxtail millet improvement for the traits studied. Furthermore, pairs of accessions showing the minimum and maximum values for these traits were examined for their Jaccard's similarity coefficient values (Supplementary Table 1) based on SSR markers used in the present study. The following pairs of accessions showed 27–

https://doi.org/10.1017/S0021859618000382 Published online by Cambridge University Press

73% variation between them based on Jaccard's similarity coefficient values (IC338633 and IC357343, 0.53); (IC406534 and IC337300, 0.27); (IC337327 and IC337335, 0.432); (IC355800 and IC337300, 0.567); (IC383568 and IC355794, 0.567); (IC338633 and IC337300, 0.40) and (IC337318 and IC338639, 0.73) and may be useful in breeding programmes for improving dietary fibre, carbohydrate, fat, protein, lysine, thiamine and thousand grain weight, respectively. Hence, molecular profiling may be useful to select genotypes for nutritional breeding. Selection and utilization of available genetic variability for nutritional quality based on morpho-physiological traits and molecular markers may be an easy and useful approach for food and nutritional security in resource-poor regions as well as crop improvement (Trivedi *et al.*, 2015).

Conclusion

A wide gene pool with known nutritional traits having diversity at the molecular level might be decisive for continued improvement of a crop through breeding. Results reveal considerable diversity and correspondence of molecular clustering and nutritional traits that can be utilized for designing further strategies, tailoring nutritionally rich varieties.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0021859618000382

Acknowledgements. Authors are grateful to the Director, ICAR-NBPGR, Pusa Campus, New Delhi for providing necessary facility and a keen interest in the study.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of interest. None.

Ethical standards. Not applicable.

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