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# Diversity and trait-specific sources for productivity and nutritional traits in the global proso millet (*Panicum miliaceum* L.) germplasm collection



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# A R T I C L E I N F O

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# ABSTRACT

Proso millet is an important short-duration crop that adapts well to varied climatic conditions and is grown worldwide for food, feed and fodder purposes. Owing to a lack of genetic improvement, the crop has experienced no yield improvement and provides low income to farmers. In this study, 200 accessions of proso millet originating in 30 countries were evaluated in two rainy seasons to assess phenotypic diversity for morpho-agronomic and grain nutritional traits and to identify high grain-yielding and grain nutrient-rich accessions. Proso millet diversity was structured by geographical region, by country within region, and by racial group. Race patentissimum showed high diversity and ovatum low diversity, and diverged widely from each other. The lowest divergence was observed between races compactum and ovatum. Eighteen high grain-yielding, 10 large-seeded, and 26 two or more grain nutrients-rich accessions were identified, and highly diverse pairs of accessions within and between trait groups were identified. They included IPm 9 and IPm 2661 for high grain yield and large seed size; and IPm 2069, IPm 2076, and IPm 2537 for high Fe, Zn, Ca, and protein contents. IPm 2875 had a seed coat that is readily removed by threshing. This study provides valuable information to proso millet researchers about agronomic and nutritional traits in accessions that could be tested for regional adaption and yield for direct release as cultivars, and could be used in breeding for developing high grain-yielding and nutrient-rich cultivars.

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# 1. Introduction

E-mail address: h.upadhyaya@cgiar.org (H.D. Upadhyaya). Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS. Small millets, including finger millet (Eleusine coracana (L.) Gaertn.), foxtail millet (Setaria italica (L.) P. Beauv.), proso millet (Panicum miliaceum L.), kodo millet (Paspalum scrobiculatum L.), little millet (Panicum sumatrense Roth. ex. Roem. & Schult.), and barnyard millet (Echinochloa crus-galli (L.) P. Beauv. and E. colona (L.) Link) are well adapted to diverse climatic conditions and play an important role in food and nutritional security in rural

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households in regions where these crops are grown. Proso millet is one of the important small millets, commonly known as broomcorn millet, common millet, hog millet, Russian millet, and by other names in different regions. Vavilov [1] suggested China as the center of diversity of proso millet, while Harlan [2] suggested that proso millet was probably domesticated in China and Europe. Proso millet grows at a wide range of altitudes, with a short growth cycle of 6 to 12 weeks, and requires little water for growth and development. It is grown in northern China, Mongolia, Republic of Korea, southeastern Russia, Afghanistan, Pakistan, India, and southern Europe. The cultivation area of proso millet is 0.82 m ha in Russia, 0.7 to 1.0 m ha in China [3], 0.5 m ha in India [4] and 0.20 m ha in USA [5]; however, the area in proso millet cultivation is declining owing to a shift to cultivation of major crops that give much higher yields and profit.

Proso millet is used for feeding birds and as livestock feed in developed countries and for food in some parts of Asia [6]. In the USA, most of the proso millet crop is used for birdfeed and in cattle-fattening rations [5]. Nutritionally, proso millet grains are rich in protein, vitamins, minerals, and micronutrients including iron, zinc, copper and manganese, compared to other staple cereals [7]. The protein content of proso millet (12.5%) is comparable with that of wheat and its grains are richer in essential amino acids (leucine, isoleucine, and methionine) than those of wheat [8, 9]. The husked grain is nutritious and is eaten whole, boiled, or cooked like rice (Oryza sativa L.). Sometimes it is ground to make roti (flatbread). Starch is the main carbohydrate in the grain and is similar to corn starch; it is suitable as a sizing agent in the textile industry. Green plants are excellent fodder for cattle and horses and are also used as hay. Proso millet can be a substrate for distilled liquors and beers and is used to make fermented beverages in Africa and Asia. Proso millet has been receiving growing interest from food industries in Europe and North America because of its mild flavor, light color, glutenfree quality, and potential health benefits [3].

Grain yield enhancement is one of the main objectives of proso millet breeding programs; however, limited cropimprovement research efforts and production and processing technologies result in low yields and farmer profit. Cultivars of proso millet have been developed mostly by selection from landraces and a few by hybridization followed by selection (http://www.aicrpsm.res.in/Releasevarities.html) [5]. Globally, over 29,000 germplasm accessions of proso millets have been conserved in genebanks, with the major collections of proso millet germplasm accessions held in Russia, China, Ukraine, and India [10]. In the ICRISAT genebank, 849 accessions of proso millet are held, and a core collection (106 accessions) representing the entire collection has been established [11]. Assessment of proso millet germplasm diversity for various morpho-agronomic and grain quality traits and their use in crop improvement programs would help in breeding highyielding cultivars, would contribute to crop and diet diversification, and would reduce micronutrient and protein malnutrition. No comprehensive study of yield and grain nutritional trait diversity in proso millet is available. The present study was initiated to assess the phenotypic diversity of the global proso millet germplasm collection for morpho-agronomic and grain nutritional traits, to assess association of grain yield and

contributing traits with grain nutritional traits, and to identify high grain-yielding and grain nutrient-rich accessions for use in proso millet improvement.

# 2. Materials and methods

#### 2.1. Experimental details

A total of 200 accessions including 106 from a core collection [11] originating in 30 countries, representing five races of proso millet (60.5% miliaceum, 12.5% compactum, 12.0% contractum, 8.5% patentissimum, and 6.5% ovatum), were included in this study. These accessions were selected using the cluster information of proso millet which was used to assemble core collection [11]. This set represents 24% of the entire collection and 22% to 27% of each race of proso millet germplasm conserved at the ICRISAT genebank. These accessions were evaluated for morpho-agronomic traits during the 2015 and 2016 rainy seasons at ICRISAT, Patancheru, Telangana, India (17°51′N, 78°27′E, 545 m a.s.l.). Accessions were planted in the third week of July in both years, in an alpha design with two replications. The experiments were planted on red soils (Alfisols) and seed was sown on ridges 60 cm apart. Each accession occupied a single row of 4 m length, with a plant-to-plant spacing of 10 cm. Diammonium phosphate was applied at the rate of 100 kg ha<sup>-1</sup> as a basal dose to supply nitrogen and phosphorus. In addition, 100 kg ha<sup>-1</sup> of urea was applied as top dressing. The precision fields at the ICRISAT center have uniform fertility and a gentle slope of 0.5%. Irrigation and hand weeding were applied as needed.

#### 2.2. Data collection

Observations for eight qualitative and 14 agronomic traits were recorded following the descriptors of *P. miliaceum* [12]. Data for all qualitative traits (growth habit, culm branching, sheath pubescence, ligule pubescence, leaf pubescence, inflorescence shape, seed color, and apiculus color), and three agronomic traits namely days to 50% flowering, days to maturity, and grain yield were recorded on a plot basis. The agronomic traits namely plant height, basal tiller number, flag leaf blade length, flag leaf blade width, flag leaf sheath length, peduncle length, panicle exsertion, inflorescence length, number of nodes per main stem, and inflorescence primary branch number were recorded on the main culms of five representative plants in a plot. Bulked seeds of each accession were used to determine 100-seed weight. The measure of grain yield per plot was converted into grain yield kg ha<sup>-1</sup>.

For grain nutritional content estimation, accessions were harvested at maturity and care was taken to avoid contamination of grains with dust and metal particles during their cleaning. A random well cleaned grain sample (unhusked) from each accession was used to estimate grain protein, calcium (Ca), iron (Fe), and zinc (Zn) contents at the Charles Renard Analytical Laboratory, ICRISAT. Grain Ca, Fe, and Zn contents were assessed by nitric acid and hydrogen peroxide digestion followed by inductively coupled plasma optical emission spectrometry [13]. Protein content in grain samples was determined by sulfuric acid-selenium digestion followed by total nitrogen (N) estimation in a SKALAR SAN<sup>++</sup> SYSTEM autoanalyzer and protein % calculation as N%  $\times$  6.25 conversion factor [14].

#### 2.3. Statistical analysis and trait-specific source identification

The 14 agronomic and four grain nutritional traits were analyzed separately for each rainy season and pooled over the two rainy seasons using residual maximum likelihood (REML) [15] in GenStat 17 (https://www.vsni.co.uk/) considering genotypes as random and season as fixed. The significance of season was tested using Wald's statistic [16]. Variance components due to genotype ( $\sigma^2_g$ ) and genotype × season ( $\sigma^2_{gs}$ ) and their standard errors (SE) were estimated.

Best linear unbiased predictors (BLUPs) [17] were obtained for agronomic and grain nutritional traits for each accession for individual seasons as well as pooled over the two seasons. On the basis of BLUPs derived from the pooled seasons, the range, mean and variances were estimated. Mean performances of races for the agronomic and grain nutrient traits were compared using the Newman-Keuls test [18, 19] and the homogeneity of variances among races were tested using Levene's [20] procedure using the R packages agricolae [21] and car [22], respectively. Broad-sense heritability  $(h_{\rm h}^2)$  was estimated for individual seasons and combined over the two seasons for each trait, to estimate the fraction of the total variation among individuals that is attributable to genetic variation, and categorized as low (<0.30), moderate (0.30 to 0.60), or high (>0.60). Principal component analysis (PCA) was performed to estimate the relative importance of different traits in capturing the variation. Genetic correlations were estimated to determine trait associations at the genetic level using META-R 6.0 [23]. The Shannon–Weaver diversity index H' [24] was used as a measure of the phenotypic diversity of eight qualitative, 14 agronomic, and four grain nutritional traits using GenStat 17 (https://www.vsni.co.uk/). Gower's [25] dissimilarity matrix was constructed using morpho-agronomic and grain nutritional traits using the R package cluster [26], and accessions were clustered by neighbor joining [27] using the software DARwin 6.0.14 [28]. On the basis of proso millet accessions grown in two rainy seasons, accessions with high grain yield, large seeds, and high grain Fe, Zn, Ca, and protein were identified, and Gower's distance matrix was used to identify the most diverse pairs of accessions for potential use in proso millet improvement.

# 3. Results

#### 3.1. Variance components and heritability

The REML analysis showed that variances due to genotype  $(\sigma_g^2)$  were significant for all 14 agronomic and four grain nutritional traits in the individual rainy seasons and pooled over the two seasons, indicating the presence of high levels of variation in the proso millet germplasm accessions (Table 1). Except for basal tiller number, panicle exsertion, and protein content, all traits showed significant genotype × season interaction  $(\sigma_{gs}^2)$  indicating that accession interacted with

season. Wald's statistics for season was significant for all traits except peduncle length, indicating the significant influence of the season on expression of these traits. The estimates of  $h^2_b$  indicated high heritability of all the traits in individual years (0.75 for basal tiller number to 0.98 for inflorescence length in 2015; 0.60 for 100-seed weight to 0.97 for inflorescence length and grain yield in 2016) (Table 1). In the combined analysis over both seasons, basal tiller number (0.37) and Fe content (0.51) showed moderate heritability, whereas the remaining traits showed high heritability.

#### 3.2. Proso millet diversity and structure

#### 3.2.1. Qualitative traits

The frequency distributions of different phenotypic classes of the eight qualitative traits showed large variation (Table S1). In the full set, decumbent growth habit (68.0%), high culm branching (49.5%), medium sheath pubescence (41.5%), sparse and medium ligule pubescence (43.0% and 41.5%, respectively), sparse leaf pubescence (52.0%), diffuse sparse and diffuse dense inflorescence shape (29.5% and 23.0%, respectively), light brown-colored seed (37.0%), and straw apiculus color (62.5%) were the predominant classes. However, frequencies of qualitative traits varied among races. The majority of accessions in compactum (84.0%), contractum (54.2%), miliaceum (71.1%), and ovatum (77.0%) had decumbent growth habit, whereas the accessions of race patentissimum had erect growth habit (52.9%). High culm branching in compactum (60.0%) and miliaceum (55.4%) and medium culm branching in ovatum (61.5%) were the most prevalent, whereas accessions of race contractum and patentissimum had all three classes of culm branching (high, medium and low) at 29.0% to 38.0%. The majority of contractum (50.0%), miliaceum (43.8%), and ovatum (53.8%) accessions had medium sheath pubescence, whereas compactum and patentissimum accessions had sparse (44.0%) and dense (41.2%) sheath pubescence, respectively. All races had sparse leaf and ligule pubescence in higher frequency except miliaceum, which had medium ligule pubescence in higher frequency. For inflorescence shape, arched dense inflorescence in contractum (70.8%), elliptic dense (48.0%) and elliptic sparse (40.0%) inflorescence in compactum, diffuse sparse (46.3%) and diffuse dense (36.4%) inflorescence in miliaceum, globose dense (61.5%) and globose sparse (30.8%) inflorescence in ovatum and arched sparse (70.6%) inflorescence in *patentissimum* were in high proportion. Light brown seed was in higher proportion among accessions of each race, followed by straw and white colored seed, and the majority of accessions in each race and in the full set had straw apiculus color (Table S1). The H' revealed that in the full set, seed color had the highest diversity (0.72) followed by inflorescence shape (0.50) and apiculus color the lowest (0.29) (Table 2). Seed color had the highest H' (0.59 to 0.71) in all races, and growth habit in compactum (0.23) and inflorescence shape and apiculus color in all five races had the lowest H'. A low H' of inflorescence shape within each race is because accessions were classified into races based on inflorescence shape [29]. The mean H' was 0.46 in the full set (n = 200), and the race miliaceum (0.42) had the highest H' among races.

Table 1 – Variance components due to genotype ( $\sigma_{g}^{2}$ ) and genotype × season interaction ( $\sigma_{gs}^{2}$ ), and broad-sense heritability ( $h_{b}^{2}$ ) for agronomic and grain nutritional traits of proso millet accessions evaluated in two rainy seasons at ICRISAT, Patancheru, India.

Trait	In	dividua	l season		Combined over two seasons						
	2015	2015		;	$\sigma^2_{g}$	$\sigma^2_{\rm gs}$	Wald statistic season	$h^2_{b}$			
	$\sigma^2_{g}$	$h^2_{b}$	$\sigma^2_{g}$	$h^2_{b}$							
Days to 50% flowering	24.3**	0.96	36.2**	0.96	26.6**	3.6**	30.6**	0.92			
Basal tiller number	0.5 **	0.75	0.6**	0.84	0.2**	0.4	49.3 **	0.37			
Plant height (cm)	543.8**	0.97	355.3**	0.93	414.1**	32.1 **	577.4**	0.93			
Number of nodes per main stem	0.8**	0.88	0.6**	0.84	0.6**	0.1 **	337.5**	0.86			
Flag leaf blade length (mm)	4308.5*	0.93	3189.1**	0.92	3293.2**	425.4**	413.8 **	0.90			
Flag leaf blade width (mm)	7.5 **	0.79	7.3**	0.77	6.7 **	0.6*	446.6**	0.83			
Flag leaf sheath length (mm)	242.3**	0.91	212.5 **	0.89	201.1**	17.7 **	481.3**	0.90			
Peduncle length (mm)	2873.8*	0.90	2483.3**	0.90	2573.6**	101.6 **	200.6	0.93			
Panicle exsertion (mm)	2500.8**	0.90	2139.5 **	0.90	2266.0**	58.1	66.5**	0.93			
Inflorescence length (mm)	5195.3**	0.98	3954.2**	0.97	4308.0**	256.4**	233.7 **	0.96			
Inflorescence primary branch number	4.1**	0.79	4.4**	0.76	3.9**	0.5 *	359.5 **	0.82			
Days to maturity	24.3**	0.96	40.6**	0.92	27.6**	4.2**	66.3**	0.88			
Grain yield (kg ha <sup>-1</sup> )	612783 **	0.97	89225**	0.97	204346**	148681 **	267.6**	0.73			
100-seed weight (g)	0.01**	0.85	0.004 **	0.60	0.001**	0.001**	11.5 **	0.65			
Fe (mg kg <sup>-1</sup> )	63.6**	0.76	46.8**	0.69	26.3**	28.7 **	8.4**	0.51			
Zn (mg kg <sup>-1</sup> )	14.0***	0.83	31.0**	0.83	16.1**	6.2**	29.2**	0.74			
Ca (mg kg <sup>-1</sup> )	1837.6**	0.94	1052.8**	0.92	1005.1**	434.1**	581.4**	0.79			
Protein (%)	1.2**	0.83	3.5**	0.85	2.5 **	0.2	266.3**	0.86			
* Significant at P < 0.05.											

Significant at  $P \ge 0.05$ .

\*\* Significant at  $P \le 0.01$ .

#### 3.2.2. Agronomic traits

A wide range of variation was observed for agronomic traits, in the full set as well as within each race (Table 3). Days to 50% flowering varied from 19 to 47 days after sowing (DAS), basal tiller number from 1.9 to 4.9, flag leaf blade width from 11 to 24 mm, peduncle length from 99 to 287 mm, panicle exsertion from 15 to 199 mm, number of nodes per main stem from 2 to 6, and days to maturity from 49 to 79 DAS; and none of these traits showed a significant difference among races. Plant height, flag leaf blade length, flag leaf sheath length, inflorescence length, inflorescence primary branch number, 100-seed weight, and grain yield varied among races. Accessions of patentissimum, miliaceum, and contractum were tall (average 73-78 cm) and produced long panicles (average 213-234 mm), and differed significantly from compactum and ovatum (averaging 48-58 cm plant height and 108-160 mm inflorescence length). Flag leaf blade length varied from 95 to 370 mm, flag leaf sheath length from 54 to 127 mm, and inflorescence primary branch number from 9 to 20, and these traits did not differ significantly among compactum, contractum, miliaceum and patentissimum, whereas all four races differed significantly from race ovatum, which had short flag leaf blade length (average 161 mm) and flag leaf sheath length (average 72 mm) and low inflorescence primary branch number (average 12) and 100-seed weight (average 0.47 g). Grain yield varied from 15 to 2334 kg ha<sup>-1</sup>, with a mean of 736 kg ha<sup>-1</sup>. Among races, accessions of patentissimum, miliaceum and contractum produced highest average yield (746 to 880 kg ha<sup>-1</sup>) and differed significantly from race ovatum, which produced lowest grain yields (average 286 kg ha<sup>-1</sup>). The variances were heterogeneous for days to 50% flowering, plant height, flag leaf blade length, flag leaf blade width, flag leaf sheath length,

inflorescence length, number of nodes per main stem, days to maturity and grain yield among races (Table 3). The H' for the 14 agronomic traits indicated that panicle exsertion had high H' in races contractum (0.59), ovatum (0.55), and patentissimum (0.57), whereas compactum had the highest H' value for inflorescence primary branch number (0.60) and miliaceum for flag leaf blade width (0.62) and 100-seed weight (0.62) (Table 2). Among races, miliaceum had the highest H' (0.57).

### 3.2.3. Grain nutritional traits

Proso millet accessions showed a wide range of variation for grain nutrient content: Fe, 41–73 mg kg<sup>-1</sup>; Zn, 26–47 mg kg<sup>-1</sup>; Ca, 91–241 mg kg<sup>-1</sup>; protein, 11%–19%. Mean grain nutritional contents were Fe, 54 mg kg<sup>-1</sup>; Zn, 36 mg kg<sup>-1</sup>; Ca, 165 mg kg<sup>-1</sup>; and protein, 14%. Accessions of *ovatum* had highest grain nutrients: Fe, 60 mg kg<sup>-1</sup>; Zn, 40 mg kg<sup>-1</sup>; Ca, 193 mg kg<sup>-1</sup>; and protein, 16%, and differed significantly from *contractum*, *miliaceum*, and *patentissimum*, whereas *compactum* had comparable grain nutrients and did not differ significantly from *ovatum* with respect to Fe and Ca contents. The variances were homogeneous among races for Fe, Zn, and Ca contents and heterogeneous for protein content (Table 3). Grain nutritional traits had high H' ranging from 0.62 (for Fe, Zn, and Ca contents) to 0.64 (protein %) in the full set, and race *miliaceum* had high H' (0.61).

#### 3.2.4. Phenotypic distance and racial structure

Gower's phenotypic distance matrix among 200 accessions based on 26 traits (eight qualitative traits, 14 agronomic traits and four grain nutritional traits) was estimated. The average distance was 0.270 among 19,900 pairs of accessions (n = 200), and varied from 0.037 (between IPm 390 and IPm 361, both belonging to race miliaceum and originating in India) to 0.618

	- 11					<u> </u>
Trait	Full set (n = 200)	compactum	contractum	miliaceum	ovatum	patentissimum
Qualitative traits						
Growth habit	0.35	0.23	0.44	0.33	0.30	0.42
Culm branching	0.45	0.40	0.47	0.43	0.37	0.48
Sheath pubescence	0.47	0.47	0.45	0.46	0.43	0.47
Ligule pubescence	0.44	0.44	0.40	0.44	0.37	0.45
Leaf pubescence	0.43	0.41	0.39	0.44	0.34	0.33
Inflorescence shape	0.50	0.26	0.20	0.24	0.12	0.24
Seed color	0.72	0.66	0.63	0.71	0.59	0.59
Apiculus color	0.29	0.27	0.24	0.30	0.00	0.26
Mean H' across traits	0.46	0.39	0.40	0.42	0.32	0.41
Agronomic traits						
Days to 50% flowering	0.60	0.48	0.54	0.58	0.50	0.42
Basal tiller number	0.38	0.34	0.39	0.34	0.45	0.37
Plant height (cm)	0.59	0.54	0.51	0.57	0.37	0.52
Flag leaf blade length (mm)	0.60	0.47	0.53	0.61	0.45	0.38
Flag leaf blade width (mm)	0.56	0.52	0.53	0.62	0.44	0.29
Flag leaf sheath length (mm)	0.61	0.47	0.54	0.61	0.47	0.49
Peduncle length (mm)	0.59	0.55	0.58	0.59	0.53	0.52
Panicle exsertion (mm)	0.58	0.54	0.59	0.58	0.55	0.57
Inflorescence length (mm)	0.59	0.48	0.52	0.59	0.30	0.54
Number of nodes per main stem	0.54	0.48	0.53	0.53	0.30	0.56
Inflorescence primary branch number	0.59	0.60	0.53	0.60	0.49	0.50
100-seed weight (g)	0.62	0.56	0.55	0.62	0.41	0.52
Days to maturity	0.61	0.51	0.58	0.60	0.34	0.52
Grain yield (kg ha <sup>-1</sup> )	0.55	0.53	0.58	0.56	0.52	0.51
Mean H' across traits	0.57	0.51	0.54	0.57	0.44	0.48
Grain nutritional traits						
Fe (mg kg <sup>-1</sup> )	0.62	0.56	0.53	0.62	0.57	0.54
$Zn (mg kg^{-1})$	0.62	0.58	0.57	0.63	0.44	0.54
Ca (mg kg <sup>-1</sup> )	0.62	0.59	0.59	0.62	0.50	0.54
Protein (%)	0.64	0.57	0.55	0.55	0.57	0.56
Mean H' across traits	0.62	0.58	0.56	0.61	0.52	0.55

Table 2 – Shannon-Weaver diversity indices (H') of qualitative, agronomic and grain nutritional traits in a full set (n = 200) and five races of proso millet evaluated in two rainy seasons (2015 and 2016) at ICRISAT. Patancheru, India.

(between IPm 2577 and IPm 381, both belonging to race miliaceum, and originating in Russia and India, respectively). Among races, patentissimum had highest average distance (0.309, with range 0.100-0.551), followed by contractum (0.262, range 0.072-0.511), miliaceum (0.258, range 0.037-0.618), and compactum (0.228, range 0.049-0.448), whereas ovatum had the lowest diversity (average distance 0.187, range 0.082-0.368) (data not shown). The average distance between accessions of patentissimum and ovatum was high (0.324), whereas the lowest divergence was between races compactum and ovatum (0.233) (Table S2). An unweighted neighbor joining tree based on Gower's phenotypic distance grouped accessions into two major clusters (C1 and CII) (Fig. 1). Accessions were clustered largely according to geographical region and country within region (Fig. 1-A) and racial group (Fig. 1-B). Accessions originating in Asia were largely in CI, while those from Europe and the Americas were in CII, and accessions from Russia, India, Mexico, Republic of Korea and Syria were distinctly clustered (Fig. 1-A). Accessions of miliaceum, contractum, and patentissimum were distributed in both clusters, whereas accessions of compactum and ovatum were in CII (Fig. 1-B).

#### 3.2.5. PCA and trait association

PCA revealed the importance of the first three PCs, which explained about 76% of total variance among agronomic and

grain nutritional traits (data not shown). Days to 50% flowering, plant height, flag leaf blade length, flag leaf sheath length, inflorescence length, number of nodes per main stem, days to maturity, grain yield, and protein content contributed largely to PC1, which explained about 52% of total variance. Genetic correlation coefficients among 14 agronomic and four grain nutritional traits indicated that all of the agronomic traits were positively and significantly correlated with grain yield except panicle exsertion and peduncle length, both showing significant negative correlations with grain yield (Table 4). All four grain nutrient traits showed significantly negative correlations with grain yield; however, all of them were positively correlated with one another and also with panicle exsertion and peduncle length, except for peduncle length with Fe.

#### 3.3. Trait-specific sources and their agronomic performance

#### 3.3.1. Agronomic traits

Grain yield and 100-seed weight are the important agronomic traits of focus in proso millet improvement. Grain yield of proso millet accessions varied from 15 to 2334 kg ha<sup>-1</sup>, with a mean of 736 kg ha<sup>-1</sup>. Accessions that produced grain yields >1500 kg ha<sup>-1</sup> were considered as high grain-yielding, so that 18 accessions with grain yield ranging from 1601 to

Table 3 – Range, mean, and variance of agronomic and grain nutritional traits in proso millet germplasm evaluated in two rainy seasons (2015 and 2016) at ICRISAT, Patancheru, India.

Trait <sup>¶</sup>	Range								Homoge	neity of variance				
	Full set	compactum	contractum	miliaceum	ovatum	patentissimum	Full set	compactum	contractum	miliaceum	ovatum	patentissimum		F-value
DFL	19–47	22–36	20–40	19–47	23–32	21–42	30	28 a	30 a	31 a	27 a	30 a	5.7**	
BT	1.9-4.9	2.3-4.7	1.9-4.0	2.3–4.8	2.2-4.9	2.5-4.8	3.3	3.1 a	3.2 a	3.3 a	3.0 a	3.4 a	0.63 <sup>ns</sup>	
PLHT	24–127	37–98	41–127	24–123	34.8–75.5	40–127	78	58 b	73a	74 a	48 b	78 a	6.3**	
FLBL	95–370	154–298	139–370	95–341	129–234	124–360	231	202 a	226 a	236 a	161 b	231 a	6.7 **	
FLBW	11–24	13–19	13–21	11–22	11–17	11–24	15	16 a	17 a	16 a	15 a	15 a	2.8*	
FLSL	54–127	66–118	68–118	54–122	63–86	58–127	89	83 a	91 a	92 a	72 b	89 a	3.1*	
PEDL	99–287	116–281	130–281	99–287	125–257	108–268	202	183 a	207 a	193 a	178 a	202 a	0.5 <sup>ns</sup>	
PEX	15–199	40–186	38–199	15–196	44–171	16–192	113	100 a	116 a	101 a	107 a	113 a	0.3 <sup>ns</sup>	
INFL	73–391	99–301	127–389	73–347	78–222	108–391	234	160 b	213 a	226 a	108 c	234 a	6.4**	
NN	2.0-6.0	2.32-4.9	2.31-6.0	2.2-5.8	2.6-4.2	2.3–5.6	4.0	3.6 a	3.9 a	4.0 a	3.4 a	4.0 a	3.4*	
INF-PBN	9–20	10–18	11–19	9–19	10–15	10–20	14	14 a	14 a	14 a	12 b	14 a	2.1 <sup>ns</sup>	
HSW	0.34-0.66	0.38-0.62	0.40-0.64	0.39–0.66	0.42-0.58	0.34-0.60	0.51	0.49 ab	0.51 a	0.51 a	0.47 b	0.51 a	0.8 <sup>ns</sup>	
DM	49–79	54–69	51–72	50–79	55–65	51–75	61	60 a	62 a	62 a	59 a	61 a	6.2**	
GYKH	15–2334	62-1208	46-1627	34–2306	117–572	15–2334	736	491 ab	746 a	814 a	286 b	880 a	5.2**	
Fe (mg kg <sup>-1</sup> )	41–73	49–63	47–63	41–66	45–73	42–60	54	57 a	53 b	53 b	60 a	52 b	1.7 <sup>ns</sup>	
Zn (mg kg <sup>-1</sup> )	26–47	30–46	27–47	26–46	37–43	30–45	36	38 ab	35 c	35 c	40 a	37b c	1.3 <sup>ns</sup>	
Ca (mg kg <sup>-1</sup> )	91–241	136–224	101–207	91–240	165–241	97–215	165	186 a	158 b	161 b	193 a	150 b	1.2 <sup>ns</sup>	
Protein (%)	11–19	12–17	12–17	12–18	13–18	11–19	14	15 b	14 b	14 b	16 a	15 b	2.5*	

<sup>¶</sup> DFL, days to 50% flowering; BT, basal tiller number; PLHT, plant height (cm); NN, number of nodes per main stem; FLBL, flag leaf blade length (mm); FLBW, flag leaf blade width (mm); FLSL, flag leaf sheath length (mm); PEDL, peduncle length (mm); PEX, panicle exsertion (mm); INFL, inflorescence length (mm); INF-PBN, inflorescence primary branch number; HSW, 100-seed weight (g); DM, days to maturity; GYKH, grain yield (kg ha<sup>-1</sup>); Fe, iron (mg kg<sup>-1</sup>); Ca, calcium (mg kg<sup>-1</sup>).

<sup>#</sup> Means of races were tested following the Newman-Keuls test [19, 20]. Mean followed by the same letters are not significant at  $P \le 0.05$  and means followed by different letters are significant at  $P \le 0.05$ . Homogeneity of variances among clusters was tested using Levene's test [21].

\* Significant at  $P \le 0.05$ .

\*\* Significant at  $P \le 0.01$ .

<sup>ns</sup> Non-significant.



Fig. 1 – Unweighted neighbor-joining clustering of proso millet accessions based on Gower's distance. (A) Accessions are color coded by region (pink, Asia; green, Europe; blue, the Americas; black, unknown origin and regions (Africa and Oceania) with only two accessions) and (B) accessions are color coded by race (pink, compactum; green, miliaceum; blue, contractum; red, patentissimum; brown, ovatum).

Trait	Grain yield	Fe	Zn	Ca	Protein
	(kg ha <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(%)
Days to 50% flowering	0.98 <sup>**</sup>	-0.71**	-0.74**	-0.69**	-0.80**
Basal tiller number	0.56 <sup>**</sup>	-0.78**	-0.44**	-0.55**	-0.72**
Plant height (cm)	0.87 <sup>**</sup>	-0.70**	-0.66**	-0.66**	-0.79**
Flag leaf blade length (mm)	0.98 <sup>**</sup>	-0.75**	-0.82**	-0.71**	-0.86**
Flag leaf blade width (mm)	0.39 <sup>**</sup>	-0.21**	-0.44**	-0.19**	-0.40**
Flag leaf sheath length (mm)	0.58 <sup>**</sup>	-0.51**	-0.60**	-0.48**	-0.65**
Peduncie length (mm) Panicle exsertion (mm) Inflorescence length (mm) Number of nodes per main stem Inflorescence primary branch number 100-seed weight (g) Days to maturity Fe (mg kg <sup>-1</sup> ) Zn (mg kg <sup>-1</sup> ) Ca (mg kg <sup>-1</sup> ) Protein (%)	-0.25 -0.45** 0.86 <sup>**</sup> 0.64 <sup>**</sup> 0.51 <sup>**</sup> 1.00 <sup>**</sup> -0.88 <sup>**</sup> -0.92 <sup>**</sup> -0.83 <sup>**</sup>	0.07 0.22 ** -0.76 ** -0.64 ** -0.33 ** -0.48 ** -0.72 ** 0.80 ** 0.62 ** 0.69 **	0.27 0.46 -0.70 -0.70 -0.55 -0.33 -0.72 ** 0.56 ** 0.74 **	0.30 0.46 ** -0.65 ** -0.70 ** -0.42 ** -0.60 ** -0.68 **	0.22 0.42 ** -0.78 ** -0.86 ** -0.65 ** -0.44 ** -0.80 **
** Significant at $P \le 0.01$ .					

Table 4–Genetic correlation coefficients of agronomic and grain nutritional traits with grain yield in proso millet germplasm evaluated in two rainy seasons (2015 and 2016) at ICRISAT, Patancheru, India.

2334 kg ha<sup>-1</sup> were selected (Table 5). Days to 50% flowering of these high-yielding accessions varied from 33 to 42 DAS and they matured in 65 to 75 DAS. High grain-yielding accessions belonged largely to *miliaceum* (13 accessions), followed by *patentissimum* (3 accessions) and *contractum* (2 accessions), originating mostly in India (11 accessions), with two accessions from Sri Lanka and one each from Pakistan and Nepal. For large-seeded accessions, 10 accessions (100-seed weight ranging from 0.60 to 0.66 g), significantly larger than the trial mean of 0.50 g were identified. Two high-yielding accessions (IPm 2661 and IPm 9) also had significantly large seeds and produced grain yields of 1601 and 2334 kg ha<sup>-1</sup>, respectively. Altogether, 26 accessions, and two high grain-yielding and large-seeded accessions) were identified.

#### 3.3.2. Grain nutritional traits

Assessment of grain nutritional content of proso millet accessions resulted in identification of grain nutrient- rich accessions with contents significantly higher than the trial mean. These included 12 accessions for Fe (63.3–73.2 mg  $kg^{-1}$ ), 27 accessions for Zn (40.6–46.7 mg  $kg^{-1}$ ), 56 accessions for Ca (185.5–241.2 mg kg<sup>-1</sup>), and 27 accessions for protein (16.1%– 19.1%) (Table S3). All of the selected accessions had significantly higher grain nutrient content than the best high grainyielding accession, IPm 9. The search for accessions with two or more grain nutrients among these nutrient-rich accessions resulted in identification of 28 accessions (Table 6). Days to 50% flowering of the accessions with multiple grain nutrients varied from 19 to 29 DAS and they matured in 49 to 61 DAS. However, grain yields of these accessions were low (average 233; range 15–917 kg ha<sup>-1</sup>). These accessions belonged mostly to race miliaceum (11 accessions) followed by ovatum (6 accessions), compactum (5 accessions), patentissimum (4 accessions), and contractum (2 accessions), and most originated in Russia (8 accessions) (Table 6), whereas 11 accessions were of unknown origin and one accession each was from Afghanistan, China, Hungary, Republic of Korea, Syria, Turkey, the United Kingdom, Ukraine, and Kyrgyzstan.

#### 3.3.3. Genetic distances among trait-specific sources

Phenotypic distances between pairs of accessions within the sources for agronomic traits (high yield and large seed size) and accessions rich in multiple grain nutrients, and between accessions with agronomic traits and those with multiple nutrients were estimated. Average distance was 0.279 among agronomically superior accessions, 0.213 among multiple grain nutrient-rich accessions, and 0.383 between accessions with agronomic traits and multiple grain nutrients. The 10 most diverse pairs of accessions were identified within each trait group (high grain yield, multiple grain nutrients) and between high grain-yielding and multiple-nutrient accessions (Table S4).

# 4. Discussion

Small millets' presence in the food basket has been declining over the years, mainly because of the increased availability of rice and wheat (*Triticum aestivum* L.) and other staple foods, a lack of crop improvement efforts in developing high yielding cultivars, and a lack of modern technologies for processing and utilization [10]. Over the last few years, there has been increasing recognition of small millets' nutrient composition and benefits as healthy food. Considering their diverse adaption and agronomic and health benefits, small millets could be an alternate or supplementary crop to enlarge the food basket to ensure food, feed, and nutritional security. Proso millet is one of the under-researched and underutilized small millets, and a potential climate-smart and nutrient-rich crop. With its short growing season, proso millet can be planted late as a catch crop when main crops fail [3].

Table 5 – Proso millet germplasm accessions identified as high grain-yielding and large seeds and their agronomic performance based on evaluation in two rainy seasons (2015 and 2016) at ICRISAT, Patancheru, India.														
Accession	Country of origin	Race	DF <sup>¶</sup>	BT	PLHT	INFL	INF-PBN	HSW	DM	GHKH	Fe	Zn	Ca	Protein
IPm 9	India	patentissimum	38	3.3	100*	296*	14	0.60*	69	2334*	47.2	33.2	118.9	12.2
IPm 2784	Sri Lanka	miliaceum	36	3.3	99*	275	15	0.52	67	2306 *	61.4	34.9	104.5	12.7
IPm 2621	India	miliaceum	38	3.2	101*	300*	15	0.58	70	2257*	46.8	29.6	117.8	12.0
IPm 2802	India	miliaceum	38	3.8	85	269*	12	0.56	69	2108 *	47.0	33.2	91.1	12.2
IPm 390	India	miliaceum	37	4.8	97	283	14	0.58	69	2026	48.5	34.0	104.3	12.2
IPm 361	India	miliaceum	39	4.1	99*	288	14	0.58	71	1919*	51.4	31.7	116.1	12.3
IPm 2824	India	patentissimum	39	4.8*	107*	314 *	14	0.58	71	1877 *	52.5	31.7	96.6	10.8
IPm 2783	Sri Lanka	miliaceum	33	3.5	98*	281*	14	0.56	65	1858*	50.7	36.3	111.2	12.8
IPm 366	India	miliaceum	37	3.3	97*	293*	14	0.54	69	1795 *	50.3	32.3	117.1	12.1
IPm 2620	India	miliaceum	38	3.9*	100*	299*	14	0.54	70	1794*	48.4	32.3	148.2	12.6
IPm 2660	Nepal	patentissimum	42	2.5	127 *	391*	20	0.56	75	1786*	54.1	35.2	101.4	13.4
IPm 2685	Pakistan	miliaceum	37	3.0	102*	295*	16 <sup>*</sup>	0.56	68	1731*	48.2	26.7	93.5	11.9
IPm 2158	Unknown	miliaceum	37	2.6	101*	280*	17*	0.56	69	1717 *	51.1	31.7	113.1	13.3
IPm 388	India	miliaceum	38	4.2*	94*	281*	13	0.58	70	1691*	50.7	33.0	95.5	12.5
IPm 2700	India	miliaceum	38	3.1	96*	299*	14	0.54	69	1646*	44.9	29.0	119.2	12.1
IPm 384	India	miliaceum	38	2.6	98*	282*	14	0.52	69	1645*	48.0	34.3	117.4	12.9
IPm 1545	Unknown	contractum	37	3.4	77	187	16*	0.52	69	1627 *	47.7	30.1	123.5	12.6
IPm 2661	Nepal	contractum	40	2.8	127 *	389*	19 <sup>*</sup>	0.64*	72	1601*	52.6	32.2	101.1	12.9
IPm 362	India	miliaceum	47	4.5 *	82*	254*	12	0.66*	79	1446*	45.2	32.8	102.1	12.6
IPm 2826	Romania	miliaceum	29	3.0	72	243*	15	0.64*	60	878	46.5	31.1	151.2	12.4
IPm 381	India	miliaceum	47	4.7 *	98*	289*	14	0.63*	75	1196*	47.3	33.6	93.7	12.9
IPm 2575	Russia	compactum	24*	2.4	54	148	13	0.62*	56*	337	53.2	39.4	175.6	16.9*
IPm 2273	Unknown	contractum	24*	2.7	56	150	11	0.60*	57*	350	50.5	35.8	172.8	16.1*
IPm 2769	Ukraine	compactum	24*	3.2	56	139	11	0.60*	56*	217	55.9	40.8*	196.4*	16.6*
IPm 2780	Turkey	miliaceum	23*	3.1	50	140	13	0.60*	56*	180	56.0	36.7	171.9	14.9
IPm 2037	Unknown	contractum	20*	3.0	52	134	11	0.60*	51*	173	62.6	45.4	207.4*	16.5 *
Trial mean			30	3.0	70	207	14	0.50	62	736	53.9	36.0	164.7	14.4
Trial range			19–47	2.0-5.0	24–127	73–391	9–20	0.34-0.66	49–79	15–2334	41.4-73.2	26.2-46.7	91.1-241.2	10.8–19.1
LSD 0.05			2.25	0.75	10.21	22.2	2.28	0.09	3.55	203	9.25	4.59	20.8	1.57
CV (%)			5.46	16.53	10.53	7.75	11.85	13.07	4.17	19.8	12.38	9.22	9.11	7.89

<sup>¶</sup> DF, days to 50% flowering; BT, basal tiller number; PLHT, plant height (cm); INFL, inflorescence length (mm); INF-PBN, inflorescence primary branch number; HSW, 100-seed weight (g); DM, days to maturity; GYKH, grain yield (kg ha<sup>-1</sup>); Fe, iron (mg kg<sup>-1</sup>); Zn, zinc (mg kg<sup>-1</sup>); Ca, calcium (mg kg<sup>-1</sup>); protein (%).

\* Significant at  $P \le 0.05$ .

Table 6 – Proso millet accessions identified as sources for two or more grain nutrients and their agronomic performance, based on germplasm accessions evaluated in two rainy seasons (2015 and 2016) at ICRISAT, Patancheru, India.														
Accession	Country of origin	Race	DF <sup>¶</sup>	BT	PLHT	INFL	INF-PBN	HSW	DM	GYKH	Fe	Zn	Ca	Protein
IPm 2069	Unknown	ovatum	27	3.5	46	85	12	0.42	58	175	73.2*	43.0*	222.5*	17.4*
IPm 2076	Unknown	ovatum	28	3.3	48	85	14	0.44	59	388	68.8	41.1*	208.8	16.4*
IPm 2537	Russia	compactum	23*	2.8	58	145	13	0.52	56*	274	63.3*	46.5*	213.9*	16.5 *
IPm 2005	Unknown	patentissimum	21*	3.0	56	170	12	0.54	51*	109	58.0	42.6*	209.9*	16.3*
IPm 2037	Unknown	contractum	20*	3.0	52	134	11	0.60*	51*	173	62.6	45.4 <sup>*</sup>	207.4*	16.5*
IPm 2053	Unknown	miliaceum	27	2.7	44	101	12	0.44	58	229	64.6*	40.7 *	177.3	17.3*
IPm 2077	Unknown	ovatum	27	2.9	35	78	10	0.46	58	117	66.2*	42.2*	175.2	17.8*
IPm 2278	Unknown	miliaceum	25*	2.4	35	119	10	0.48	57*	326	64.1*	41.3*	142.7	17.2*
IPm 2528	Russia	patentissimum	21*	2.6	40	108	10	0.50	52*	15	59.8	43.9 <sup>*</sup>	215.3*	19.1*
IPm 2689	Afghanistan	compactum	28	3.5	60	158	14	0.42	60	576	60.8	40.6*	188.6*	16.2*
IPm 2769	Ukraine	compactum	24*	3.2	56	139	11	0.60*	56*	217	55.9	40.8*	196.4 *	16.6*
IPm 2903	Syria	miliaceum	19*	2.8	50	165	11	0.48	50*	60	61.4	40.9*	211.2*	17.8*
IPm 2004	Unknown	contractum	21*	1.9	47	127	11	0.58	52*	46	59.0	46.7*	181.3	17.1*
IPm 2062	Unknown	miliaceum	28	2.9	45	130	14	0.40	57*	372	64.1*	34.2	218.6*	15.5
IPm 2083	Unknown	ovatum	27	2.2	38	88	12	0.42	58	159	58.6	40.2	215.3*	17.3*
IPm 2123	Unknown	ovatum	28	2.3	47	94	12	0.46	59	307	66.1*	37.4	187.3	15.9
IPm 2507	Russia	miliaceum	26*	3.1	53	179	14	0.42	58	348	55.5	41.5 *	193.3 *	14.9
IPm 2540	Russia	miliaceum	20*	3.1	63	204	15	0.54	52*	212	64.0*	41.3*	150.3	15.7
IPm 2552	Russia	ovatum	23*	3.1	49	95	10	0.50	55	148	60.0	42.9*	165.9	18.5
IPm 2555	Russia	patentissimum	21*	3.6	54	150	12	0.34	54*	130	51.7	45.2 <sup>*</sup>	153.4	16.5 *
IPm 2594	Russia	miliaceum	22*	2.6	47	142	13	0.45	52*	90	53.5	39.9	235.4	17.9*
IPm 2601	Russia	miliaceum	20*	2.9	59	176	11	0.58	50*	113	65.6*	39.4	192.5 *	15.8
IPm 2697	Turkey	miliaceum	26*	3.0	32	102	9	0.48	59	60	57.6	45.9 <sup>*</sup>	182.1	16.4*
IPm 2721	Hungary	miliaceum	24*	2.8	55	172	13	0.49	56*	287	64.0*	37.3	215.2*	15.7
IPm 2735	UK	miliaceum	19*	4.0*	49	162	13	0.48	49*	69	58.4	42.7*	177.6	17.3*
IPm 2745	Kyrgyzstan	patentissimum	23*	2.7	54	155	15	0.48	55*	164	54.7	35.3	214.8	17.7 *
IPm 2782	China	compactum	26*	2.6	60	145	13	0.56	58	455	62.9	41.3*	224.5*	15.3
IPm 2875	Republic of Korea	compactum	29	3.1	79	248*	13	0.46	61	917	48.8	39.8	213.1*	16.4 *
Trial mean			30	3.0	70	207	14	0.5	62	736	53.9	36.0	164.7	14.4
Trial range			19–47	2.0-5.0	24–127	73–391	9–20	0.34-0.66	49–79	15–2334	41.4–73.2	26.2-46.7	91.1-241.2	10.8–19.1
LSD <sub>0.05</sub>			2.25	0.75	10.21	22.2	2.28	0.09	3.55	203	9.25	4.59	20.8	1.57
CV (%)			5.46	16.53	10.53	7.75	11.85	13.07	4.17	19.8	12.38	9.22	9.11	7.89

<sup>1</sup> DF, days to 50% flowering; BT, basal tiller number; PLHT, plant height (cm); INFL, inflorescence length (mm); INF-PBN, inflorescence primary branch number; HSW, 100-seed weight (g); DM, days to maturity; GYKH, grain yield (kg ha<sup>-1</sup>); Fe, iron (mg kg<sup>-1</sup>); Zn, zinc (mg kg<sup>-1</sup>); Ca, calcium (mg kg<sup>-1</sup>); protein (%). \* Significant at  $P \le 0.05$ .

Knowledge of diversity and structure of germplasm resources is important for their use in crop improvement programs. The characteristics and diversity of proso millet accessions and races were assessed using morpho-agronomic and grain nutritional traits revealed a wide range of variation. Overall, accessions of ovatum were low grain-yielding, and characterized by short plants with short flag leaf blade length, flag leaf blade sheath length, and inflorescence length, and medium culm branching and sheath pubescence, compared to other races, and had globose dense or globose sparse inflorescence. Accessions of compactum were slightly high yielding, tall and with long panicles compared to ovatum, while flag leaf blade length, flag leaf sheath length and inflorescence primary branch numbers were higher than in race ovatum and did not show significant difference from other races, and produced high culm branches and elliptic dense and elliptic sparse inflorescences. Accessions of contractum, miliaceum and patentissimum did not show significant difference for the agronomic traits studied, however average grain yield of patentissimum was higher than that of other races. The majority of patentissimum accessions produced dense sheath pubescence and arched sparse inflorescence, while contractum had medium sheath pubescence and arched dense inflorescence and miliaceum had medium sheath pubescence and diffuse dense and diffuse sparse inflorescence.

Among the races, miliaceum had the highest H' for all the traits studied including qualitative, agronomic and grain nutritional traits, and all the five races had comparatively rich diversity. Estimation of pairwise distance between accessions based on 26 traits including eight qualitative, 14 agronomic, and four grain nutritional traits revealed that the average distance of accessions within race patentissimum was high (0.309), whereas ovatum had the lowest distance (0.182), and these races diverged widely from each other. The lowest divergence was observed between races compactum and ovatum. Population structure as revealed by neighbor-joining clustering indicated that the accessions of proso millet were structured largely according to geographical region, country within region, and racial group. Accessions originating in Asia and Europe were distinctly grouped, accessions from Asia showed high diversity (average distance 0.268) relative to those from Europe (average distance 0.225), and high diversity was observed between accessions of Asia and Europe (average distance 0.301). This finding supports independent origins of proso millet in Europe and Asia, or may correspond to a domestication within Asia followed by a spread westward across the Eurasian steppe [30]. Accessions of miliaceum, contractum, and patentissimum were distributed in both clusters, whereas accessions of compactum and ovatum were present in the same cluster, supporting the observation that these two races were less diverged from each other than with other races. Rajput and Santra [31] assessed genetic diversity and structure of 90 proso millet accessions and reported that they were structured partially according to geographical origin.

Among the millet species produced worldwide, proso millet is the most important species traded in the world market, and the USA is among the top producers [5]. The USA generally exports 15–20% of its annual millet production to over 70 countries, primarily as feed, and the largest export markets for USA proso millet include the Netherlands, the United Kingdom, Canada, and Japan [5]. The current marker for proso millet is limited mostly to bird feed, but there are many other uses including for human food and alcoholic beverages, livestock and poultry feed, and biofuel ethanol [32]. Genetic improvement and cultivar develop of proso millet, as in other small millets, has been achieved largely through direct selection of promising germplasm. In the USA, of 15 cultivars of proso millet available to growers, nine were selections from adapted landraces, whereas six were developed by hybridization followed selection [5], and average yields of popular cultivars range from 1020 to 2016 kg ha<sup>-1</sup> [33]. In India, 24 cultivars have been released, of which seven were developed by hybridization followed by selection and the remaining by selection from landraces, with yields of 0.8–2.6 t ha<sup>-1</sup> (http://www.aicrpsm.res.in/Releasevarities. html). In this study, we identified 18 accessions as promising for higher grain yield (1601 to 2334 kg  $ha^{-1}$ ), with two of these (IPm 2661 and IPm 9) also having large seed size. These highyielding accessions could be tested in multiple locations to assess their adaptation and yield potential, and could be released as cultivars.

Large variation was observed for Fe (41–73 mg kg<sup>-1</sup>), Zn (26–47 mg kg<sup>-1</sup>), Ca (91–241 mg kg<sup>-1</sup>) and protein (11–19%) contents. The proso millet accessions had an average of 54 mg kg<sup>-1</sup> of Fe, 36 mg kg<sup>-1</sup> of Zn, 165 mg kg<sup>-1</sup> of Ca, and 14% of protein. On average, Fe, Zn and protein contents were higher than in the finger millet core collection (Fe 29.3 mg kg<sup>-1</sup>, Zn 19.9 mg kg<sup>-1</sup>, and protein 7.3%) [34], and Fe, protein and Ca contents were higher than in the foxtail millet core collection (Fe 49.8 mg kg<sup>-1</sup>, protein 13.5% and Ca 145.8 mg kg<sup>-1</sup>) [35]. Wang et al. [36] identified accessions with protein content >15% by screening 6515 germplasm accessions from 14 provinces of China. Proso millet accessions rich in two or more grain nutrients were identified, of which IPm 2875, originating in the Republic of Korea produced high grain yield (917 kg ha<sup>-1</sup>). In general, the seed coat of proso millet is not readily removed by threshing and the grain requires seed processing machinery for dehusking, whereas the seed coat of IPm 2875 is readily removed by threshing, a useful and economically important trait. The seeds of IPm 2875 (without seed coat) had 48.8 mg kg<sup>-1</sup> Fe, 39.8 mg kg<sup>-1</sup> Zn, 213.1 mg kg<sup>-1</sup> Ca, and 16.4% protein. All the grain nutrient-rich accessions had significantly higher grain nutrients than the high-yielding accession IPm 9. Accessions IPm 2069, IPm 2076, and IPm 2537 were rich in all four grain nutrients. Further, the 10 most diverse pairs of accessions within sources for high grain yield and multiple grain nutrients, and between high-yielding and multiple-nutrient accessions, were identified and could be used in breeding programs with agronomically superior accessions. Negative correlations of grain nutritional contents with grain yield and positive correlations among grain nutrients were observed, suggesting that exploitation of hybridization-derived variation is the next step toward combining two or more grain nutrients and desirable agronomic traits with the goal of breeding high grain-yielding and nutrient-dense proso millet cultivars. Significant influences of genotype and season (except for peduncle length), and their interaction (except for basal tiller number, panicle exsertion, and protein content) were noted. However, moderate to high heritability was observed for all the traits investigated including grain yield and grain nutrients, indicating that a large

fraction of variation was due to genetic effects. It could be useful to assess the stability of macro- and micronutrients across different soil types and soil fertility levels typical of the areas to which the cultivars are targeted. Knowledge of genotype × environment interactions aids in designing suitable breeding and selection strategies to increase grain nutrients in edible portions of grain [37].

In general, average yields of accessions belonging to races *patentissimum* and *miliaceum* were high, whereas accessions of *ovatum* produced low grain yield. The high grain-yielding accessions identified belong to either *miliaceum* (13 accessions), *patentissimum* (3 accessions), or *contractum*, suggesting that evaluation of the full set of accessions of *patentissimum*, *miliaceum*, and *contractum* maintained in genebanks would reveal additional high grain-yielding accessions. The selected high grain-yielding accessions also had high grain nutrient contents (Fe, 44.9–61.4 mg kg<sup>-1</sup>; Zn, 26.7–36.2 mg kg<sup>-1</sup>; Ca, 91.1–148.2 mg kg<sup>-1</sup>; protein 10.8% to 13.4%), though not significantly higher than the trial means. Accessions of races *ovatum* and *compactum* are potentially superior sources for high grain nutrients compared to other races, indicating the importance of these races for grain nutritional trait improvement.

# 5. Conclusions

Large variation was found in proso millet germplasm for morpho-agronomic and grain nutrient traits (Fe, Zn, Ca, and protein). Diversity assessment indicated that race patentissimum had high diversity and race ovatum low diversity. High divergence was observed between patentissimum and ovatum, and the lowest divergence between races compactum and ovatum. The diversity of proso millet was structured according to geographical region, country within region, and racial group. This study supports independent origins of proso millet in Europe and Asia, or the crop may also have originated by domestication within Asia and then spread westward across the Eurasian steppe. Trait-specific sources including 18 accessions for higher grain yield, 10 large-seeded accessions, and 26 accessions rich in multiple grain nutrients, and highly diverse pairs of accessions, were identified for potential use in proso millet improvement. This study provides valuable information to proso millet researchers, and the trait specific sources identified could be tested for their adaptation in diverse locations for grain yield and grain nutritional content stability, for direct release as cultivars, and could be used in breeding for developing high grain-yielding and nutrient-rich cultivars. Cultivation and consumption of proso millet would not only reduce micronutrient and protein malnutrition but also contribute to crop and diet diversification.

Proso millet researchers can obtain seed samples of these accessions from the ICRISAT genebank for research purposes via a Standard Material Transfer Agreement.

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### **Conflict of interest**

The authors hereby state that there is no conflict of interest with regard to this manuscript.

#### Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2018.04.002.

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