Biochemical markers of environmental stress tolerance in finger millet [*Eleusine coracana* (L.) Gaertn.] germplasm of Central Himalayan Region

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Received: 16 July 2021 / Accepted: 8 May 2022 / Published online: 13 June 2022 © The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract Availability of the germplasm of suitable crops for cultivation in environmental stress prone and resource poor terrains is crucial for food security in these regions. Rich diversity of millets, which are highly tolerant to extreme weather conditions, is obtainable in the Central Himalayan Region (CHR). However, biochemical evaluation of available germplasm for food security is a major apprehension yet to be addressed properly. In this backdrop expeditions were conducted to tap and evaluate the germplasm variability of finger millet [*Eleusine coracana* (L.) Gaertn.]. Germplasm accessions (314) having traits of agronomic importance were collected and evaluated for three consecutive years. In addition to set of biochemical traits, yield was recorded to find out suitable germplasm for large scale cultivation/breeding programme. At flowering stage variability in antioxidants level of leaves such as glutathione and ascorbate ranged from 105.27 to 423.63 mmol g\(^{-1}\) FW and 4.89–10.21 mmol g\(^{-1}\) FW respectively. Extensive varibleness in the activity of enzymes which are important for multiple abiotic stress tolerance viz., catalase (115–855 mmol H\(_2\)O\(_2\) decomposed min\(^{-1}\) mg\(^{-1}\) protein), peroxidase (1.24–6.35 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein), superoxide dismutase (1236–2963 enzyme U mg\(^{-1}\) protein), glutathione reductase (0.53–2.14 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein), ascorbate peroxidase (1.86–7.04 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein), monodehydroascorbate reducatase (1.11–4.41 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein), dehydroascorbate reductase (0.34–1.36 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein) was also recorded.

Worldwide Abiotic/environmental stresses hamper agricultural production. Enzymatic and non-enzymatic defence system precisely controls this situation. Hence, biochemical evaluation might be an appropriate strategy for identification of suitable germplasm to combat the problem of food security in such areas.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10722-022-01405-7.
Keywords  Finger millet · Genetic diversity · Glutathione · Ascorbate · Biomarkers

Introduction

Globally, judicious use of fertilizers and plant protection chemicals, although significantly improved food grain availability; however in turn created a peril to sustainable food production system. Therefore, current focus of agricultural research is towards sustainability, organic production and climate compliant germplasm/varieties to cater the current needs and be fitting for future obligations. CHR is known for its biological richness as well as vulnerability to natural disasters. Unpredictable extreme weather, climate events and natural disasters widespread in this region have the prospective to distress the growth and development of plants and damage agro-biodiversity. As compared to other regions, impact of climate change was found to be more in CHR such as warming in Himalayan Region is about 2–3 times advanced than worldwide average of 0.74 °C during the last 100 years (Du et al. 2004; IPCC 2007) which is adversely affecting plant processes. Although, plants examine their environs continuously and adjust metabolic systems accordingly to optimize yield, however, in different genotypes degree of plasticity varies to a large extent. Therefore, for sustainable food production in such regions, identification, selection and availability of suitable plant genetic resources is a challenge.

As a fast growing cereal crop, it reaches maturity within 3 to 6 months (Dida and Devos 2006). It is commonly found at altitudes between 1000 and 2000 m asl in eastern and southern Africa and up to 2500 to 3000 m asl in the Himalayas (Dida and Devos 2006; FAO 2012). Finger millet [Eleusine coracana (L.) Gaertn.] is grown in more than two dozen countries and contributes approximately 12% millet area round the world (ICRISAT 2008). It is usually grown in rainfed areas, problem soils, abiotic stress prone regions where other crops are not performing well. These areas are mostly in Africa and Asia where food and nutritional security is still a major problem. Finger millet has been recognized as a very much nutritious food for the people of weaker section in the society as well as immuno-endangered people (Takan et al. 2012). In spite of comparatively stable yield in adverse environmental conditions, rich diversity of finger millet has not yet been utilized for crop breeding programmes, not well documented, meagrely researched and potential need to be harnessed properly (Upadhyaya 2008). Reason for yield stability in unfavourable environmental conditions is yet to be worked out to harness the untapped potential. In natural condition plants are open to the different environmental factors and forced to sustain in stressful conditions due to their sessile nature. This in turn adversely affects production and productivity (Shao et al. 2008). Survival and successful reproduction under adverse environmental condition is a complex happening and crucial for sustainable agricultural production and food security. It is well coordinated and in harmony with the set of physiological, cellular and molecular functions of plants (Ahuja et al. 2010). Plants survive in stressful environment at physiological cost (Mas-sad et al. 2012). This becomes a major limitation for growth, development and finally it causes more than 50% loss of yield for main crops (Bray et al. 2000). However, available variability with respect to tolerance of environmental conditions might be harnessed. Disclosure of plants to harsh environmental condition leads to oxidative stress. This causes creation of reactive oxygen species (ROS). ROS interrupts cellular homeostasis and affect cell viability (Alscher et al. 1997; Bartels and Sunkar 2005).

Cellular oxidation–reduction is synchronized with the increase/decrease of ROS and this has vital role in signaling of proper response to changes in the surrounding environmental conditions (Considine et al. 2015). Variation in regulation of this redox cycle in different accessions leads variation in the tolerance of germplasm. Apparently, indistinguishable environmental stresses exist in the field condition which leads to oxidative stress and production of reactive oxygen species (ROS) in plants. Particularly in CHR within plant species variability in production and removal of ROS exists in the germplasm. Certain enzymes viz., superoxide dismutases, catalases, ascorbate peroxidases, glutathione peroxidases as well as some compounds such as ascorbate and glutathione take away ROS (Mittler 2002; Mittler et al. 2004, 2011). However, relationship between the status of these enzymes/antioxidants, performance of plants and yield stability has not been investigated properly particularly in minor millets such as finger millet. Hence, this may be employed as biomarker for
selection of desired genotypes to improve agricultural production in stress prone areas. Finger millet, suffers from limited funding for research, lack of agronomic recommendations and changing farming systems that increase likelihood of genetic erosion. Identification of biomarkers for stress tolerance as well as for selection of suitable varieties for different regions with varying agro climatic conditions is yet lacking. Thus the outcome of the study may be useful for identification and selection of suitable genotypes for conservation, crop improvement, large scale cultivation and improving production of finger millet as well as gene bank management (Fig. 1).

Materials and methods

Plant material

To collect the untapped genetic diversity of finger millet available in *Eleusine coracana* (L) Gaertn] available in the CHR, exploration expeditions were

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**Fig. 1** Graphical Abstract

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conducted in the different remote and inaccessible areas of this region (Fig. 2a–c). Collection was done from altitudinal range of 225–2250 m amsl and a total of 314 accessions having traits of agronomic importance were collected.

Evaluation of the collected germplasm was done in the field, in rain fed condition at an experimental site located at 29° 24′ 28.7″ N latitude, 79° 30′ 47.2″ E longitude and 1480 m asl altitude. Four checks (two improved varieties released for this region i.e., VL-146, VL-149 and two local accessions widely cultivated in the region i.e., Almora Local and Pithoragarh Local) were used for comparison to evaluate the collected germplasm. Experiments were conducted during June–October (Kharif season) for three successive years i.e., 2011–2013 in augmented block design (ABD). Ten representative plants of each accession were tagged in each block. Five tagged plants were used for recording the observations (biochemical estimations) and remaining five plants for yield analysis (Trivedi et al. 2015).

Biochemical analysis

Catalase (EC 1.11.1.6) activity has been determined by consumption of hydrogen peroxide (Rao et al. 1996) and peroxidase (EC 1.11.1.7) activity by the method of Cordemener et al. (1991). 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 radical (O$_2^-$) has been determined following the method of Wang and Luo (1990).

Glutathione content [reduced glutathione (GSH) and oxidized glutathione (GSSG)] has been determined enzymatically using the method of Griffith (1980). Determination of glutathione by this method is based on the specificity of glutathione reductase. Total glutathione and GSSG contents were determined directly and GSH after subtraction of GSSG

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**Fig. 2** a Map of India (left upper); b Central Himalayan Region i.e., Uttarakhand (distribution map) (right upper); c ICAR-NBPGR, Regional Station Bhowali i.e., Location of experimental site (right lower) with amplification.
from total glutathione. To determine GSSG, GSH of the sample was derivatized by adding 8 µL of 2-vinylpyridine to 200 µL of metaphosphoric extract that has been neutralized with 72 µL of 1 M triethanolamine. After vortexing for 30 s, the mixture was allowed to incubate for 1 h at 25 °C. An aliquot of the extract (40 µL of metaphosphoric extract and 150 µL of the 2-vinylpyridine derivatized extract) was added to the reaction medium for total glutathione and GSSG determination respectively which consists of phosphate buffer (50 mM, pH 7.5), EDTA (2.5 mM), DTNB (1 mM), GR (0.5 unit, baker yeast type III, Sigma), NADPH (0.1 M) in a final volume of 1 mL at 25 °C. Reaction was started with the addition of the NADPH, the increase in absorbance at 412 nm was monitored for 3 min at 25 °C. Calibration curves were drawn using standards of GSH (1.6–80 lM) and GSSG (0.8–40 lM) prepared in 5% (w/v) metaphosphoric acid. Glutathione reductase (GR) (EC 1.6.4.2) and glutathione S-transferase (GT) (EC 2.5.1.18) activity was measured by Smith et al. (1988) and Drozda et al. (1985) respectively.

Ascorbate content [both reduced (AsA) and oxidized (DAsA)] 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 DAsA after reduction and subtraction of AsA from total ascorbate. For AsA determination, the metaphosphoric acid extract was neutralized with 25 µL of 1.5 M triethanolamine, vortexed and potassium phosphate buffer (150 µL, 0.15 M, pH 7.4) as well as H2O (150 µL) were added. Ten percent (w/v) trichloroacetic acid (300 µL), 44% (v/v) phosphoric acid (300 µL), 4% (w/v) 2,2’-dipyridyl (300 µL 70% ethanol) and 3% (w/v) FeCl3 (150 µL) were added successively to the mixture. After vortexing, samples were incubated at 37 °C for 60 min and absorbance was recorded at 525 nm. For determination of total ascorbate, triethanolamine and potassium phosphate buffer were added to the metaphosphoric extract as described above and then 75 µL dithiothreitol (DTT) 10 mM was added to the sample instead of water. The mixture was incubated for 15 min at 25 °C to reduce DAsA to AsA. Surplus DTT was removed by adding 75 µL of 0.5% (w/v) N-ethylmaleimide. After mixing, the samples were incubated for 2 min at 25 °C and determination of AsA proceeded as above. Standards of AsA and DAsA (50–600 µM) prepared in 5% (w/v) metaphosphoric acid were used for calibration. Monodehydro ascorbate reductase (MDHAR) (EC 1.6.5.4) and Dehydro ascorbate reductase (DHAR) (EC 1.8.5.1) activity was assayed according to method given by Hossain et al. (1984) and Hossain and Asada (1984) respectively. Yield in terms of dry grain weight was recorded after harvesting.

**Yield analysis**

Ten representative plants of each accession were tagged in each block for recording observations. Five tagged plants were used for recording the observations (biochemical estimations) and remaining five plants were used for estimation of yield. Leaves of plants tagged for yield estimation were kept intact for precise yield estimation. Plant to plant and row to row spacing of 20 cm and 30 cm respectively was maintained. Yield of five representative plants of each accession in each block was recorded and average yield is expressed as yield per plant.

**Statistical analysis**

The statistical analysis was performed using statistical software SAS 9.3. PROC GLM model was used for data analysis which analyses data within the framework of general linear models. The GLM procedure uses the method of least squares to fit general linear models (Rathore et al. 2004; Bairwa et al. 2015). Model: Total variance = (variance between blocks) + (variance within blocks) or simply, denoting X the data matrix (318×17); 314 variety plus 4 checks compare over 17 parameters i.e., VAR(X) = B(X) + W(X).

**Results**

Catalase enzyme is responsible for the degradation of hydrogen peroxide, its activity ranged from 115 to 855 μmol hydrogen peroxide decomposed min⁻¹ mg⁻¹ protein (Fig. 3a). Likewise, peroxidase activity varied from 1.24 to 6.35 μmol substrate min⁻¹ mg⁻¹ protein (Fig. 3b). Dismutation of superoxide radicals (O₂⁻) to molecular oxygen (O₂) and hydrogen peroxide (H₂O₂) is catalyzed by superoxide dismutase. Activity of this important enzyme
Fig. 3  a Catalase activity (mmol hydrogen peroxide decomposed min$^{-1}$ mg$^{-1}$ protein). Best check variety/accession for catalase activity is VL-149, accession with minimum value is IC317429 and accession with maximum value is IC392487. b Peroxidase activity (m mol substrate min$^{-1}$ mg$^{-1}$ protein). Best check variety/accession for peroxidase activity is VL-146, accession with minimum value is IC356039 and accession with maximum value is IC337320. c Superoxide dismutase activity (enzyme U mg$^{-1}$ protein). Best check variety/accession for superoxide dismutase activity is VL-149, accession with minimum value is IC279406 and accession with maximum value is IC355815. d Super oxide free radical ($O_2^-$) formation (nmol hydrogen peroxide formed mg$^{-1}$ protein). Best check variety/accession for super oxide free radical ($O_2^-$) formation is Pithoragarh Local, accession with minimum value is IC355771 and accession with maximum value is IC337308.
was found to vary 1236–2963 enzyme U mg\(^{-1}\) protein (Fig. 3c). As well, superoxide free radical was also found to vary 0.63–4.29 nmol hydrogen peroxide formed mg\(^{-1}\) protein (Fig. 3d).

Total glutathione content in the collected gene pool were found to vary from 105.27 to 423.63 mmol g\(^{-1}\) FW. In the best available genotype (best check) glutathione content were 296.41 mmol g\(^{-1}\) FW (Fig. 4a), which is only 69.96% of maximum value available in the collected germplasm. Plants maintain major part of antioxidant in the reduced form, however, drastic variability was found in reduced glutathione content among genotypes which varied 96.22 to 387.20 mmol g\(^{-1}\) FW (Fig. 4b), ensuing variability in oxidized glutathione content was also found i.e., oxidized glutathione content ranged 9.18 to 36.94 mmol g\(^{-1}\) FW (Fig. 4c). Variation in glutathione reductase (GR) activity was in accordance with the reduced glutathione content. GR activity varied 0.53 to 2.14 m mol substrate min\(^{-1}\) mg\(^{-1}\) protein (Fig. 4d).

Similarly, variation in the total ascorbate content among genotypes was 4.89 to 10.21 mmol g\(^{-1}\) FW (Fig. 5a), whereas ascorbic acid content varied 4.24 to 8.60 mmol g\(^{-1}\) FW (Fig. 5b). Dehydroascorbic acid (oxidized form of ascorbic acid) contents were found to vary 0.68 to 1.66 mmol g\(^{-1}\) FW (Fig. 5c). In different accessions, noteworthy variability was found in the ascorbate peroxidase activity which ranged from 1.86 to 7.04 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein (Fig. 5d). Activity of monodehydroascorbate reductase, an enzymatic component of the glutathione-ascorbate cycle was found to vary 1.11 to 4.41 mmol substrate min\(^{-1}\) mg\(^{-1}\) protein (Fig. 5e). Similarly, dehydroascorbate reductase activity varied 0.34 to 1.36 m mol substrate min\(^{-1}\) mg\(^{-1}\) protein (Fig. 5f).

Ample variation in the grain yield of collected germplasm was found which ranged 4.39 to 12.82 g plant\(^{-1}\) (Fig. 6). Plants need optimal temperature and moisture during sowing, germination and at seedling establishment stage. However, irregular temperature and precipitation pattern at the experimental site (Table 1) exposes plants to multiple environmental stresses including radical variation in the soil moisture level in this mountainous terrain. In the year 2011 there was 459 mm rain fall during the month of June while in the year 2012 it was only 84.4 mm, again during the year 2013 there was 524.5 mm rain-fall. Plants face similar fluctuations in environmental variables during the whole life span. Temperature variation influences most of the plant activities including seed germination, plant growth, development, crop maturation and yield. Considerable variation in the mean monthly minimum and maximum temperature was recorded during the life span of crop; amplitude of diurnal variation was quite large.

By the use of clustering data sets are partitioned into groups such that the similarity within a group is larger than among the groups. Cluster analysis was done by Ward’s method (Cophen. Corr.: 0.8406) to divide the observations into homogeneous and distinct groups, two main clusters were formed. These clusters were further divided into five sub clusters. Hence, k-means clustering was done to divide all the 318 accessions into five clusters (Table 2, Fig. 7). Accessions with similar traits were found to group together. One hundred twenty four accessions grouped in cluster one have very close similarity in the traits used in biochemical evaluation, this cluster includes two check accessions also, which are widely cultivated in the region. Amazingly, two local accessions widely cultivated in the region have similar traits, used as check (Almora Local and Pithoragarh Local) group together in cluster 1 and two improved varieties released for cultivation in the region (VL-146, VL-149), used as check group together in cluster 3. Cluster 5 has minimum number of accessions i.e., 31; these all accessions show close similarity in the enzymes activity as well as antioxidant levels. Accessions present in a cluster may be useful for selection of similar genotypes for a particular trait as well as for breeding.

It is evident from principal component analysis and percentage contribution of each component to the total variation (Table 3) that first four variables contributed 99.99802 percent of the total variability. First component accounted for 86.218%, second principal component contributed 9.7035%, third principal component 4.0733% and fourth principal component contributed 0.00322% of the total variability. Moreover,
**Fig. 4** a Total glutathione content (mmol g⁻¹ FW). Best check variety/accession for total Glutathione Content is Pithoragarh Local, accession with minimum value is IC281479 and accession with maximum value is IC281460. b Reduced glutathione content (mmol g⁻¹ FW). Best check variety/accession for Reduced Glutathione Content is Pithoragarh Local, accession with minimum value is IC281479 and accession with maximum value is IC392526. c Oxidized Glutathione Content (mmol g⁻¹ FW). Best check variety/accession for Oxidized Glutathione Content is Pithoragarh Local, accession with minimum value is IC281479 and accession with maximum value is IC281460. d Glutathione reductase activity (mmol substrate min⁻¹ mg⁻¹ protein). Best check variety/accession for Glutathione Reductase Activity is Pithoragarh Local, accession with minimum value is IC266857 and accession with maximum value is IC281460.
Fig. 5  a Total ascorbate content (mmol g⁻¹ FW). Best check variety/accession for Total Ascorbate Content is Almora Local, accession with minimum value is IC275038 and accession with maximum value is IC281419. b Ascorbic Acid Content (mmol g⁻¹ FW). Best check variety/accession for Ascorbic Acid is Almora Local, accession with minimum value is IC279585 and accession with maximum value is IC282792. c Dehydroascorbic acid content (mmol g⁻¹ FW). Best check accessions for Dehydroascorbic Acid are Almora Local, accession with minimum value is IC279469 and accession with maximum value is IC281448. d Ascorbate Peroxidase Activity (mmol substrate min⁻¹ mg⁻¹ protein). Best check variety/accession for Ascorbate Peroxidase Activity is Pithoragarh Local, accession with minimum value is IC281747 and accession with maximum value is IC361360. e Monodehydroascorbate Reductase Activity (mmol substrate min⁻¹ mg⁻¹ protein). Best check variety/accession for Monodehydroascorbate Reductase Activity is Pithoragarh Local, accession with minimum value is IC279487 and accession with maximum value is IC273556. f Dehydroascorbate Reductase Activity (mmol substrate min⁻¹ mg⁻¹ protein). Best check variety/accession for Dehydroascorbate Reductase Activity is Pithoragarh Local, accession with minimum value is IC356397 and accession with maximum value is IC361360.
from the ANOVA of the Augmented Block Design analysis (Table 4) it is obvious that there is significant variability in biochemical traits of finger millet diversity available in the Central Himalayan Region which can be harnessed for crop improvement.
From scree plot of the principal components (Fig. 8), it is evident that only four principal components are considerably contributing towards diversity. However, PCA score plot (Fig. 9) depicts grouping of germplasm in 5 main groups. Due to slight variation in traits total 8 groups were found. However, Discriminant Analysis of Principal Components (DAPC), which is a multivariate method designed to identify and describe clusters of genetically related individuals clearly show 5 clusters (Figs. 10, 11, 12). When curve plot was drawn all varieties were grouped in five clusters represented by 05 curves over discriminant function in DAPC analysis (Fig. 12).

Discussion

The CHR of India is facing a great challenge to produce more and more from diminishing per capita arable land where intensity and frequency of abiotic/environmental stresses is comparatively more than other regions. Climate change, deforestation and increasing anthropogenic activities are affecting sustainable traditional agricultural system of the region. Plant growth, development and production of economic yield depend largely on the environmental conditions during different phases of growth and development. However, under natural condition in the field, plants face multiple environmental stresses. In the fluctuating environmental conditions in the field, accessions of a crop which survive, sustain the adverse condition and produce economic yield might have specific biochemical regulation. In most of the cases experiments designed for stress tolerance are conducted in the laboratory. Usually, such experiments consider plant responses to one stress at a time (Todaka et al. 2012; Qin et al. 2011). However, the response to multiple stresses is much more complex (Fujita et al. 2006). Hence, in the present investigation, experiments were conducted in the field to assess and compare the relative performance of whole
finger millet germplasm of CHR of India and screen out suitable accessions (Figs. 13, 14).

Following the exposure of plant to environmental stress, reactive oxygen species (ROS) are generated quickly (Laloi et al. 2004; Foyer and Noctor 2005). This leads to reprogramming of the genetic machinery, in turn causes an increase in tolerance to abiotic/environmental stress. More expression of the gene for

### Table 2 K-Mean clustering of the germplasm

| S. No. | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|--------|-----------|-----------|-----------|-----------|-----------|
| 1      | IC261946  | IC281432  | IC337282  | IC361318  | IC266775  |
| 2      | IC261948  | IC281435  | IC337298  | IC361332  | IC273356  |
| 3      | IC261950  | IC281450  | IC337299  | IC381467  | IC279406  |
| 4      | IC261962  | IC281462  | IC337302  | IC383631  | IC279422  |
| 5      | IC261968  | IC281465  | IC337311  | IC385876  | IC279469  |
| 6      | IC261969  | IC281473  | IC337315  | IC391402  | IC279503  |
| 7      | IC261972  | IC281476  | IC337320  | IC391434  | IC281437  |
| 8      | IC261979  | IC281482  | IC337321  | IC391462  | IC281439  |
| 9      | IC261998  | IC281485  | IC337326  | IC392484  | IC281460  |
| 10     | IC266735  | IC281754  | IC337331  | IC392516  | IC281477  |
| 11     | IC266759  | IC281756  | IC338637  | IC392548  | IC281488  |
| 12     | IC266909  | IC281758  | IC338644  | IC392564  | IC281743  |
| 13     | IC266941  | IC281763  | IC338646  | IC393023  | IC282787  |
| 14     | IC273998  | IC281766  | IC338649  | IC393029  | IC282792  |
| 15     | IC274001  | IC281768  | IC341035  | IC393030  | IC282795  |
| 16     | IC274172  | IC281770  | IC341363  | IC393034  | IC316036  |
| 17     | IC275038  | IC282790  | IC355774  | IC393044  | IC317429  |
| 18     | IC279342  | IC282797  | IC355809  | IC393046  | IC337226  |
| 19     | IC279414  | IC316025  | IC355813  | IC393049  | IC337252  |
| 20     | IC279585  | IC316027  | IC355814  | IC393061  | IC337308  |
| 21     | IC279610  | IC316030  | IC355815  | IC436959  | IC337323  |
| 22     | IC279830  | IC316035  | IC355816  | IC436973  | IC337325  |
| 23     | IC279843  | IC316037  | IC355817  | IC441160  | IC337337  |
| 24     | IC281415  | IC316040  | IC355818  | IC444225  | IC338562  |
| 25     | IC281419  | IC316048  | IC355819  | IC469853  | IC338643  |
| 26     | IC281422  | IC317454  | IC355821  | IC469877  | IC345586  |
| 27     | IC281423  | IC317458  | IC356000  | IC538018  | IC345599  |
| 28     | IC281424  | IC317321  | IC356400  | IC538079  | IC356413  |
| 29     | IC281426  | IC337237  | IC356402  | IC538090  | IC361360  |
| 30     | IC281429  | IC337244  | IC356411  | IC538090  | IC361360  |
| 31     | IC281431  | IC337248  | IC356412  | IC538090  | IC392487  |
| 32     | IC281434  | IC337250  | IC356419  | IC538090  | IC392487  |
| 33     | IC316043  | IC355788  | IC469865  | IC538090  | IC392487  |
| 34     | IC337269  | IC469865  | IC538090  | IC392487  | IC392487  |
| 35     | IC356413  | IC469865  | IC538090  | IC392487  | IC392487  |
| 36     | IC356414  | IC469865  | IC538090  | IC392487  | IC392487  |
| 37     | IC418381  | IC469865  | IC538090  | IC392487  | IC392487  |
| 38     | IC538058  | IC469865  | IC538090  | IC392487  | IC392487  |
| 39     | IC469890  | IC469890  | IC538090  | IC392487  | IC392487  |
Fig. 7 Dendogram of K-mean clustering of varieties/accessions
catalase enzyme activity protects leaves against ROS (Zelitch et al. 1991), while catalase deficient plants are more sensitive to various stresses (Willekens et al. 1997). More than seven fold variability was found in the catalase activity of different accessions which will lead to considerable variation in the environmental stress tolerance of the germplasm. Catalase lowers oxidative damage by converting hydrogen peroxide to water and oxygen (Scandalios et al. 1993). On the other hand, taking away 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). The inconsistency in the activity of peroxidases in different accessions may be hypothesized as either due to difference in induction by substrate that may be H₂O₂ 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 environmental stress. Under stress conditions, the quantitative and qualitative profiles of peroxidases are usually altered.

Table 3 Contribution of the first four principal component axes to variation in finger millet [Eleusine coracana (L) Gaertn] based on biochemical traits

| S. No. | Parameter                                                                 | PC 1     | PC 2     | PC 3     | PC 4     |
|-------|---------------------------------------------------------------------------|----------|----------|----------|----------|
| 1     | Catalase activity (mmol hydrogen peroxide decomposed min⁻¹ mg⁻¹ protein) | 0.091528 | 0.99566  | −0.01635 | −0.00219 |
| 2     | Peroxidase activity (m mol substrate min⁻¹ mg⁻¹ protein)                  | 0.000218 | −0.00134 | 0.002594 | −0.05182 |
| 3     | Superoxide dismutase activity (enzyme U mg⁻¹ protein)                     | 0.99579  | −0.09143 | 0.006329 | 0.000808 |
| 4     | Super oxide free radical (O₂⁻) formation (nmol hydrogen peroxide formed mg⁻¹ protein) | −0.00043 | −0.0014  | 0.001497 | 0.006939 |
| 5     | Total glutathione (mmol g⁻¹ FW)                                           | −0.00371 | 0.012934 | 0.73645  | 0.30714  |
| 6     | Reduced Glutathione (mmol g⁻¹ FW)                                         | −0.00302 | 0.010679 | 0.67323  | −0.40352 |
| 7     | Oxidized Glutathione (mmol g⁻¹ FW)                                        | −0.00075 | 0.002389 | 0.063256 | 0.71181  |
| 8     | Glutathione Reductase (mmol substrate min⁻¹ mg⁻¹ protein)                  | −1.72E⁻⁰⁵ | 5.70E⁻⁰⁵ | 0.003717 | 0.00229  |
| 9     | Total Ascorbate (mmol g⁻¹ FW)                                             | 0.000624 | −0.00188 | 0.000213 | −0.0637  |
| 10    | Reduced Content (mmol g⁻¹ FW)                                             | 0.000566 | −0.00179 | 0.00035  | −0.08039 |
| 11    | Dehydroascorbic Acid (mmol g⁻¹ FW)                                        | 2.11E⁻⁰⁵ | −0.00031 | 0.000297 | 0.00224  |
| 12    | Ascorbate Peroxidase (mmol substrate min⁻¹ mg⁻¹ protein)                   | 0.000697 | 0.00031 | 0.006458 | 0.1471   |
| 13    | Monodehydroascorbate Reductase (m mol substrate min⁻¹ mg⁻¹ protein)       | 0.00039  | 0.000134 | 0.004039 | 0.09881  |
| 14    | Dehydroascorbate Reductase (m mol substrate min⁻¹ mg⁻¹ protein)           | 0.000131 | 2.84E⁻⁰⁵ | 0.001254 | 0.029074 |
| 15    | Yield Plant⁻¹ (g)                                                         | 0.000433 | −0.00117 | 0.00178  | −0.43835 |
| 16    | Eigen values of the covariance matrix                                      | 263.070  | 29.6076  | 12.4287  | 9.82739  |
| 17    | Contribution (percent)                                                     | 86.218   | 95.9215  | 99.9948  | 99.99802 |
| 18    | Cumulative contribution (percent)                                          | 86.218   | 95.9215  | 99.9948  | 99.99802 |
Such modifications are evidence of fundamental role played by these enzymes in the defence mechanism (Jouili et al. 2011).

Besides the variability in the activity of antioxidant enzymes, drastic variability in the redox state of antioxidant pool size was also found. Variation in the total glutathione content of various genotypes indicates variability in the capacity of these accessions to overcome environmental stress. Glutathione (GSH), an antioxidant, exerts number of functions in plants (Paranhos et al. 1999). To safe guard the cells against the toxic effects of the free radicals, to keep free-radical scavenging ascorbate in its reduced i.e., active form by participation in the ascorbate–glutathione cycle are major functions of glutathione (Zhang and Kirkham 1996). In stress tolerance, function of GSH as a reductant in ascorbate regeneration is well known ((Aono et al. 1995; Mano et al. 1997) however, chemotyping of finger millet is yet lacking to screen the large population of germplasm. In addition, drastic variability in the glutathione reductase (EC 1.6.4.2)
activity was also found which sustains the reduced status of GSH via ascorbate–glutathione pathway. Accessions found to be comparatively tolerant to environmental stress conditions have higher glutathione reductase activity that might be more availability of its substrate i.e., GSSG during stress condition as well as higher demand of its product GSH to sustain. Product GSH is important for a broad range of functions in the cell, for example, cell division (Rebhun et al. 1976), amino acid transport through membranes (Meister 1981), regulation of enzymatic activity (Holmgren 1979) etc. Environmental stress tolerance in genotypes having more GSH might be due to regulation of related enzymes at different levels.

Similarly, considerable variability in the ascorbate and ascorbate recycling enzymes (i.e., monodehydroascorbate (MDA) reductase and dehydroascorbate (DHA) reductase) activity was also found which increases in stress condition (Knorzer

Fig. 11 Distribution of 318 varieties (314 accessions and 4 checks) in 05 clusters with highlighted centroids (cross) in DAPC analysis of data matrix 318×17

Fig. 12 Varieties in five clusters represented by 05 curves over discriminant function-1 in DAPC analysis

Fig. 13 Yield LS-Mean showing distribution of variation in the yield of varieties/accessions

Fig. 14 Yield comparison for treatments
et al. 1996) and helps to stress tolerance. Recycling through these enzymes is essential for survival of plants. In absence of ascorbate recycling, \( H_2O_2 \) would deplete the available ascorbate pool via ascorbate peroxidase within minutes (Hossain et al. 1984). Substantial variability in the activity of ascorbate peroxidase (APX) enzyme that detoxifies peroxides using ascorbate as a substrate was recorded. This is a key enzyme in the ascorbate–glutathione cycle. This cycle is the main hydrogen peroxide detoxification method in plants (Asada 1992). APX and ascorbate–glutathione cycle is crucial in ROS scavenging in chloroplast, cytosol, mitochondria and peroxisomes (Mittler et al. 2004; Shigeoka et al. 2002). Glutathione dependent DHA reductase activity recycles DHA to ascorbate (Hossain and Asada 1984). DHA is an indispensable component of ascorbate turnover as well as the ascorbate redox system (Foyer and Mullineaux 1998). An increase of DHA reductase activity as well as accumulation of DHA is biochemical indicators of oxidative stress in plant metabolism (Wise 1995). Considerable increase in the DHA and corresponding increase in the DHA activity itself explicate the stress condition prevailing in the CHR and urgent need to screen the available germplasm to promote for large scale cultivation to ascertain the food security in this as well as other such areas.

In accordance with the present findings changes in oxidative stress related enzymes and redox state of antioxidant pool was found to be related with stress tolerance in proso millet \([Panicum miliaceum]\) (Trivedi et al. 2015) and barnyard millet \([Echinochloa frumentacea]\) (Trivedi et al. 2017) also. Enormous variability has been recorded in antioxidant pool as well as activities of oxidative stress enzymes which indicate potential of genotypes to deal with the stress condition. Stress compliant genotypes have been found to have more reduced glutathione as well as ascorbate. Reduced form of antioxidants in different accessions seems to be a suitable biomarker for chemotyping and germlasm screening particularly in this crop. In accordance with the clustering of whole germplasm into five clusters, germplasm within a cluster having similar traits, appropriate germplasm may be selected for cultivation in different areas prone to environmental/abiotic stresses/cultivation in different agro climatic zones (Boyle and Montgomery, 1996; Jombart et al., 2010). Quick sensitivity of plants to abiotic stresses and appropriate estimation of biochemical adjustments in response to stress are decisive to ensure future food security. Biochemical evaluation might be suitable approach for selection of suitable accessions for developing climate resilient varieties. Since the CHR has witnessed several climate cycles in the geological past, the available germplasm should have experienced several changes, hence evaluation of whole germplasm is important to find out superior available to deal with present/future challenges.

**Conclusion**

Plants adjust redox status of antioxidants as an adaptation to stress and activity of related enzymes shifts accordingly. Measurement of redox status of antioxidants rather than total antioxidant pool might be an appropriate approach to find out genotypes for cultivation in stress prone regions. In addition corresponding variability in the activity of stress related enzymes found to be a distinguishing indicator of stress tolerance. Genotypes having more reduced antioxidants (glutathione and ascorbate) have consistently better performance and have comparatively stable yield. Redox state of antioxidants might be a suitable biomarker for chemotyping and harnessing the unexploited genetic diversity of finger millet for improving agricultural production and food security. In different regions agricultural systems are challenged by climate change and abiotic/environmental stresses. Hence, sustainable and judicious utilization of untapped genetic diversity might help farmers to adapt the change and sustain their livelihoods.

**Acknowledgements** Authors are thankful to Director, ICAR—National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi for providing necessary facility and keen interest in the study. This manuscript is dedicated to Prof. A. Hemantarajan, who left for heavenly abode on May 31, 2021 at the age of 66, after a courageous battle with post-covid severe lung infection.

**Funding** This work was done under ICAR-NBPGR institute project number PGR/PGC-BUR-BHO-01.01.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.
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