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CLIMATE CHANGE AND AGRICULTURE RESEARCH PAPER

Evaluation of barnyard millet diversity in central Himalayan region for environmental stress tolerance

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SUMMARY

The mountain ecosystem of the Central Himalayan Region is known for its diversity of crops and their wild relatives. In spite of adverse climatic conditions, this region is endowed with a rich diversity of millets. Hence, the aim of the present study was to explore, collect, conserve and evaluate the diversity of barnyard millet (*Echinochloa frumentacea*) to find out the extent of diversity available in different traits and the traits responsible for abiotic stress tolerance, and to identify trait-specific accessions for crop improvement and also for the cultivation of millets in the region as well as in other similar agro-ecological regions. A total of 178 accessions were collected and evaluated for a range of morpho-physiological and biochemical traits. Significant variability was noted in days to 50% flowering, days to 80% maturity, 1000 seed weight and yield potential of the germplasm. These traits are considered to be crucial for tailoring new varieties for different agro-climatic conditions. Variations in biochemical traits such as lipid peroxidation (0·552–7·421 nmol malondialdehyde formed/mg protein/h), total glutathione (105·270–423·630 mmol/g fresh weight) and total ascorbate (4·980–9·880 mmol/g fresh weight) content indicate the potential of collected germplasm for abiotic stress tolerance. Principal component analysis also indicated that yield, superoxide dismutase activity, plant height, days to 50% flowering, catalase activity and glutathione content are suitable traits for screening large populations of millet and selection of suitable germplasm for crop improvement and cultivation. Trait-specific accessions identified in the present study could be useful in crop improvement programmes, climate-resilient agriculture and improving food security in areas with limited resources.

INTRODUCTION

In their natural habitat plants are exposed to a wide array of environmental factors. Being sessile in nature, plants are forced to adapt to various environmental stresses, which causes a wide range of alterations at cellular and molecular level. This in turn reduces production and productivity of plants (Shao *et al.* 2008). Survival and successful reproduction in a stressful environment is a complex phenomenon and decisive for food security from the perspective of climate change. It is synchronized and regulated by physiological, cellular and molecular activities of plants (Ahuja *et al.* 2010). Plant survival in stressful environments has a physiological cost (Massad *et al.* 2012), which becomes a major constraint for growth and development and can reduce yield by >50% in major crops (Bray *et al.* 2000). Frequent fluctuations in temperature and uncertain precipitation limit productivity and cause loss of diversity. Indian agriculture is highly dependent on the spatial and temporal distribution of monsoon rainfall (Kumar *et al.* 2004). As per future projections, heavy rains or drought are equally probable in the future (Allen & Ingram 2002) and this will increase the range as well as intensity of various stresses. In order to maintain sustainable agriculture

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under these conditions, there is a strong need to develop new alternatives (Khush 1999) such as exploration and identification of diverse germplasm with known traits, which can provide a practical solution to alleviate the problem of water limitation. In this context exploration, collection and evaluation of untapped diversity from different ecological regions is of paramount importance.

Early perception of stress signals by plants and immediate response is important for environmental stress tolerance. After recognition of stress signals, basal mechanisms operative within plants (Andreasson & Ellis 2010) lead to an activation of complex signalling cascades of tolerance, varying from one stress to another (Abu Qamar *et al.* 2009). In most cases, experiments designed for stress tolerance consider plant responses to individual stress (Qin *et al.* 2011; Todaka *et al.* 2012); however, the response to multiple stresses is much more complex (Fujita *et al.* 2006). Although, research on multiple stresses has been trying to simulate natural conditions, however in the field, conditions are not manually controlled and Space bar should be used between one stress can strongly influence the primary stress defence response of plants (Fujita *et al.* 2006).

As a consequence of exposure to environmental stress, reactive oxygen species (ROS) increase in cells (Laloi *et al.* 2004; Foyer & Noctor 2005) and leads to reprogramming of gene expression resulting in an increase in plant tolerance. This also minimizes the biological damage caused by the stress (Fujita *et al.* 2006). In plants grown in stressful environments, ROS have been perceived as destructive and harmful compounds. Although low levels are mostly responsible for regulating plant stress responses, high levels of ROS lead to cell death (Choudhury *et al.* 2013). Therefore, the ROS status of plants, and their enzymic and non-enzymic defence mechanism in relation to response to environmental stresses may be utilized to screen germplasm for environmental stress tolerance.

'Millet' is a collective term used to refer to a diverse group of small-seeded annual C_4 Panicoid grasses such as barnyard millet (*Echinochloa frumentacea*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*) and proso millet (*Panicum miliaceum*). These are cultivated as food and fodder crops in temperate, sub-tropical and tropical regions across the globe (Dwivedi *et al.* 2012; Lata *et al.* 2013) and have remarkable nutritional properties. Barnyard millet (*Echinochloa spp.*), also known as billion-dollar grass, madira, jhangora or sawan, is the second most important millet crop after finger millet, both in terms of acreage and production in the Central Himalayan Region (CHR). In India, the area under small millets has been steadily decreasing during the last three decades (FAO 2014) and in recent years the pace of decline has been much faster (Joshi 2013). Millet cultivation areas have shrunk nearly 42% over the last 50 years between 1956 and 2006; all millet growing areas in India have moved towards other crops (Sateesh 2010). There are several factors responsible for this decline: the availability of alternative crops with greater market value, such as rice and pulse crops, as well as lack of government support may be attributed as main reasons. In CHR, barnyard millet is a mainstay of the diet and cultural systems of hill people (Kumar *et al.* 2007). It is the fastest growing crop among all millets and can be harvested in a short period of 9 weeks. The crop is known for its good yield and high nutritional value (Prabha *et al.* 2010). Despite its significance, barnyard millet has largely been an under-researched crop compared with the main staple cereals. Millets are considered as minor cereal crops of only regional importance; hence little attention has been given to collection, conservation and evaluation of available diversity for use in crop improvement. Since millets are grown in low-input, rain-fed agricultural systems, they tend to suffer from a range of environmental stresses that become major constraints for crop production and yield. The area under study in the current work is prone to vagaries of weather, i.e. frequent fluctuation in temperature, erratic rainfall, drought, hailstorms, etc. Therefore, evaluation of whole germplasm in the field may be a practical strategy to screen trait-specific germplasm for crop improvement and climate resilience as well as cultivation in this and other such agro-ecological areas.

MATERIALS AND METHODS

Morpho-physiological traits

In total 178 accessions of barnyard millet having unique traits of agronomic importance were collected from altitudinal range of 175–2250 m a.s.l. in the CHR (Fig. 1) and evaluated in the field under rain-fed conditions at an experimental site located at 29°24′ N, 79°30′ E, 1480 m a.s.l. Experiments were conducted during the *Kharif* season (June–October) for three

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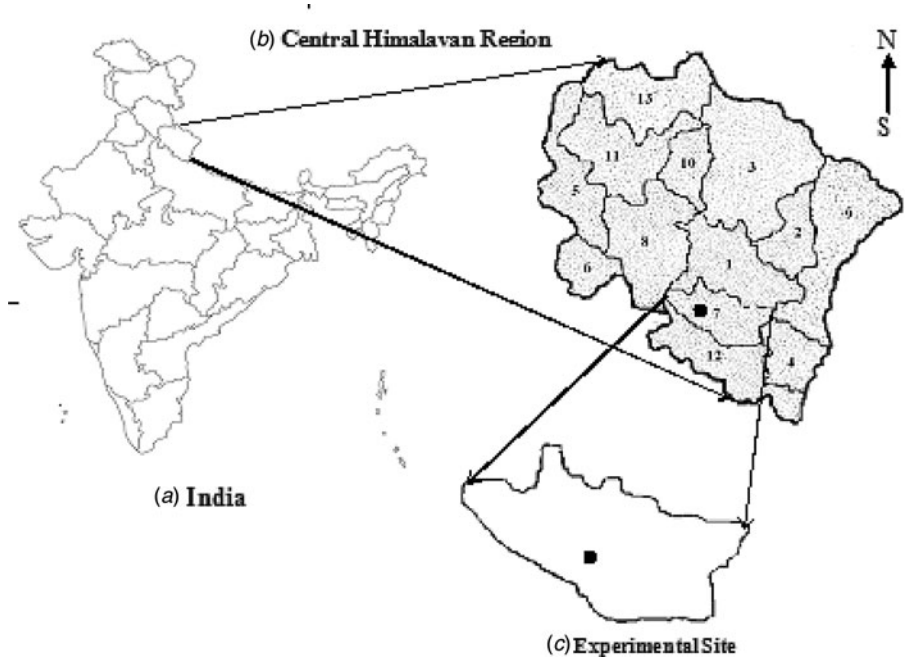

Fig. 1. Map of Central Himalayan Region and experimental site.

consecutive years, i.e. 2011–2013, in an augmented block design (ABD). Five representative plants of each accession were tagged in each block for recording observations. Data for various morphological traits were recorded following the procedure described by Trivedi *et al.* (2015).

Biochemical analysis

Fresh leaf tissues taken at the flowering stage were extracted in 80% acetone for spectroscopic estimation of chlorophyll (Strain *et al.* 1971) and carotenoid content (Duxbury & Yentsch 1956). Lipid peroxidation was measured by the thiobarbituric acid test as described by Dhindsa & Matowe (1981). The methods described by Bostock *et al.* (1992) and Cordewener *et al.* (1991) were used for measuring lipoxygenase (EC 1·13·11·12) and peroxidase (EC 1·11·1·7) activity, respectively. Catalase (EC 1·11·1·6) and superoxide dismutase (SOD) (EC 1·15·1·1) activity was determined by the methods described by Rao *et al.* (1996) and Beauchamp & Fridovich (1971), respectively. The method of Wang & Luo (1990) was followed for determination of superoxide radical ($O_2^{-\cdot}$) generation rate. Glutathione contents [reduced glutathione (GSH) and oxidized glutathione (GSSG)] were determined enzymatically using the method of Griffith (1980). Glutathione reductase (GR) (EC 1·6·4·2) was measured following Smith *et al.* (1988). Both reduced (AsA) and oxidized (DAsA) ascorbate content were determined as described by Knörzer *et al.* (1996), adapted from the bipyridyl method of Masato (1980). Monodehydroascorbate reductase (MDHAR) (EC 1·6·5·4) and dehydroascorbate reductase (DHAR) (EC 1·8·5·1) activity were assayed according to the method of Hossain *et al.* (1984) and Hossain & Asada (1984), respectively. The activity of ascorbate peroxidase (APX, EC 1·11·1·11) was assayed by the method of Nakano & Asada (1987).

Statistical analysis

The statistical analysis for principal component, clustering graph (Ward 1963) and k-means clustering was performed using statistical software SAS 9·3. Principal component analysis (PCA) was done to identify a smaller number of uncorrelated variables to explain the maximum amount of variance with the fewest number of principal components. Clustering was done to partition groups of data points into a small number of clusters.

RESULTS

Flag leaf length of germplasm collected from CHR was found to vary from 130·0 mm in IC282785 to 353·4 mm in IC261999. Similarly, flag leaf width varied from 10·0 mm in IC282785 to 47·2 mm in

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IC355786. Peduncle length ranged from 20·0 mm in IC282785 to 124·0 mm in IC469750. Approximately three-fold variability was found in plant height, which ranged from 546·6 mm in IC279563 to 1612·3 mm in IC273988. Ear length, which is directly related to seed production, was found to vary from 111·5 mm in IC337349 to 237·2 mm in IC382642. Days to 50% flowering and days to 80% maturity, crucial parameters related to the life span of a crop, were found to vary from 48·12 in IC279535 to 88·00 in IC279391 and from 96·00 in IC273927 to 132·66 in IC317641, respectively. Thousand seed weight ranged from 2·03 g in IC355791 to 5·80 g in IC261959. Similarly, yield per plant was found to vary from 0·25 g in IC279408 to 9·25 g in IC273927 (Table 1).

Chlorophyll content at the flowering stage was found to vary from 1·024 mg/g FW (fresh weight (FW)) in IC418409 to 6·859 mg/g FW in IC548696. Similarly, carotenoid content at the flowering stage ranged from 0·563 mg/g FW in IC261951 to 6·325 mg/g FW in IC337304. Lipid peroxidation, the most easily ascribed symptom of membrane damage, ranged from 0·552 nmol malondialdehyde (MDA) formed/mg protein/h in IC391472 to 7·421 nmol MDA formed/mg protein/h in IC338652. Lipoxynase activity ranged from 0·124 mmol substrate/min/mg protein in IC282785 to 4·023 mmol substrate/min/mg protein in IC279391. Activity of catalase, which splits toxic hydrogen peroxide into oxygen and water, was found to vary from 109·00 mmol hydrogen peroxide decomposed/min/mg protein in IC261951 to 855·00 mmol hydrogen peroxide decomposed/min/mg protein in IC340999. Activity of peroxidases, which are involved in many physiological processes in plants including responses to abiotic stresses, was found to vary from 1·236 mmol substrate/min/mg protein in IC281760 to 6·355 mmol substrate/min/mg protein in IC548613. Superoxide dismutase activity, which is known to control oxidative stress in plants, was found to vary from 1123·00 enzyme U/mg protein in IC261951 to 2963·00 enzyme U/mg protein in IC279703. Superoxide free radical (a toxic compound) was found to range from 0·452 nmol hydrogen peroxide formed/mg protein in IC261951 to 4·285 nmol hydrogen peroxide formed/mg protein in IC469893.

The lowest contents of total, reduced and oxidized glutathione (105·270, 96·217 and 9·180 mmol/g FW, respectively), as well as the lowest level of glutathione reductase activity (0·532 mmol substrate/min/mg protein) were all found in IC355792. Similarly, IC355775 had maximum total, reduced and oxidized glutathione content (423·630, 387·20 and 36·941 mmol/g FW, respectively) as well as glutathione reductase activity (2·139 mmol substrate/min/mg protein).

The least amounts of total ascorbate (4·980 mmol/g FW), ascorbic acid (4·235 mmol/g FW) and dehydroascorbic acid (0·676 mmol/g FW) were found in IC261971, IC548641 and IC418409, respectively. The highest amounts of total ascorbate (9·880 mmol/g FW) and dehydroascorbic acid (1·657 mmol/g FW) were found in IC355769, whereas the highest amount of ascorbic acid (8·600 mmol/g FW) was found in IC393054. The activity of ascorbate peroxidase, which detoxifies peroxides such as hydrogen peroxide using ascorbate as a substrate, was found to vary from 1·860 enzyme U/mg protein in IC355803 to 7·040 enzyme U/mg protein in IC355796. Monodehydroascorbate reductase activity varied from 1·106 mmol substrate/min/mg protein in IC391472 to 4·41 mmol substrate/min/mg protein in IC355796. Dehydroascorbate reductase activity varied from 0·330 mmol substrate/min/mg protein in IC261951 to 1·359 mmol substrate/min/mg protein in IC355796 (Table 2).

Cluster analysis was done by Ward's method to divide observations into homogeneous and distinct groups, in which two main clusters were formed. These clusters were further divided into seven subclusters. Hence, *K* means clustering was done to divide all 178 accessions into seven clusters (Table 3). Accessions with similar traits were found to group together. Three accessions grouped in Cluster 1 have very close similarity in morpho-physiological traits. Cluster 7 has the maximum number of accessions, i.e. 48; these accessions all show close similarity in morpho-physiological traits within the cluster. Accessions within a cluster may be useful for selection of similar genotypes for a particular trait, as well as for breeding programmes.

It is evident from PCA and percentage contribution of each component to the total variation (Table 4) that the first four variables contributed 99·94% of the variability (55·24, 38·24, 5·10 and 1·36% of the total variability from the first, second, third and fourth principal components, respectively). It is obvious from the scree plot of the principal components (Fig. 2) that only four principal components contribute considerably towards diversity.

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Table 1. *Variability in morphological traits, flowering and yield of barnyard millet (*Echinochloa frumentacea*) accessions of Central Himalayan Region*

S. no.	Parameter	Minimum value	Maximum value	Average value	Standard error (S.E.)	CV (%)
1.	Flag leaf length (mm)	130·0 (IC282785)*	353·4 (IC261999)*	228·8	0·22	12·6
2.	Flag leaf width (mm)	10·0 (IC282785)*	47·2 (IC355786)*	21·1	0·04	23·8
3.	Peduncle length (mm)	20·0 (IC282785)*	124·0 (IC469750)*	59·6	0·11	25·1
4.	Plant height (mm)	547 (IC279563)*	1612 (IC273988)*	1208	1·4	15·5
5.	Ear head length (mm)	111·5 (IC337349)*	237·2 (IC382642)*	165·3	0·15	12·4
6.	Days to 50% flowering	48·1 (IC279535)*	88·0 (IC279391)*	62·0	0·63	13·5
7.	Days to 80% maturity	96·0 (IC273927)*	132·7 (IC317641)*	109·7	0·45	5·4
8.	1000 seed weight (g)	2·0 (IC355791)*	5·8 (IC261959)*	3·4	0·05	19·4
9.	Yield/plant (g)	0·3 (IC279408)*	9·3 (IC273927)*	3·9	0·14	47·5

* Accession numbers having maximum or minimum value are given within parenthesis in the respective columns on the right side of the value.

DISCUSSION

The area under study is characterized by considerable diversity in millet types. However, uneven distribution of precipitation and abrupt changes in temperature upsets biochemical processes at cellular and molecular levels, which impedes normal life processes. This in turn affects plant growth and development; plants growing in adverse climatic conditions differ in shape and size compared with those growing in normal/favourable conditions. Vegetative growth under stress conditions, particularly shoot growth, decreases due to slower cell division and growth (Schuppler *et al*. 1998). Significant variation in leaf dimensions in the present study indicates the variable ability of different accessions to adapt to the surrounding environment. This is supported by the studies of Sisó *et al*. (2001) and Pandey & Nagar (2002), who suggested that modifications in leaf shape and size are early symptoms of plant adaptation to growth habitat. It also provides a link between various environmental factors and leaf functions (Roche *et al*. 2004). In addition, plant height is an appropriate determinant of a plant's ability to compete for light (Falster & Westoby 2003), particularly in dense fields of cultivated crops. Plant height is also an important part of life-history traits (Moles & Leishman 2008). Approximately three-fold variation in plant height of the germplasm studied here indicates rich diversity in this particular trait and the ability of plants to adjust to environmental conditions. In adverse environmental conditions, several pheno-morphological traits relate to the competitive ability of a crop to survive and yield optimally, such as plant height (Lindquist *et al*. 1998), leaf angle (Sankhala *et al*. 2004) and crop maturity (Begna *et al*. 2001). Substantial variability found in these traits helps the germplasm to survive and produce seeds in otherwise adverse agro-climatic conditions. These traits are central in determining how a species lives, grows and reproduces. Life-span as well as different phenophases of a crop is affected by surrounding conditions. In plants, switchover from the vegetative to reproductive phase is a crucial turning point, which is under the control of a complex genetic network that integrates information from various endogenous and environmental cues (Amasino 2010). Among the environmental factors that may affect plant growth, only a few appear to be specifically monitored to control flowering (Amasino 2010; Srikanth & Schmid 2011). The shift from vegetative to reproductive phase ensures that plants set their flowers at an optimum time for pollination, seed development and dispersal (Cockram *et al*. 2007). Difference in flowering time of germplasm can be used to increase yield and extend agricultural flexibility as well as the eco-geographical range of crops (Cockram *et al*. 2007). Noteworthy variation in days to 50% flowering and days to 80% maturity of germplasm was found in the current study. Collected germplasm has an inherent ability to grow and produce economic yield in environments entirely different from the site of collection. Considerable variability in yield attributes and yield seems to be due to the net result of direct and indirect effects of the component characters from which grain yield is derived (Prasanna *et al*. 2013).

Furthermore, alteration in the activity of antioxidant enzymes is an adaptation to stress and a defence

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Table 2. *Variability in biochemical traits of barnyard millet (*Echinochloa frumentacea*) genepool of Central Himalayan Region*

S. no.	Parameter	Minimum value	Maximum value	Average value	Standard error (S.E.)	CV %
1.	Chlorophyll content at flowering (mg/g FW)	1·0 (IC418409)*	6·9 (IC548696)*	3·8	0·11	38·2
2.	Carotenoids content at flowering (mg/g FW)	0·6 (IC261951)*	6·3 (IC337304)*	2·3	0·07	43·1
3.	Lipid peroxidation (nmol MDA formed/mg/ protein/h)	0·6 (IC391472)*	7·4 (IC338652)*	3·6	0·13	49·2
4.	Lipoxynase activity (mmol substrate/min/mg protein)	0·1 (IC282785)*	4·0 (IC279391)*	2·0	0·06	37·9
5.	Catalase activity (mmol hydrogen peroxide decomposed/min/mg protein)	109 (IC261951)*	855 (IC340999)*	427	13·4	41·9
6.	Peroxidase activity (mmol substrate/min/mg protein)	1·2 (IC281760)*	6·4 (IC548613)*	3·3	0·09	36·1
7.	Superoxide dismutase activity (enzyme U/mg protein)	1123 (IC261951)*	2963 (IC279703)*	1997	36·7	24·5
8.	Super oxide free radical (O_2.–) formation (nmol hydrogen peroxide formed/ mgprotein)	0·5 (IC261951)*	4·3 (IC469893)*	2·2	0·07	42·9
9.	Total glutatione (mmol/g FW)	105 (IC355792)*	424 (IC355775)*	229	5·1	29·6
10.	Reduced glutathione (mmol/g FW)	96 (IC355792)*	387 (IC355775)*	209	4·6	29·6
11.	Oxidized Glutathione (mmol/g FW)	9·2 (IC355792)*	36·9 (IC355775)*	19·9	0·44	29·6
12.	Glutatione reductase (mmol substrate/min/mg protein)	0·5 (IC355792)*	2·1 (IC355775)*	1·2	0·03	29·6
13.	Total ascorbate content (mmol/g FW)	5·0 (IC261971)*	9·9 (IC355769)*	7·3	0·10	17·6
14.	Ascorbic acid (mmol/g FW)	4·2 (IC548641)*	8·6 (IC393054)*	6·2	0·08	17·6
15.	Dehydroascorbic acid (mmol/g FW)	0·7 (IC418409)*	1·7 (IC355769)*	1·1	0·02	20·2
16.	Ascorbate Peroxidase (enzyme U/mg protein)	1·9 (IC355803)*	7·0 (IC355796)*	4·3	0·11	34·4
17.	Monodehydroascorbate reductase (mmol substrate/min/mg protein)	1·1 (IC391472)*	4·4 (IC355796)*	2·6	0·07	34·6
18.	Dehydroascorbate reductase (mmol substrate/ min/mg protein)	0·3 (IC261951)*	1·4 (IC355796)*	0·8	0·02	35·0

MDA, malondialdehyde.

* Accession numbers having maximum or minimum value are given within parenthesis in the respective columns on the right side of the value.

process. Significant variability in the lipid peroxidation of different accessions denotes the symptom most easily attributed to oxidative damage (Zhang & Kirkham 1996). It also indicates that plants experience oxidative stress (Jagtap & Bhargava 1995), but adjust to survive and produce an economic yield. Variability in lipoxygenase activity may be due to variability in the hydroperoxidation of polyunsaturated fatty acids. Remarkably, eight-fold variability in catalase activity indicates considerable variability of germplasm to tolerate stress conditions. Catalase lowers oxidative damage by converting hydrogen peroxide to water and oxygen (Scandalios *et al.* 1997). Up-regulation of the gene for catalase enzyme activity protects leaves against ROS (Zelitch *et al.* 1991); in contrast, catalase-deficient plants are more sensitive to various stresses (Willekens *et al.* 1997). Moreover, ascorbate peroxidase is a key enzyme in the ascorbate-glutathione cycle, the main hydrogen peroxide-detoxification system found in plant chloroplasts (Asada 1992). Ascorbate peroxidase also plays a role in ROS scavenging in cytosol, mitochondria and peroxisomes (Noctor & Foyer 1998; Asada 1999; Shigeoka *et al.* 2002; Mittler *et al.* 2004). In agreement with a previous report, significant variation in peroxidase activity was found in different accessions due to environmental stress conditions prevailing in CHR (Shigeoka *et al.* 2002). Up-regulation of the peroxidase activity confirms the major role played by these enzymes in defence mechanisms (Jouili *et al.* 2011).

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Table 3. *K-means clustering of germplasm*

S. no.	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
1.	IC261984	IC261959	IC279593	IC261971	IC261951	IC279391	IC262002
2.	IC273927	IC261995	IC279792	IC261999	IC261986	IC279428	IC273988
3.	IC338653	IC262006	IC281416	IC279495	IC279408	IC279493	IC279605
4.		IC279563	IC281760	IC279535	IC279519	IC279481	IC279703
5.		IC279752	IC337304	IC281418	IC279542	IC299482	IC279726
6.		IC281420	IC337305	IC317629	IC279579	IC282785	IC281456
7.		IC281452	IC338634	IC337238	IC281445	IC317522	IC281464
8.		IC281741	IC338648	IC337314	IC316050	IC317641	IC281757
9.		IC281748	IC338652	IC337324	IC337349	IC337276	IC281762
10.		IC317574	IC355784	IC337333	IC340999	IC337292	IC337319
11.		IC355773	IC355796	IC338635	IC355775	IC337295	IC337334
12.		IC355781	IC356395	IC338642	IC355791	IC337316	IC337336
13.		IC355806	IC356406	IC355769	IC355804	IC337317	IC338638
14.		IC392501	IC391472	IC355770	IC355807	IC337328	IC338651
15.		IC393043	IC393054	IC355786	IC383686	IC337329	IC341011
16.		IC393051	IC406556	IC355792	IC391404	IC337332	IC341061
17.		IC469871	IC436950	IC355793	IC391425	IC341101	IC341350
18.		IC469879	IC436977	IC355797	IC391478	IC355778	IC355779
19.		IC548664	IC444161	IC355805	IC548641	IC355780	IC355795
20.		IC548681	IC538037	IC356396	IC548645	IC355803	IC355799
21.		TA-100	IC548658	IC356410	PSM/A/B-46	IC356404	IC355801
22.			IC548696	IC383387	PSM/A/B-51	IC383551	IC382642
23.				IC391367	PSM/A/B-56	IC383673	IC383598
24.				IC392481	PSM/A/B-65	IC391494	IC391440
25.				IC393045		IC391509	IC392507
26.				IC548671		IC392527	IC392517
27.				RRA/M/B/H-5		IC393021	IC392568
28.						IC418380	IC392624
29.						IC444187	IC393031
30.						IC548613	IC393033
31.						IC548621	IC393037
32.						IC548635	IC393040
33.						IC548699	IC393041
34.							IC406555
35.							IC418391
36.							IC418409
37.							IC469750
38.							IC469753
39.							IC469881
40.							IC469889
41.							IC469893
42.							IC469897
43.							IC538047
44.							IC538089
45.							IC548697
46.							IC548710
47.							IC548718
48.							IC717442

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Table 4. *Contribution of the first four principal component axes to variation in barnyard millet (*Echinochloa frumentacea*) based on morpho-physiological and biochemical traits*

S. no.	Parameter	PC 1	PC 2	PC 3	PC 4
1.	Flag leaf length (mm)	0·000032	0·000003	−0·00036	−0·00188
2.	Flag leaf width (mm)	0·000047	0·000036	−0·00039	0·000385
3.	Peduncle length (mm)	0·000674	0·000024	−0·0006	0·001591
4.	Plant height (mm)	0·009075	0·004028	−0·00022	0·03267
5.	Ear head length (mm)	0·000525	0·00056	−0·00036	−3·4 E-^{05}
6.	Days to 50% flowering	0·002949	0·001065	−0·00415	0·00291
7.	Days to 80% maturity	−0·00292	−0·00033	−0·00265	−0·0084
8.	1000 grain weight (g)	−0·00011	−0·00019	−0·00035	−0·00018
9.	Yield/plant (g)	0·001977	−0·0001	−1·1E-05	−0·00096
10.	Yield kg/ha	0·991377	−0·1306	0·001661	−0·0048
11.	Chlorophyll content at flowering (mg/g FW)	0·000083	0·000367	−0·00077	−0·00081
12.	Carotenoids content at flowering (mg/g FW)	−0·00024	−7·3E−05	0·001029	−0·00091
13.	Lipid peroxidation (nmol MDA formed/mg/protein/h)	0·000135	0·000141	−0·00002	−0·00079
14.	Lipoxynase (mmol substrate/min/mg protein)	−0·00013	−9·2E−05	−0·00031	0·001172
15.	Catalase activity (mmol hydrogen peroxide decomposed/min/mg protein)	0·002893	0·034834	0·999358	−0·00579
16.	Peroxidase activity (mmol substrate/min/mg protein)	−0·00021	0·000275	−0·00003	0·001203
17.	Superoxide dismutase activity (enzyme U/mg protein)	0·130566	0·990771	−0·03486	0·008776
18.	Super oxide free radical (O_2.−) formation (nmol hydrogen peroxide formed/mg protein)	−9·7E-05	0·000035	0·000472	0·000837
19.	Total glutatione (mmol/g FW)	0·002431	−0·00682	0·00449	0·736126
20.	Reduced glutathione (mmol/g FW)	0·002222	−0·00624	0·004104	0·67282
21.	Oxidized glutathione (mmol/g FW)	0·000212	−0·0006	0·000392	0·064189
22.	Glutatione reductase (mmol substrate/min/mg protein)	0·000012	−3·4E-05	0·000023	0·003718
23.	Total ascorbate content (mmol/g FW)	0·00014	0·000125	−9E-06	0·000122
24.	Ascorbic acid (mmol/g FW)	0·000134	0·000111	−2·9E-05	0·000042
25.	Dehydroascorbic Acid (mmol/g FW)	0·000005	0·000015	0·000019	0·000079
26.	Ascorbate peroxidase (enzyme U/mg protein)	0·000096	−4·1E-05	0·000921	0·001745
27.	Monodehydroascorbate reductase (mmol substrate/min/mg protein)	0·000047	−3·4E-05	0·000566	0·001015
28.	Dehydroascorbate reductase (mmol substrate/min/mg protein)	0·000016	−2E-06	0·000182	0·000354
29.	Eigen values of the covariance matrix	344671·8	238584·7	31803·99	8460·489
30.	Contribution (%)	55·24	38·24	5·10	1·36
31.	Cumulative contribution (%)	55·24	93·48	98·58	99·94

MDA, malondialdehyde.

However, scavenging of superoxide by SOD is an important mechanism to cope with stress conditions (Bowler *et al.* 1992). A small quantity of superoxide free radical was also found which may be scavenged simultaneously by SOD. Besides, variability in the activity of antioxidant enzymes and variation in the antioxidant pool is a distinctive symptom of stress tolerance. Plants adjust antioxidant levels as an adaptation to stress and a defence process. Differences in total glutathione content of various accessions might be due to 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). In spite of frequent fluctuations in environmental conditions and erratic rainfall, the collected germplasm was able to survive and produce economic yield. This might be due to the protection of cells against the toxic effects of free radicals and the ability to keep free-radical scavenging ascorbate in its reduced, i.e. active form, by glutathione (Zhang & Kirkham 1996). Significant variation in glutathione reductase (EC 1·6·4·2) activity indicates that it sustains the reduced status of glutathione via the ascorbate-glutathione

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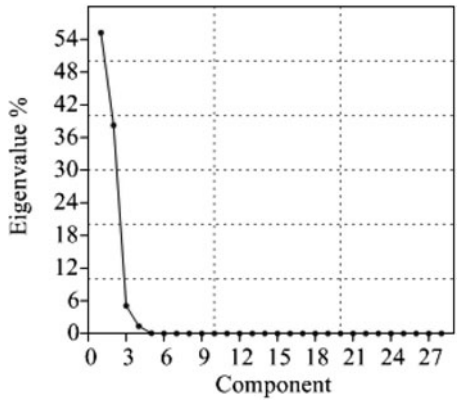

Fig. 2. Scree plot of principal components.

pathway. The activity of glutathione reductase is crucial for stress tolerance because its substrate GSSG, as well as its product GSH, is important for several cellular functions, such as cell division (Rebhun *et al.* 1976), amino acid transport through membranes (Meister 1981) and regulation of enzymatic activity (Holmgren 1979). Correspondingly, variability in the ascorbate and ascorbate-recycling enzymes, i.e. monodehydroascorbate reductase and dehydroascorbate reductase, was also found, which is in agreement with the previous findings of Knörzer *et al.* (1996). These enzymes help to maintain the redox pool of ascorbate and in turn improve stress tolerance (Kim *et al.* 2014). In accordance with earlier reports (Shigeoka *et al.* 2002; Trivedi *et al.* 2015), APX activity was found to increase in response to stress conditions in the field. Although all the accessions were grown at one experimental site, enormous variability was recorded in antioxidant pool size and activity of oxidative stress enzymes, which indicates the potential of accessions to cope with the stress conditions. Based on the clustering of whole germplasm into seven groups having similar traits, appropriate germplasm may be selected for cultivation in areas prone to environmental/abiotic stresses; also climate-compliant accessions may be identified for cultivation in different agro-climatic zones. Rapid perception of abiotic stresses by plants and appropriate estimation of phenological and biochemical adjustments in response to stress are critical to ensure future food security. Based on morpho-physiological evaluation, suitable accessions might be selected for developing climate-resilient varieties.

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