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ORIGINAL PAPER

Genetic variability in proso millet [*Panicum miliaceum*] germplasm of Central Himalayan Region based on morpho-physiological traits and molecular markers

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Abstract Sixteen representative accessions of proso millet [*Panicum miliaceum*] having distinct traits of agronomic importance were collected from altitudinal range of 510–2,695 m asl in the Central Himalayan Region (CHR) of India. Considerable diversity was found in morpho-physiological traits viz., leaf length (16.80–32.00 cm), leaf width (1.7–2.1 cm), plant height (57.00–134.00 cm), days to 50 % flowering (34–54), days to 80 % maturity (111–144), and 1,000 seed weight (0.68–1.86 g). Collected accessions have been evaluated for a battery of biochemical parameters viz., chlorophyll, carotenoids, lipid peroxidation, cellular hydrogen peroxide, activity of nitrate reductase, lipoxygenase, catalase, peroxidase, superoxide dismutase along with super oxide free radical formation, glutathione (total, reduced, and oxidized), glutathione reductase, glutathione S-transferase, ascorbate (total, reduced, and oxidized), monodehydroascorbate reductase, and didehydroascorbate reductase. The sets of 12 genic-SSRs (simple sequence repeats), 54 ISSR (inter simple sequence repeats), and 40 SRAP (sequence related amplified polymorphism) markers were used to study the level of genetic diversity, and Nei's gene diversity value of 0.20 was obtained with both ISSR and SRAP markers. SRAP markers showed higher average number of polymorphic bands, % polymorphism, polymorphic information content (PIC), and Shannon information index compared to ISSR markers; genic-SSRs showed no allelic variation. Cluster analysis shows close groupings of germplasm based on morpho-physiological traits as well as molecular markers. The diverse germplasm identified based on molecular markers with considerable diversity in morpho-physiological traits may be utilized for development of climate resilient cultivars.

Keywords Chlorophyll · Glutathione · Ascorbate · ISSR · SRAP · Genic-SSRs

Introduction

Weather extremities are highly prevalent in Central Himalayan Region (CHR). Unpredictable extreme weather and climate events have the potential to upset the normal life processes of plants and cause great uncertainty in evaluation of the production and productivity. Rich diversity of flora in general and minor millets in particular is available in Central Himalayas, i.e., Uttarakhand state of India. Millet which is one of the oldest foods known to human and possibly the first cereal grain to be used for domestic purposes is the main cereal crop grown in the region. Millets, i.e., under-utilized food grains, have a vast scope for supporting the commercially grown crops by reducing pressure on their availability as well as a cheap source of nutrients and can be grown at low management cost (Sankhala et al. 2004).

Plants are exposed to different abiotic/environmental and biotic stresses and to cope with these stresses

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reactive oxygen species (ROS) are produced in mitochondria and chloroplast. Increased concentration of reactive oxygen species (ROS) can damage cellular membranes, membrane-bound structures, enzymes, and DNA especially in mitochondria and chloroplasts and can ultimately impair plant growth and survival (Noctor and Foyer 1998; Mittler 2002). Enzymic and non-enzymic antioxidant molecules are responsible to regulate the concentration of ROS (Apel and Hirt 2004; Smirnoff 2005). In order to remove H_2O_2 efficiently, plants contain two antioxidant compounds glutathione (GSH) and ascorbic acid (Noctor and Foyer 1998) and different enzymes such as catalases (CAT) (Vandenabeele et al. 2004) and glutathione peroxidases (GPX) (Rodriguez et al. 2003). Moreover, to scavenge superoxide (O_2^-) and highly toxic hydroxyl radical ($HO^{\cdot}$), plants require the coordinated activity of an enzyme superoxide dismutase (SOD) (Kliebenstein et al. 1998). The functions of these enzymes are well coordinated in plants which are critical for the controlling biochemical processes such as growth, development, hormonal signaling, and stress responses (Smirnoff 2005; Ashraf 2009). Therefore, in order to insure food security in such a fragile ecosystem, it is important to collect available diversity and evaluate morphological and biochemical traits associated with tolerance against abiotic stresses and productivity. Further molecular characterization of these accessions will add complementary information to morpho-physiological data and help in the selection of superior germplasm. Molecular markers play an important role and have been used in genotyping and evaluation of the genetic diversity, population structure, and tagging of traits of economic importance using association mapping approaches in millets (Arya et al. 2013; Pandey et al. 2013; Gupta et al., 2014). In proso millet, only few reports (Karam et al. 2004; Karam et al. 2006) are available on molecular characterization. Hence, there is a need to develop molecular markers in this crop or use the available genomic resources available in other millets (Gupta et al. 2012; Muthamilarasan and Prasad 2014) for molecular characterization.

Withstanding adverse climatic conditions, germplasm available in this region is able to survive and produce at par with released varieties cultivated in optimal climatic conditions and inputs. Despite this meager work is on records with respect to collection and evaluation of proso millet germplasm of this region for tolerance against abiotic/environmental stresses. Therefore, it has been presumed that there might be some morpho-physiological, biochemical, and molecular adaptations carried though the processes of evolution which enable this crop to yield optimally even in adverse environmental conditions. Utility of molecular markers in germplasm characterization, transferability, and phylogenetics has been already demonstrated (Kumari et al. 2013).

Generating genomic resources in terms of molecular markers is imperative in molecular breeding for crop improvement (Muthamilarasan et al. 2014). Evaluation of germplasm may provide some clue about these adaptations and answers to certain questions unanswered so far. Hence, the study has been carried out with the aim to collect and screen the intra-specific genetic variability of proso millet for different morpho-physiological, biochemical, and molecular traits which might be useful for breeding, research as well as for climate resilient agriculture.

Materials and methods

Morpho-physiological traits

Sixteen accessions of proso millet having unique traits of agronomic importance were collected from altitudinal range of 510–2,695 m asl in the Central Himalayan Region (CHR) (Fig. 1) and evaluated in the field condition at an experimental site, located at 29°24′28.7″N latitude, 79°30′47.2″E longitude and 1480 m asl altitude where unexpected variation in temperature (Fig. 2a–c) rainfall (Fig. 3) and relative humidity (Fig. 4) is of common occurrence. Experiments were conducted during Kharif season (June–October) for three consecutive years, i.e., 2011–2013 in randomized block design (RBD) with three replications. Five representative plants of each accession were tagged in each replication for recording observation.

Data have been recorded for various morphological traits such as leaf length (cm) and leaf width (cm) of flag leaf at flowering stage. Plant height (cm) has been recorded at harvest while days of 50 % flowering and days to 80 % maturity have been recorded when approximately 50 % plants were in flowering and 80 % plants were yellowing/senescing, respectively. Thousand seeds weight (g) has been recorded after harvesting and threshing.

Fresh leaf tissues at flowering have been extracted in 80 % acetone for the spectroscopic estimation of chlorophyll (Strain et al. 1971) and carotenoid (Duxbury and Yentshe 1956) content. Nitrate reductase (EC 1.17.99.4) activity in leaves has been determined in vivo (Nicholas et al. 1976) at flowering. To measure lipid peroxidation, the thiobarbituric acid test, which determines malondialdehyde (MDA) as an end product of lipid peroxidation (Heath and Packer 1968) has been used as per the pro-

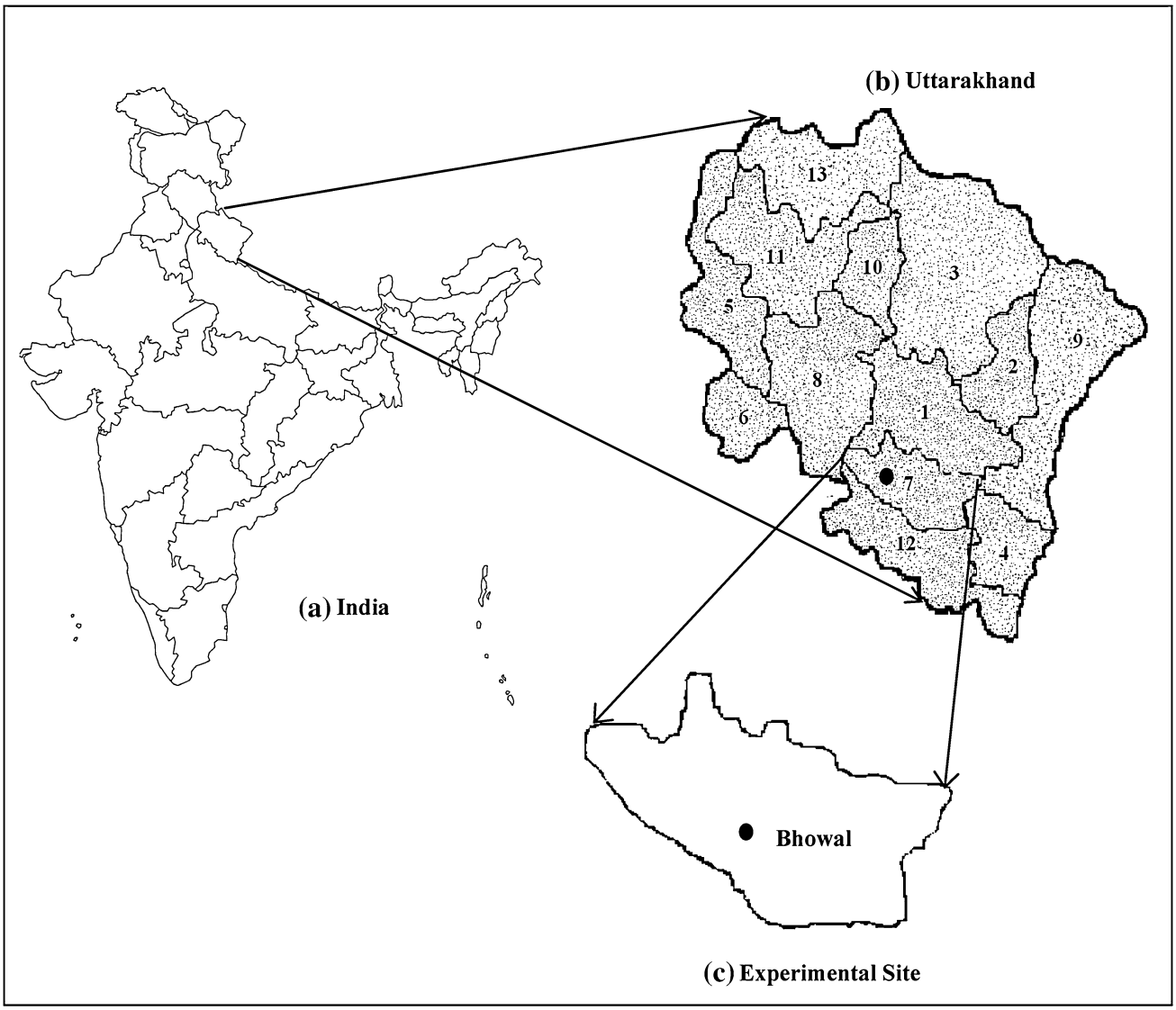

Fig. 1 **a** Map of India (*left upper*), **b** Central Himalayan Region, i.e., Uttarakhand (distribution map) (*right upper*), **c** NBPGR, Regional Station Bhowali, i.e., location of experimental site (*right lower*) with amplification

cedure described by Dhindsa and Matowe (1981). Lipoxygenase (EC 1.13.11.12) and peroxidase (EC 1.11.1.7) activity has been measured by the method of Bostaock et al. (1992) and Cordemener et al. (1991), respectively. Cellular hydrogen peroxide has been measured by fluorimetric assay with homovanillic acid according to Ishikawa et al. (1993). Catalase (EC 1.11.1.6) activity has been determined by the consumption of hydrogen peroxide (Rao et al. 1996). Superoxide dismutase (SOD) (EC 1.15.1.1) activity, the basis of which is its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971), has been assayed as per the procedure described by Stewart and Bewley (1980). The generation rate of superoxide radicals (O_2^-) has been determined following the method of Wang and Luo (1990).

Glutathione content [reduced (GSH) and oxidized (GSSG) glutathione] has been determined enzymatically using the method of Griffith (1980). Total glutathione and GSSG contents were determined directly and GSH after subtraction of GSSG from total glutathione. Glutathione reductase (GR) (EC 1.6.4.2) and glutathione S-transferase (GT) (EC 2.5.1.18) activities were measured by Smith et al. (1988) and Drotar et al. (1985), respectively.

Both reduced (AsA) and oxidized (DAsA) ascorbate contents were determined as described by Knorzer et al. (1996), adapted from the bipyridyl method of Okamura (1980). In this method, AsA is determined directly and

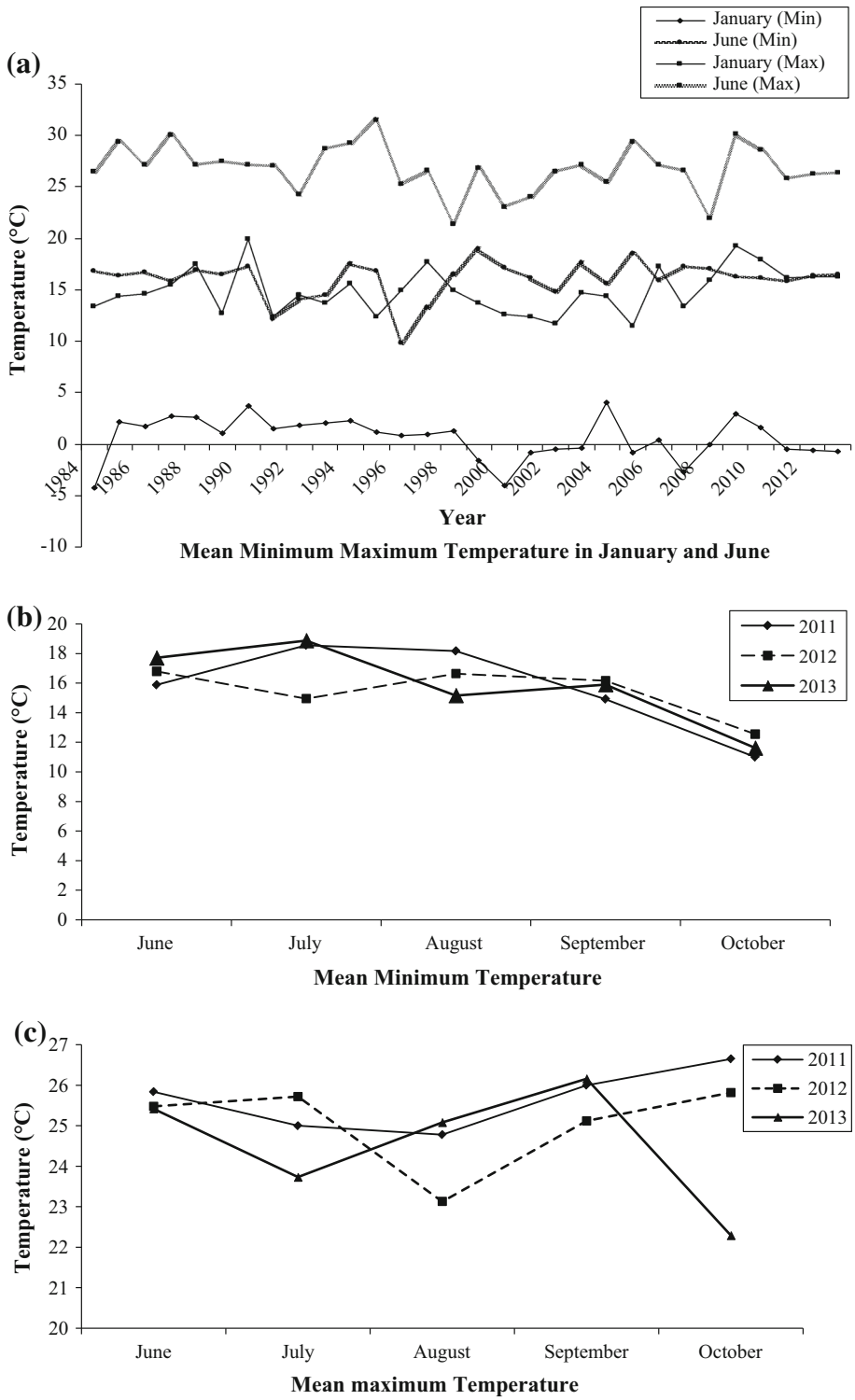

Fig. 2 **a** Annual variation in mean monthly temperature of January and June at experimental site. **b** Mean monthly variation in minimum temperature during June to October at experimental site. **c** Mean monthly variation in maximum temperature during June to October at experimental site

DAsA after reduction and subtraction of AsA from total ascorbate. Monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4) and Dehydroascorbate reductase (DHAR) (EC 1.8.5.1) activity was assayed according to the method of Hossain et al. (1984) and Hossain and Asada (1984), respectively.

Molecular markers analysis

DNA Extraction

DNA was extracted from bulked (30) seeds of proso millet for each accession using mini CTAB method (Saghai-

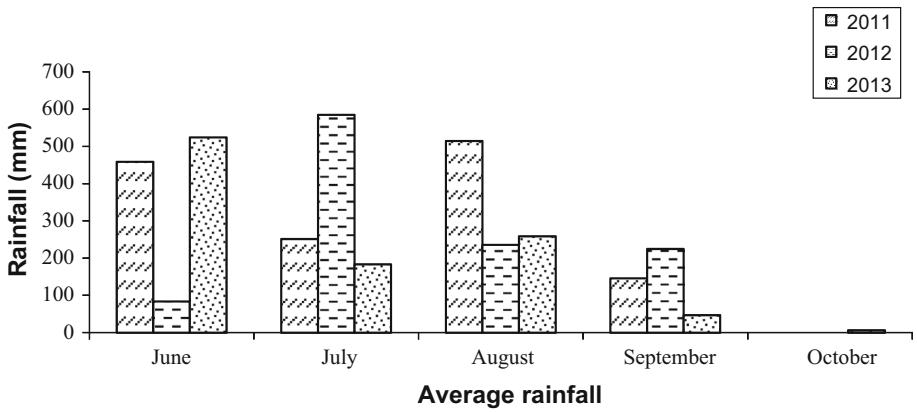

Fig. 3 Average rainfall during June to October at experimental site

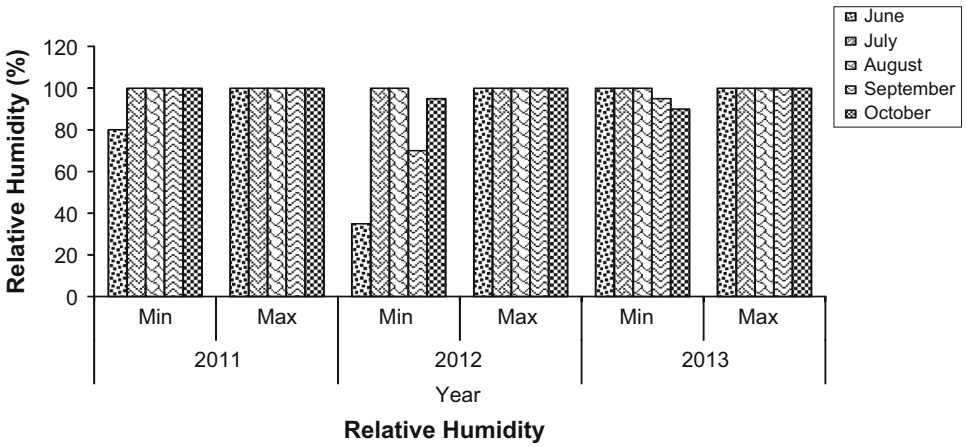

Fig. 4 Relative Humidity during June to October at experimental site

Maroof et al. 1984). Quantity of purified DNA was estimated using NANODROP 1000 (Thermo Scientific) spectrophotometer, and a working solution of 20 ng/µl was prepared for PCR reactions.

SSR Analysis

SSR profiling was carried out using the following PCR components: 100 ng of genomic DNA, 3.0 mM $MgCl_2$, 1.0 U *Taq* DNA polymerase, 1× PCR buffer without $MgCl_2$, 1.0 µM each (forward and reverse primer), and 0.2 mM dNTP mix. For SSR analyses, 93 foxtail millet primer pairs (Kumari et al. 2013) were tested at the following PCR amplification (Eppendorf thermo cycler) conditions: initial denaturation at 94 °C for 3 min followed by 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. Then thirty five 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. Out of twenty three primer pairs, which showed amplification at the above touchdown cycle, eleven were used for genotyping of 16 proso millet accessions, and PCR products were separated in 3 % metaphor agarose gel.

ISSR Analysis

ISSR primers-based PCR amplification was carried out with 60 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U *Taq* DNA polymerase, 1× PCR buffer without $MgCl_2$, 1.0 µM ISSR primer, and 0.2 mM dNTP mix. PCR thermo cycling conditions used were as follows: denaturation at 94 °C for 5 min, thirty five cycles of denaturation at 94 °C for 1 min, primer annealing at temperature (48–55 °C) specific to each primer for 1 min and primer extension at 72 °C for 2 min, and final extension step at 72 °C for 7 min. Amplification products were separated in 1.6 % regular agarose gel.

SRAP analysis

PCR amplification of SRAP primers (Li and Quiros, 2001) was performed using 80 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U *Taq* DNA polymerase, 1x PCR buffer without $MgCl_2$, 1.0 µM SRAP forward and reverse primer each,

and 0.2 mM dNTP mix. Thermo cycling conditions used for PCR were as follows: denaturation at 94 °C for 5 min, five cycles of denaturation at 94 °C for 1 min, primer annealing at 35 °C for 1 min, and primer extension at 72 °C for 2 min, thirty five cycles of denaturation at 94 °C for 1 min, primer annealing at 50 °C for 1 min and primer extension at 72 °C for 1 min, and final extension step at 72 °C for 5 min. A regular agarose gel (2.5 %) was used for separating PCR products.

Bands were scored as 0 or 1 binary data based on the absence or presence of bands, respectively. UPGMA (unweighted pair-group method with arithmetic averages) cluster analysis was done using the program NTSYSpc. ver. 2.1 (Rohlf 2000). Other parameters like PIC value was also calculated using the formula $2f_i(1 - f_i)$, where f_i is the frequency of bands present and $(1 - f_i)$, is the frequency of bands absent (Roldan-Ruiz et al. 2000). Genetic parameters like Nei's gene diversity statistics were estimated using POPGENE version 1.32 (Yeh et al. 1999).

Ward's minimum variance method was used for cluster analysis based on morpho-physiological and biochemical traits. Data for each parameter were evaluated for statistical significance using two-way analysis of variance (ANOVA) to compare the means considering accession and altitude as independent variables. The variation between two groups was assessed by computation of least significant difference taking 't' values for error *df* at 5 % level of significance.

Results

Frequent temperature fluctuations occur in CHR region (Fig. 2), which might have impact on genetic diversity of crops. Approximately, two-fold diversity was found in leaf length of different accessions growing at different altitudinal ranges which ranged 16.8–32.0 cm (Table 1). Similarly, leaf width ranged 1.7–2.1 cm; comparatively, more diversity has been recorded in plant height which ranged 58–134 cm. Days to 50 % flowering were found to vary 34–54 and days to 80 % maturity 111–144. Furthermore, weight of 1,000 seeds was found to vary 0.68–1.86 g.

Chlorophyll and carotenoid content at flowering stage ranged 3.29–4.49 mg g^{-1} fresh weight and 0.94–1.26 mg g^{-1} fresh weight, respectively (Table 2). Similarly, diversity in nitrate reductase activity in different accessions ranged 2.59–3.54 µ mol substrate min^{-1} mg^{-1} protein. Lipid peroxidation which is most easily ascribed symptom of abiotic stresses ranged 1.2–7.8 nmol MDA formed mg^{-1} protein h^{-1}. Approximately, three-fold diversity was found in lipoxygenase activity which ranged 0.85–2.53 µmol substrate min^{-1} mg^{-1} protein. These enzymes are common in plants where they may be involved in diverse aspects of physiological functions. Activity of catalase, enzyme responsible for the degradation of hydrogen peroxide, ranged from 64 to 485 µmol hydrogen peroxide decomposed min^{-1} mg^{-1} protein (Table 3). Similarly, peroxidase activity was found to vary 1.72–4.36 µmol substrate min^{-1} mg^{-1} protein. Superoxide dismutase activity was found to vary 1,066–2,630 enzyme U mg^{-1} protein which acts as an antioxidant and protects cellular components from being oxidized by reactive oxygen species (ROS). In different accessions, enormous variation was found in hydrogen peroxide generation which is a strong oxidizer. The oxidizing capacity of hydrogen peroxide is so strong that it is considered a highly reactive oxygen species. Hydrogen peroxide content in different accessions ranged 0.89–5.46 nmol hydrogen peroxide formed mg^{-1} protein. Likewise, superoxide free radical was found to vary 0.4–3.5 nmol hydrogen peroxide formed mg^{-1} protein.

From Table 4, it is apparent that total glutathione which is a major endogenous antioxidant produced by cells involved in neutralization of free radicals and reactive oxygen compounds varied from 88–222 µmol g^{-1} fresh. More variability was found in reduced glutathione (GSH) (85–214 µmol g^{-1} fresh weight) as compared to oxidized glutathione (GSSG) content (4–9 µmol g^{-1} fresh weight). In accordance with this, glutathione reductase activity was also found to vary 0.13–0.97 µmol substrate min^{-1} mg^{-1} protein. Similarly, glutathione S-transferase activity was found to vary 0.33–0.87 µmol substrate min^{-1} mg^{-1} protein.

Table 5 gives a picture of variability in ascorbate content. Ascorbate which is the predominant species at typical biological pH values ranged 4.08–7.32 µmol g^{-1} fresh weight. Ascorbic acid (reduced form) was found to vary 3.48–6.23 µmol g^{-1} fresh weight and oxidized form, i.e., dehydroascorbic acid ranged 0.62–1.11 µmol g^{-1} fresh weight. In conformity with this, monodehydroascorbate reductase, an enzyme component of glutathione ascorbate cycle was found to vary 1.56–3.87 µmol substrate min^{-1} mg^{-1} protein. Similar variability in dehydroascorbate reductase activity which reduces dehydroascorbate (DHA) to ascorbate with glutathione (GSH) as the electron donor was found to vary 0.31–0.67 µmol substrate min^{-1} mg^{-1} protein. Based on morpho-physiological and biochemical traits, cluster analysis was done using ward's minimum variance method in which accessions collected from similar agro-climatic conditions, and similar altitudinal ranges have been found to group together (Fig. 5), which indicates that these accessions are closely related. Sub-cluster I consisted of three accessions from low altitudinal regions, which are showing significant variation in terms of traits evaluated in this study. IC444094 and IC444221 collected from high altitudinal region and are thus falling in sub-cluster II are also showing significant variation in terms of traits evaluated.

Table 1 Genetic diversity in morphological parameters and yield attributes of proso millet in Central Himalayan Region

S. no	IC number	Altitude	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Days to 50 % flowering	Days to 80 % maturity	1,000 seed weight (g)
1	IC406554	510	26.9 ± 1.5	2.0 ± 0.31	95 ± 6	35 ± 3	123 ± 9	0.87 ± 0.04
2	IC383618	1280	20.0 ± 1.1	2.1 ± 0.23[a]	127 ± 8[a]	43 ± 3	130 ± 8[a]	1.17 ± 0.07[a]
3	IC383466	1420	16.8 ± 1.2	1.7 ± 0.14	76 ± 5	39 ± 3	123 ± 8	0.97 ± 0.05
4	IC444094	2050	26.4 ± 2.1	2.1 ± 0.22	72 ± 5	54 ± 4	111 ± 8	1.32 ± 0.04[a]
5	IC444221	2100	32.0 ± 2.5[a]	2.0 ± 0.21[a]	129 ± 8[a]	38 ± 2	130 ± 9	0.93 ± 0.05
6	IC356094	2150	16.9 ± 1.1	1.8 ± 0.22	132 ± 10	37 ± 3	128 ± 9	0.77 ± 0.03
7	IC340916	2160	24.5 ± 2.1	1.9 ± 0.16	83 ± 7	34 ± 2	118 ± 10	1.86 ± 0.05[a]
8	IC444091	2180	30.8 ± 2.4[a]	2.1 ± 0.17	87 ± 6	38 ± 3	124 ± 10	0.94 ± 0.04
9	IC340860	2510	22.3 ± 2.1	1.7 ± 0.13	58 ± 4	46 ± 3[a]	129 ± 9	0.68 ± 0.03
10	IC340847	2530	24.3 ± 1.9	1.9 ± 0.18	65 ± 5	48 ± 4[a]	143 ± 8[a]	0.76 ± 0.04
11	IC340856	2545	23.9 ± 2.1	1.9 ± 0.16	67 ± 5	49 ± 4[a]	144 ± 9[a]	0.92 ± 0.05
12	IC341000	2560	22.9 ± 1.6	1.8 ± 0.16	86 ± 6	38 ± 2	125 ± 10	1.03 ± 0.08
13	IC341400	2650	22.9 ± 2.1	1.7 ± 0.14	58 ± 4	50 ± 3	138 ± 9	1.68 ± 0.03[a]
14	IC362254	2650	22.1 ± 1.9	2.1 ± 0.19[a]	134 ± 9[a]	39 ± 3	127 ± 7	1.07 ± 0.08
15	IC340939	2660	26.9 ± 1.5	1.9 ± 0.17	92 ± 6	36 ± 3	134 ± 8[a]	1.03 ± 0.09
16	IC340897	2695	24.1 ± 2.2	1.9 ± 0.8	74 ± 6	39 ± 3	122 ± 9	0.94 ± 0.05

Values presented are mean ± SE

[a] Significant at $p \leq 0.05$

Table 2 Genetic diversity in total chlorophyll, carotenoids, nitrate reductase activity, lipid peroxidation, and lipoxygenase activity of proso millet in Central Himalayan Region

S. no	IC no	Chlorophyll content at flowering (mg g^{-1} FW)	Carotenoids content at flowering (mg g^{-1} FW)	Nitrate reductase activity (μmol substrate min^{-1} mg^{-1} protein)	Lipid peroxidation (nmol MDA formed/ mg protein/h)	Lipoxygenase (μmol substrate min^{-1} mg^{-1} protein)
1	IC406554	4.21 ± 0.19[a]	1.03 ± 0.04	3.18 ± 1.1	7.6 ± 0.31[a]	1.89 ± 0.09
2	IC383618	4.49 ± 0.18[a]	1.16 ± 0.03	3.25 ± 1.3[a]	4.5 ± 0.38	1.72 ± 0.08
3	IC383466	4.23 ± 0.16[a]	1.26 ± 0.05[a]	2.60 ± 0.9	5.4 ± 0.41[a]	1.65 ± 0.07
4	IC444094	4.18 ± 0.17	1.09 ± 0.04	2.80 ± 1.0	3.4 ± 0.29	1.39 ± 0.05
5	IC444221	4.34 ± 0.20[a]	1.15 ± 0.06	2.99 ± 0.8	4.2 ± 0.18	2.53 ± 0.07[a]
6	IC356094	4.26 ± 0.20[a]	1.18 ± 0.04	2.59 ± 0.9	4.8 ± 0.19	1.18 ± 0.05
7	IC340916	3.46 ± 0.16	0.95 ± 0.03	3.42 ± 1.2[a]	3.2 ± 0.15	1.54 ± 0.06
8	IC444091	3.29 ± 0.15	1.18 ± 0.05	2.88 ± 0.9	7.8 ± 0.29[a]	2.12 ± 0.09[a]
9	IC340860	3.93 ± 0.18	0.94 ± 0.04	2.87 ± 0.8	1.7 ± 0.08	1.41 ± 0.07
10	IC340847	3.38 ± 0.15	0.98 ± 0.05	2.90 ± 1.2	1.3 ± 0.06	0.85 ± 0.04
11	IC340856	3.43 ± 0.17	1.02 ± 0.05	3.16 ± 1.3	2.2 ± 0.09	0.96 ± 0.04
12	IC341000	3.71 ± 0.16	1.21 ± 0.07[a]	3.54 ± 1.3[a]	6.4 ± 0.26[a]	0.97 ± 0.04
13	IC341400	3.51 ± 0.13	1.06 ± 0.06	2.96 ± 1.2	1.8 ± 0.08	1.74 ± 0.06
14	IC362254	3.59 ± 0.15	1.05 ± 0.04	3.38 ± 1.1[a]	5.2 ± 0.26[a]	1.33 ± 0.06
15	IC340939	3.99 ± 0.19	1.12 ± 0.05	3.23 ± 1.0[a]	1.2 ± 0.05	1.22 ± 0.05
16	IC340897	4.12 ± 0.19	1.08 ± 0.05	3.09 ± 1.1	3.1 ± 0.14	1.26 ± 0.06

Values presented are mean ± SE

[a] Significant at $p \leq 0.05$

Eleven foxtail millet EST-SSR loci were used for profiling of 16 accessions of proso millet and generated 12 alleles, but no allelic variation has been observed with these loci (Fig. 6). Characteristics of the EST–SSR markers used are shown in Table 6. In view of the monomorphic results obtained with EST–SSR markers, ISSR and SRAP markers were used to evaluate molecular variation in proso millet.

Table 3 Genetic diversity in catalase, peroxidase, superoxide dismutase, hydrogen peroxide, and superoxide free radical formation of proso millet in Central Himalayan Region

S. no	IC no	Catalase activity (μmol hydrogen peroxide decomposed min^{-1} mg^{-1}protein)	Peroxidase activity (μmol substrate min^{-1} mg^{-1}protein)	Superoxide dismutase activity (enzyme U mg^{-1} protein)	Hydrogen peroxide generation (nmol hydrogen peroxide formed mg^{-1} protein)	Super oxide free radical (O_2^-) formation (nmol hydrogen peroxide formed mg^{-1} protein)
1	IC406554	366 ± 16	3.46 ± 0.168	1380 ± 35	5.12 ± 0.24	2.7 ± 0.13[a]
2	IC383618	204 ± 11	4.36 ± 0.186[a]	1940 ± 36	2.02 ± 0.09	0.7 ± 0.03
3	IC383466	432 ± 19[a]	3.99 ± 0.17	2630 ± 38[a]	4.65 ± 0.23	3.5 ± 0.13[a]
4	IC444094	445 ± 19[a]	3.89 ± 0.17	2330 ± 37[a]	4.78 ± 0.27	3.2 ± 0.11[a]
5	IC444221	375 ± 17	4.12 ± 0.19[a]	1590 ± 35	2.63 ± 0.09	0.7 ± 0.03
6	IC356094	485 ± 19[a]	4.22 ± 0.20[a]	1680 ± 33	3.36 ± 0.11	2.4 ± 0.09
7	IC340916	342 ± 17	3.44 ± 0.17	2148 ± 39[a]	3.88 ± 0.11	2.0 ± 0.04
8	IC444091	266 ± 13	1.88 ± 0.09	2140 ± 39[a]	5.46 ± 0.23[a]	2.1 ± 0.05
9	IC340860	212 ± 11	2.96 ± 0.11	1082 ± 30	2.45 ± 0.09	1.6 ± 0.06
10	IC340847	138 ± 6	2.34 ± 0.10	1066 ± 32	0.89 ± 0.08	0.4 ± 0.01
11	IC340856	64 ± 3	2.66 ± 0.09	1168 ± 33	1.12 ± 0.09	0.7 ± 0.03
12	IC341000	359 ± 15	3.66 ± 0.14	2060 ± 35	5.42 ± 0.20[a]	2.0 ± 0.08
13	IC341400	185 ± 8	2.65 ± 0.13	1268 ± 32	1.36 ± 0.10	1.6 ± 0.07
14	IC362254	78 ± 3	3.65 ± 0.12	2550 ± 39	3.32 ± 0.11	2.5 ± 0.02
15	IC340939	136 ± 7	1.72 ± 0.13	1898 ± 34	2.89 ± 0.11	1.0 ± 0.04
16	IC340897	332 ± 15	3.12 ± 0.15	1478 ± 34	3.55 ± 0.12	1.3 ± 0.05

Values presented are mean ± SE

[a] Significant at $p \leq 0.05$

Table 4 Genetic diversity in glutathione (total, reduced, and oxidized), glutathione reductase, and glutathione S-transferase content of proso millet in Central Himalayan region

S. no	IC no	Total glutathione (μmol g^{-1} FW)	Reduced glutathione (μmol g^{-1} FW)	Oxidized glutathione (μmol.g^{-1} FW)	Glutathione reductase (μmol substrate min^{-1} mg^{-1} protein)	Glutathione S-transferase (μmol substrate min^{-1} mg^{-1} protein)
1	IC406554	187 ± 9	181 ± 8	8 ± 0.37[a]	0.42 ± 0.01[a]	0.87 ± 0.043[a]
2	IC383618	216 ± 10[a]	209 ± 9[a]	9 ± 0.37[a]	0.29 ± 0.01	0.69 ± 0.03
3	IC383466	155 ± 7	148 ± 7	7 ± 0.29	0.16 ± 0.01	0.43 ± 0.02
4	IC444094	113 ± 6	109 ± 5	5 ± 0.12	0.23 ± 0.01	0.73 ± 0.03[a]
5	IC444221	222 ± 9[a]	214 ± 8[a]	9 ± 0.40[a]	0.16 ± 0.01	0.66 ± 0.03
6	IC356094	186 ± 8	179 ± 8	8 ± 0.35	0.18 ± 0.01	0.63 ± 0.03
7	IC340916	165 ± 6	159 ± 7	7 ± 0.34	0.26 ± 0.01	0.76 ± 0.04[a]
8	IC444091	108 ± 5	104 ± 5	4 ± 0.21	0.49 ± 0.02[a]	0.48 ± 0.02
9	IC340860	88 ± 4	85 ± 4	4 ± 0.17	0.14 ± 0.01	0.63 ± 0.03
10	IC340847	122 ± 6	117 ± 6	5 ± 0.22	0.17 ± 0.01	0.45 ± 0.02
11	IC340856	102 ± 5	98 ± 5	5 ± 0.26	0.13 ± 0.01	0.56 ± 0.03
12	IC341000	98 ± 4	94 ± 5	4 ± 0.18	0.97 ± 0.05[a]	0.59 ± 0.03
13	IC341400	126 ± 6	121 ± 6	6 ± 0.22	0.21 ± 0.01	0.49 ± 0.02
14	IC362254	206 ± 9	198 ± 4[a]	8 ± 0.39[a]	0.25 ± 0.01	0.55 ± 0.03
15	IC340939	145 ± 7	140 ± 7	6 ± 0.23	0.19 ± 0.01	0.33 ± 0.02
16	IC340897	112 ± 6	108 ± 5	5 ± 0.21	0.16 ± 0.01	0.85 ± 0.04[a]

Values presented are mean ± SE

[a] Significant at $p \leq 0.05$

Table 5 Genetic diversity in ascorbate (total, reduced, and oxidized), monodehydroascorbate reductase, and dehydroascorbate reductase content of proso millet in Central Himalayan region

S. no	IC no	Total ascorbate content (μmol g^{-1} FW)	Ascorbic acid (μmol g^{-1} FW)	Dehydroascorbic acid (μmol g^{-1} FW)	Monodehydroascorbate reductase (μmol substrate min^{-1} mg^{-1} protein)	Dehydroascorbate reductase (μmol substrate min^{-1} mg^{-1} protein)
1	IC406554	6.67 ± 0.33[a]	5.69 ± 0.28[a]	1.02 ± 0.05[a]	3.78 ± 0.18[a]	0.62 ± 0.03[a]
2	IC383618	5.36 ± 0.26	4.58 ± 0.22	0.81 ± 0.04	3.64 ± 0.17[a]	0.67 ± 0.03[a]
3	IC383466	7.06 ± 0.34[a]	6.12 ± 0.29[a]	1.08 ± 0.05[a]	1.96 ± 0.08	0.38 ± 0.02
4	IC444094	7.32 ± 0.34[a]	6.23 ± 0.30[a]	1.11 ± 0.04[a]	2.59 ± 0.12	0.59 ± 0.02[a]
5	IC444221	4.89 ± 0.23	4.17 ± 0.19	0.74 ± 0.03	2.58 ± 0.12	0.44 ± 0.01
6	IC356094	4.08 ± 0.19	3.48 ± 0.17	0.62 ± 0.03	3.66 ± 0.17[a]	0.41 ± 0.01
7	IC340916	4.12 ± 0.20	3.51 ± 0.17	0.62 ± 0.03	1.87 ± 0.09	0.54 ± 0.02
8	IC444091	5.89 ± 0.28	5.11 ± 0.24	0.89 ± 0.04	3.87 ± 0.18[a]	0.59 ± 0.02[a]
9	IC340860	5.22 ± 0.25	4.45 ± 0.22	0.79 ± 0.03	2.24 ± 0.11	0.39 ± 0.01
10	IC340847	4.23 ± 0.21	3.61 ± 0.17	0.64 ± 0.03	1.56 ± 0.07	0.33 ± 0.01
11	IC340856	4.56 ± 0.22	3.87 ± 0.19	0.69 ± 0.03	2.03 ± 0.09	0.41 ± 0.02
12	IC341000	6.47 ± 0.31	5.52 ± 0.27	0.98 ± 0.04	2.49 ± 0.12	0.51 ± 0.02
13	IC341400	5.88 ± 0.28	5.01 ± 0.24	0.89 ± 0.04	3.26 ± 0.15	0.47 ± 0.02
14	IC362254	5.96 ± 0.28	5.12 ± 0.24	0.91 ± 0.04	2.25 ± 0.11	0.31 ± 0.01
15	IC340939	6.54 ± 0.32	5.56 ± 0.24	0.99 ± 0.04[a]	1.98 ± 0.09	0.35 ± 0.02
16	IC340897	6.32 ± 0.29	5.38 ± 0.26	0.95 ± 0.04	2.67 ± 0.12	0.48 ± 0.02

Values presented are mean ± SE

[a] Significant at $p \leq 0.05$

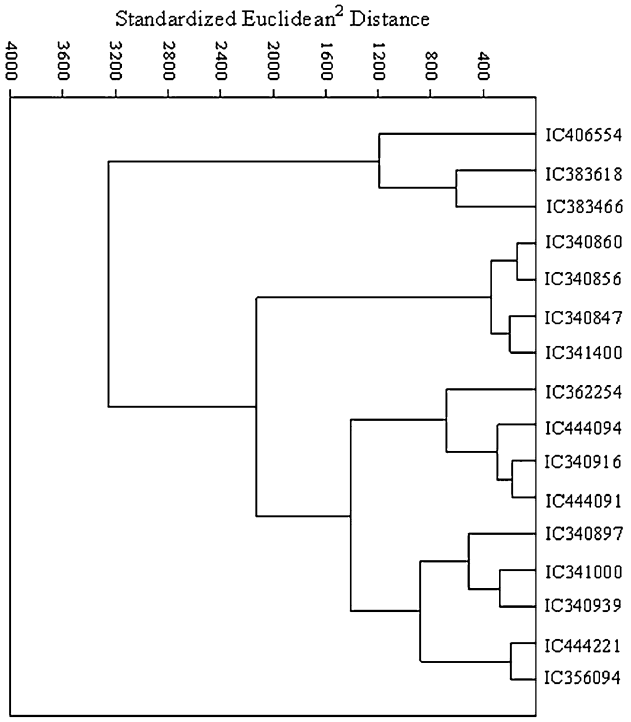

Fig. 5 Ward's Minimum Variance Dendogram

Eight ISSR primers generated a total of 54 bands with an average of 6.75 bands per primer, and the average number of polymorphic bands per primer was 3.75 in proso millet (Fig. 6). Average PIC value of 0.178 ± 0.071 and average % polymorphism of 55.56 % were reported with 54 ISSR markers. Observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), and Shannon's information index (I) values of 1.56 ± 0.50, 1.35 ± 0.40, 0.20 ± 0.21, and 0.29 ± 0.30, respectively, were observed (Table 7). Jaccard's similarity coefficient values ranged from 0.62 to 0.89 with an average of 0.79 and IC340916 and IC341000 were found as most distant proso millet accessions based on ISSR data. UPGMA cluster analysis grouped the 16 accessions into two clusters viz. I and II (IIA and IIB) (Fig. 7)

Forty SRAP markers were produced based on seven SRAP primer pairs with an average number of 5.71 bands per primer and average number of 4.0 polymorphic bands per primer. Average % polymorphism of 70 % and average PIC value of 0.119 ± 0.068 were observed with 40 SRAP markers. Genetic diversity parameters like observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), and Shannon's information index (I) values of 1.70 ± 0.46, 1.34 ± 0.39, 0.20 ± 0.20, and 0.30 ± 0.28, respectively, were found (Table 7). An average Jaccard's similarity coefficient of 0.72 (0.48 to 0.93) was observed, and IC340916 and IC341400 were identified as the two distant accessions based on SRAP data. UPGMA cluster analysis also revealed two clusters viz. I (IA and IB) and II (Fig. 8).

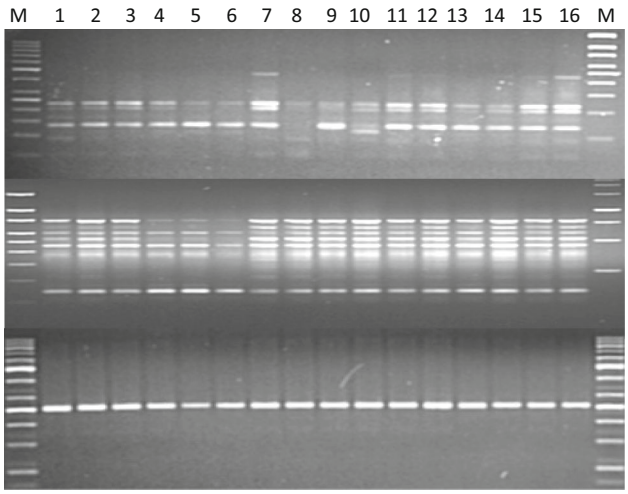

Fig. 6 Gel profile of 16 accessions of proso millet with SRAP primer Em2- me10 (*top*), ISSR primer (GA)8T, and SSR primer SieSSR65, and *M* is molecular weight marker (50, 100 bp); numbers 1 to 16 are *1* IC340847, *2* IC340856, *3* IC340860, *4* IC340897, *5* IC340916, *6* IC340939, *7* IC341000, *8* IC341400, *9* IC356094, *10* IC362254, *11* IC383466, *12* IC383618, *13* IC406554, *14* IC449091, *15* IC444094, *16* IC444221

Discussion

In Central Himalayan Region, abrupt rise or fall in temperature upsets biochemical processes at cellular level which impedes normal life processes. This in turn affects plant architecture; plants growing in adverse climatic conditions differ in shape and size from those growing in favorable conditions. Height is a major determinant of a plant's ability to compete for light (Falster and Westoby 2003). Plant height is also an important part of life-history traits (Moles and Leishman 2008). More than 2.5-fold variability in plant height of germplasm indicates rich diversity in this particular trait and ability of plants to adjust with environmental conditions.

Several pheno-morphological traits relate to competitive ability of a crop to survive and yield optimally such as plant height (Lindquist and Mortensen 1998), leaf uprightness (Sankula et al. 2004), and crop maturity (Begna et al. 2001). Considerable variability in these traits was found which helps the germplasm to survive in variable agro-climatic conditions of CHR. These traits are central in determining how a species lives, grows, and reproduces. Nevertheless, to maximize yield, it is essential to tailor the life cycle of a plant according to the agro-environments in which they are grown. The transition, from vegetative to reproductive growth as recorded in the form of days to 50 % flowering and days to 80 % maturity, is a critical developmental switch and a key adoptive trait which ensures that plants set their flowers at an optimum time for pollination, seed development, and dispersal (Cockram et al. 2007). Farmers throughout history and latterly, plant breeders have selected differences in flowering time to increase yield and extend the agricultural flexibility and eco-geographical range of crops (Cockram et al. 2007). This is obvious by the ability of collected germplasm which may be grown and produce economic yield in environments away from the site of collection. The correlation between yield and its particular components like 1000 seed weight is the net result of direct and indirect effects of the component characters through other yield attributes (Prasanna et al. 2013).

The variation in nitrate reductase activity among different accessions may be associated with difference in regulation of N transporter genes or N fluxes in roots (Britto and Kronzucker, 2002). Roots possess at least three, kinetically distinct, NO_3^- transport systems (Glass and Siddiqi 1995). The high affinity transport system(s) mediates most of the uptake activity when the N concentration is lower than 1 mM, and the low-affinity transport system is responsible for the main uptake when the N concentration is increased above 1 mM (Forde and Clarkson 1999; Williams and Miller 2001). This assumption is based on *K*m value data of rice and Arabidopsis nitrate transporters, because *K*m value of nitrate transporters of proso millet has not been calculated and reported. However, it could be speculated that the activity of the proso millet transporter may be similar to the rice nitrate transporters. It is currently assumed that the negative effect of the abiotic/environmental stresses is at least partially due to the generation of active oxygen species (AOS) and or inhibition of the system, which defends against them (Shalata and Tal 1998). Alteration in the activity of antioxidant enzymes is an adaptation to stress and a defense process. Significant variability in lipid peroxidation of different accessions has been found, which is the symptom most easily ascribed to oxidative damage (Zhang and Kirkham 1996); it is often used as an indicator of oxidative stress (Jagtap and Bhargava 1995). Alike, lipoxygenase activity was found to vary approximately three-fold which might be due to variability in lipid peroxidation of different accessions. Moreover, hydrogen peroxide is formed during various oxidase reactions and can lead to highly reactive oxygen radicals (Halliwell and Gutteridge 1989). Cellular hydrogen peroxide is involved in oxidative stress signaling (Morita et al. 1999). Hydrogen peroxide content increases in response to various environmental stresses such as chilling (Fadzilla et al. 1996), ultraviolet radiation (Murphy and Huerta 1990), heat (Dat et al. 1998), and excessive light (Karpinski et al. 1997) which are the environmental conditions prevailing in this area. Considerable diversity in this trait among different accessions shows variability in abiotic stress tolerance of germplasm. Furthermore, over expression of the gene for catalase enzyme activity protects

Table 6 Characteristics of SSR loci used for profiling 16 accessions of proso millet

SSR loci	Repeat type	Forward primer	Reverse primer	Observed size	Putative function
SieSSR18	(CT)4	ACAGGTGGGTGGGTAGAGAATA	TGTCATCAGTTCACCAACTTCC	236,170	Cell wall-associated hydrolase, partial (*Medicago truncatula*)
SieSSR23	(GTC)5	CCTTTTGTATCTACCTACCT	GTCTGCTTGTTCTCTTTAAC	407	No homology
SieSSR24	(CT)4	GAAGCATATCCCTGTCTGATCC	ACACCACAGTTACAGCAGTTGG	256	No homology
SieSSR31	(TA)4	CCACACTCAACTTATTTTCCCATC	TAGCTACCAAGTGAGGCTTGTG	217	acyl coenzyme a oxidase (*Oryza sativa* Indica Group)
SieSSR33	(AAG)4	GAAATCTGATGTGGATGGTGAG	GACAGCGGCACCAAAATTTAAA	275	No homology
SieSSR40	(GT)4	GGCATGGCCCTAACTAGATTATT	ACTGGGTCACAACCTACGTCTT	198	beta-cyanoalanine synthase (*Hordeum vulgare*)
SieSSR65	(ATGG)5	TCACAAACAACGGCATCTCT	GCACAAAAGAGCTCTGGGAC	254	hypothetical protein [*Oryza sativa* Indica Group]
SieSSR84	(AGC)7	GTTACCGTGTCCCACCAAAC	ATGATAGGAGTGGTGGCTCG	310	unknown (*Zea mays*)
SieSSR91	(TGC)7	CTGCTTCCTGTCTTCCTTGG	AAGGCGCTTCTCATATCCCT	132	S-adenosylmethionine decarboxylase (*Saccharum arundinaceum*)
SieSSR101a	(ATCA)5	GTTAACTTGGCCTTTGCCAC	TCAGAGGCAGGTTCTTTGGT	200	uncharacterized protein (*Zea mays*)
SieSSR164	(GCT)7	AGAACATAGCAATGGGGACG	TATTCCAAATGCTTGGGCTC	300	hypothetical protein (*Oryza sativa* Japonica Group)

leaves against ROS (Zelitch et al. 1991), while catalase deficient plants are more sensitive to various stresses (Willekens et al. 1997). More than seven times, variability in catalase activity among different accessions might be due to considerable variability of germplasm to tolerate stress condition. Catalase is thought to lower oxidative damage by converting hydrogen peroxide to water and oxygen (Scandalios et al. 1997). Nevertheless, scavenging of super oxide by SOD is an important mechanism to cope with stress condition (Bowler et al. 1992). Small quantity of superoxide free radical was also found which may be scavenged simultaneously by SOD. Significant variability in peroxidase activity was recorded, which is related to the generation and use of ROS (Van Huystee 1987). Plant peroxidases exist in variety of isoforms that use different reductants and are located in different cellular compartments (Campa 1991). Peroxidase (POD) activity uses H_2O_2 as substrate. The variability in the activity of peroxidases in different accessions might be due to difference in substrate i.e., H_2O_2 and/or due to difference in total protein. In plants, peroxidases are involved in numerous cellular processes such as development and stress responses. These enzymes are considered as biomarkers of biotic and abiotic stresses. Under stress conditions, the quantitative and qualitative profiles of peroxidases are generally modified. Such modulations prove the major role played by these enzymes in the defense mechanism (Jouili et al. 2011). Profile of specific peroxidases was not studied but variability in the activity indicates response of the accessions to abiotic stress available in natural condition. In addition to variability in the activity of antioxidant enzymes, variation in the antioxidant pool size was also found. Plants adjust antioxidant level as an adaptation to stress and a defense process. Difference in total glutathione content of various accessions might be due to the variability in the capacity of accessions to overcome environmental stress. The tripeptide glutathione (GSH), an antioxidant, exerts a number of functions in plants (Paranhos et al. 1999). Its major function is in the protection of cells against the toxic effects of free radicals, to keep free radical-scavenging ascorbate in its reduced, hence, active form by involvement in the ascorbate–glutathione cycle (Zhang and Kirkham 1996). Similarly, considerable variability in ascorbate and ascorbate-recycling enzymes monodehydroascorbate reductase and dehydroascorbate reductase was also found which increases in stress condition (Knorzer et al. 1996).

Table 7 Characteristics of SRAP and ISSR markers used for profiling 16 accessions of proso millet

Primer	TNB	PB	%P	na	ne	h	I	PIC
SRAP								
EM1–ME10	6	5	83.33	1.80	1.42	0.22	0.33	0.228
EM1–ME2	2	1	50.00	1.83	1.49	0.26	0.39	0.240
EM1–ME5	5	4	80.00	1.80	1.41	0.23	0.34	0.183
EM2–ME10	5	4	80.00	1.75	1.38	0.23	0.36	0.186
EM3–ME10	7	5	71.43	1.43	1.12	0.08	0.14	0.197
EM3–ME3	8	6	75.00	1.50	1.43	0.23	0.33	0.289
EM3–ME4	7	3	42.86	1.71	1.27	0.18	0.29	0.068
Total	40	28						
Average	5.71	4.0	70.00	1.70	1.34	0.20	0.30	0.199
Standard deviation				0.46	0.39	0.20	0.28	0.068
ISSR								
$(AC)_8T$	8	5	62.50	1.63	1.45	0.26	0.37	0.178
$(AG)_8C$	4	2	50.00	1.50	1.39	0.22	0.31	0.230
$(AG)_8T$	3	2	66.67	1.67	1.37	0.20	0.31	0.174
$(AGC)_4Y$	12	7	58.33	1.58	1.29	0.18	0.27	0.109
$(CA)_6R$	8	4	50.00	1.50	1.11	0.08	0.14	0.118
$(CA)_6RG$	6	5	83.33	1.83	1.77	0.40	0.56	0.306
$(CA)_6RY$	6	3	50.00	1.50	1.29	0.18	0.27	0.212
$(GA)_8T$	7	2	28.57	1.29	1.28	0.14	0.20	0.097
Total	54	30						
Average	6.75	3.75	55.56	1.56	1.35	0.20	0.29	0.178
Standard deviation				0.50	0.40	0.21	0.30	0.071

Observed number of alleles (na); effective number of alleles (ne); Nei's gene diversity (h); Shannon's information index (I); Polymorphism Information Content (PIC); Y (Pyrimidine); R (Purine)

Although all the accessions have been grown at one experimental site, great variation has been recorded in antioxidant pool size and activity of oxidative stress enzymes which indicates variability in the potential of different accessions to cope with the stress condition.

DNA-based markers play a pivotal role in expediting molecular breeding (Yadav et al. 2014). Hence, three different marker systems were used to estimate the level of genetic variability among 16 accessions of proso millet. Meager genomic resources are available as compared to foxtail millet which might be used for marker-aided breeding as well as in genetic engineering for abiotic stress tolerance (Lata et al. 2013) in this as well as related millets. Moreover, foxtail millet Marker Database (FmMDb; http://www.nipgr.res.in/foxtail.html) is available online (Muthamilarasan et al. 2013); hence, it can be used for molecular marker research in millet crops. Foxtail EST-SSR loci did not show allelic variation, so there is a need to test more number of EST–SSR markers and genomic SSRs (Gupta et al. 2012; Zhang et al. 2014) from other millets and also develop SSR genomic resources from proso millet in order to get polymorphic SSRs. All the 16 proso millet accessions could be distinguished using both ISSR and SRAP marker systems individually. SRAP markers showed higher average number of polymorphic bands, % polymorphism, observed number of alleles, Shannon's information index, and PIC value as compared to ISSR markers. But, average total number of bands and effective number of alleles were higher with ISSR as compared to SRAP markers, while average Nei's gene diversity was same for both ISSR and SRAP markers. Based on comparison with the earlier reports, it was found that % polymorphism reported in this study using SRAP markers was higher than earlier report in sorghum (Adil et al. 2014) and % polymorphism and average total number of bands generated using ISSR markers is higher compared to earlier reports (Ajithkumar and Panneerselvam 2013; Prabhu and Ganesan 2013), which might be due to the better selection of the primers and diverse germplasm representing whole Central Himalayan Region used for the present study. Average Jaccard's similarity coefficient was higher based on ISSR markers, revealing more diversity with SRAP markers compared to ISSR markers. Overall SRAP markers were found comparatively more informative than ISSR markers for molecular characterization in proso millet. UPGMA cluster analysis revealed close associations of some accessions using ISSR, SRAP markers, and morpho-physiological traits viz. IC340847, IC340856, and IC340860 (almost same altitude and days to 50 %

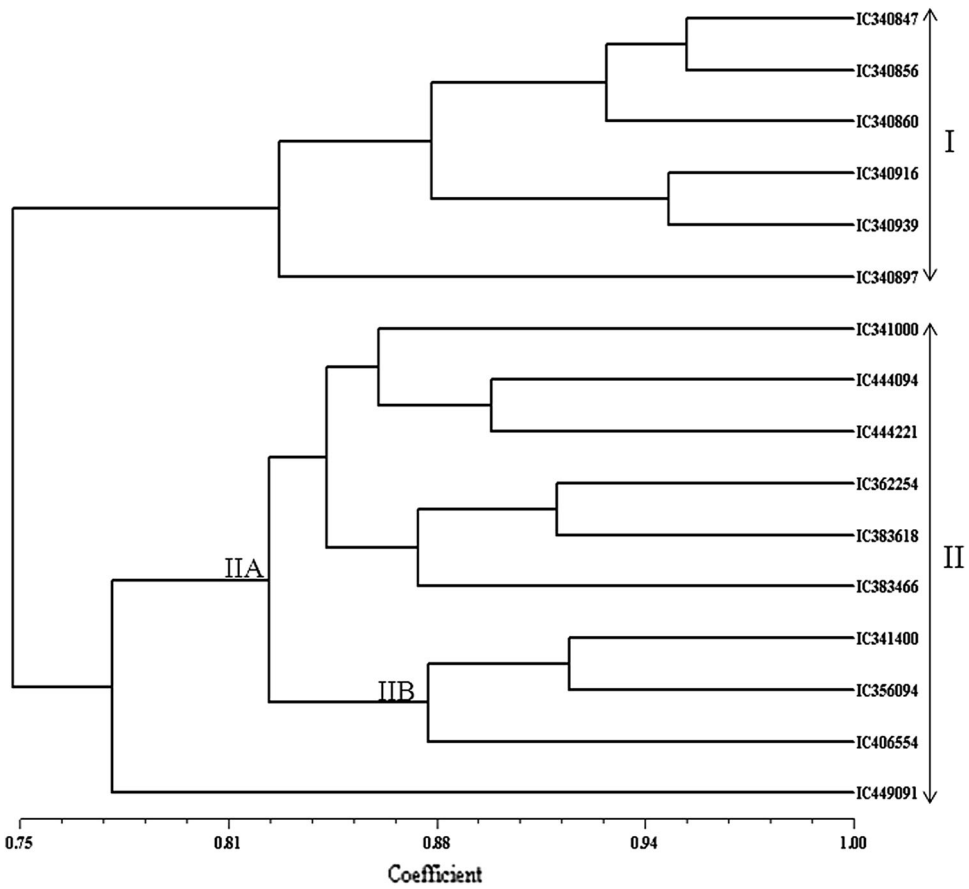

Fig. 7 UPGMA cluster analysis based on ISSR markers

flowering); IC340916, IC340939, and IC340897 (collected from similar altitude, having similarity in days to 50 % flowering and days to 80 % maturity). IC444094 and IC444221; IC341400 and IC356094 were closely grouped based on both ISSR and SRAP marker data which represent similar altitudinal distribution in natural condition. Out of three accessions which were from low altitudes (510–1,420 m asl) regions, two viz. IC383618 and IC383466 were grouped together based on both ISSR and morpho-physiological data. The distant accessions viz. IC340916 and IC341000 identified on the basis of ISSR markers were also found to significantly differ in 1000 seed weight, carotenoid content at flowering, lipid peroxidation, lipoxygenase, hydrogen peroxide generation, total glutathione, reduced glutathione, oxidized glutathione, glutathione S-transferase and glutathione reductase, total ascorbate content, ascorbic acid, and dehydroascorbic acid. Similarly, the diverse accessions viz. IC340916 and IC341400 identified based on SRAP marker data also revealed considerable variability in plant height, days to 50 % flowering, days to 80 % maturity, nitrate reductase, catalase, peroxidase, superoxide dismutase, hydrogen peroxide generation, total glutathione, reduced glutathione, monodehydroascorbate reductase, dehydroascorbate reductase activity. Accessions chosen based on morpho-physiological and molecular data may be used for developing climate pliant varieties. Morpho-physiological, biochemical, and molecular screening of proso millet may be used to understand the mechanism of stress tolerance and high water use efficiency in this crop as in other millets (Lata et al. 2011). The identified accessions may also be used for transcriptome profiling in control and stress conditions to identify the genes/transcription factors responsible for abiotic stress adaptation in proso millet. Proso millet has vast scope for supporting the commercially grown crops by reducing pressure on their availability as well as a cheap source of nutrients. It can be raised at low management cost (Sankhala et al. 2004), has remarkable ability to survive under severe drought (Beryl et al. 2012), and possesses a wealth of genetic diversity. With progressive improvement and development of methods for the measurement of the life functions of plants, it is important to study the physiology and molecular biology of such plants, so that available genetic variability may be harnessed to ensure food security in sustainable manner.

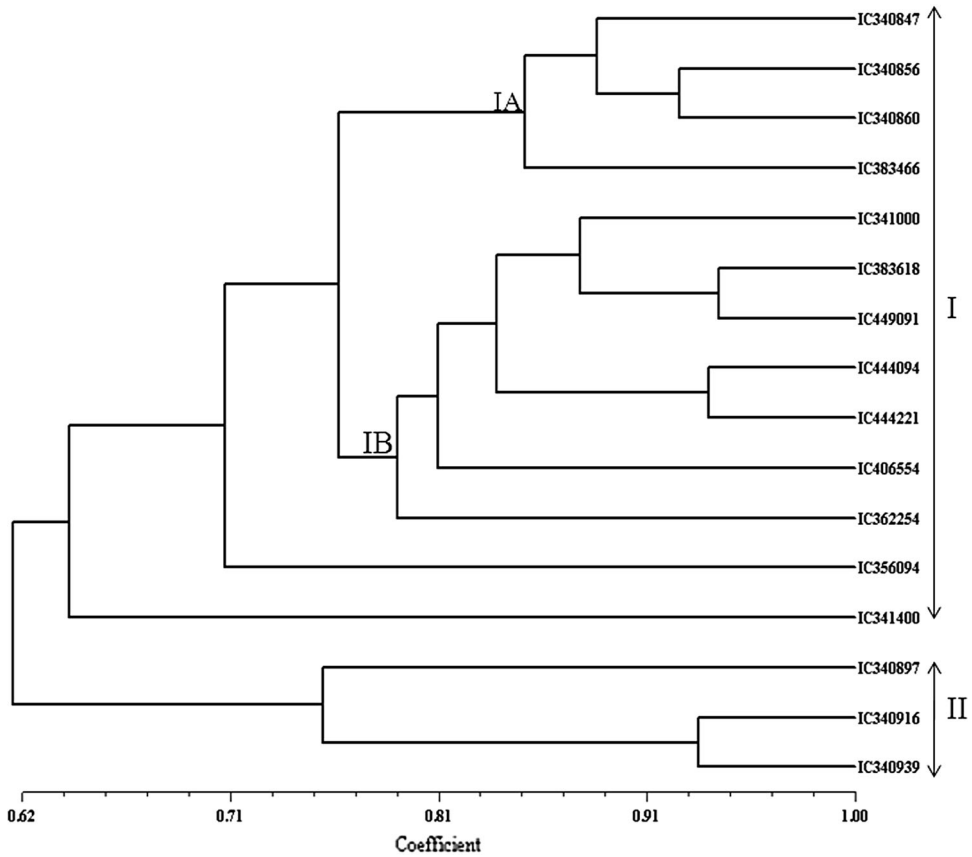

Fig. 8 UPGMA cluster analysis based on SRAP markers

Conclusion

Wide range of genetic diversity is available at morpho-physiological, biochemical, and molecular level in proso millet germplasm of CHR which might be harnessed in breeding for abiotic stresses tolerance. Trait-specific accessions identified after morpho-physiological, biochemical, and molecular evaluation might be utilized in research, crop improvement programs, and climate resilient agriculture and to improve food security and sustainability.

Author contribution statement A. K. Trivedi designed and conducted experiments, recorded and analyzed data, prepared manuscript. L. Arya have done molecular marker analysis. M. Verma have done molecular marker analysis. S. K. Verma helped in data recording and manuscript preparation. R. K. Tyagi facilitated in experimentation and final checking of manuscript. A. Hemantaranjan provided facility and helped in biochemical analysis.

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