

Article

Variability in the Global Proso Millet (*Panicum miliaceum* L.) Germplasm Collection Conserved at the ICRISAT Genebank

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Abstract: Proso millet (*Panicum miliaceum* L.), also known as common millet or broomcorn millet, is an important ancient crop mostly grown for food, feed, and fodder purposes largely in China, Russia, India, and the USA. It is an under-researched and under-utilized crop. Over 29,000 germplasm accessions have been conserved in genebanks globally. Five races (*miliaceum*, *patentissimum*, *contractum*, *compactum*, *ovatum*) have been recognized in proso millet based on panicle morphology and shape. The genebank at the International Crops Research Institute for the Semi-Arid Tropics conserves 849 accessions of proso millet originating from 30 countries and represents all five races. Characterization of these germplasm accessions revealed large variability for morpho-agronomic traits, including for days to 50% flowering (26 to 50 days), plant height (20 to 133 cm), and inflorescence length (22 to 400 mm). On average, the race *miliaceum* was tall (62 cm) with long panicles (209 mm) and *ovatum* had short plants (46 cm) with small panicles (108 mm). The average Gower's distance based on 18 morpho-agronomic traits on 841 accessions was 0.261. The race *miliaceum* had the highest among accessions within race average pairwise distance (0.254), while the distance was the lowest in *ovatum* (0.192). The races *miliaceum* and *ovatum* showed the highest divergence with each other (0.275), while the lowest divergence was observed between *compactum* and *ovatum* (0.229). Trait-specific sources were identified for early maturity, tall plants, long inflorescences, and greater seed size. The information on variability and trait-specific sources identified could potentially support proso millet improvement.

Keywords: proso millet; germplasm; diversity; race; trait-specific sources

1. Introduction

Proso millet (*Panicum miliaceum* L.), also called as common millet or broomcorn millet, is an annual herbaceous plant from the genus *Panicum*, and it has a chromosome number of $2n = 36$ with a basic chromosome number of $x = 9$. de Wet [1] classified proso millet germplasm into five races (*miliaceum*, *patentissimum*, *contractum*, *compactum*, *ovatum*) based on panicle morphology and shape (Figure S1). The race *miliaceum* resembles wild *P. miliaceum* in inflorescence morphology, characterized by large open inflorescences with suberect branches that are sparingly subdivided. The race *patentissimum* is characterized by slender and diffuse panicle branches, which is often difficult to distinguish from race *miliaceum*. Accessions with more or less compact inflorescences are classified into races *contractum*, *compactum*, and *ovatum*. Accessions in the race *contractum* have compact drooping inflorescences, the race *compactum* has cylindrical inflorescences that are essentially erect, while accessions with compact and slightly curved inflorescences that are ovate in shape belong to race *ovatum* [1]. Vavilov [2]

suggested China as the center of diversity for proso millet, while Harlan [3] opined that proso millet was probably domesticated in China and Europe. The earliest records come from the Yellow River valley site of Cishan, China dated between 10,300 and 8700 cal Before Present (BP) [4]. Evidence of proso millet also occurs at a number of pre-7000 cal BP sites in Eastern Europe, in the form of charred grains and grain impressions in pottery [5]. These two centers of earlier records suggest the independent domestication of proso millet in Eastern Europe or Central Asia, or may have also originated from a domestication within China and then spread westward across the Eurasian steppe [6].

Proso millet is grown in Asia, Australia, North America, Europe, and Africa, and is used for feeding birds and as livestock feed in developed countries and for food in some parts of Asia [7]. Proso millet is cultivated in about 0.82 million ha in Russia, 0.32 million ha in China [8], 0.20 million ha in the U.S [9], 0.03 million ha in India [10], and 0.002 million ha in Korea [11]. The U.S. is among the top producers, and exports 15–20% of its annual proso millet production to over 70 countries, primarily as feed [9]. This crop matures in 6 to 12 weeks and requires less water and adapts well to varied environmental conditions [12]. Proso millet grains are rich in protein, vitamins, minerals, and micronutrients including iron, zinc, copper, and manganese, compared to other staple cereals [13]. The protein content of proso millet is comparable to that of wheat, and its grains are richer in essential amino acids (leucine, isoleucine, and methionine) than those of wheat [14,15]. The husked grains are eaten whole, boiled, or cooked like rice (*Oryza sativa* L.), and are sometimes ground to make roti (flatbread). The starch is suitable as a sizing agent in the textile industry. Green plants are used as fodder for cattle and horses, and are also used as hay. Proso millet is used to make fermented beverages in Africa and Asia, and is receiving growing interest from food industries in Europe and North America because of its mild flavor, light color, gluten-free quality, and potential health benefits [16].

Proso millet is an under-researched and under-utilized crop. Germplasm plays an important role in crop improvement. Considerable numbers of proso millet germplasms have been conserved in genebanks globally. Information about variability in the germplasm collection of a given species for important traits, including yield and quality, enables their utilization in crop improvement programs. The genebank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) conserves 849 accessions of proso millet originating from 30 countries. This study aims to assess the racial characteristics and geographical distribution, as well as the diversity of the proso millet germplasm collection conserved at the ICRISAT genebank for various morpho-agronomic traits.

2. Materials and Methods

2.1. Experiment Details

The genebank at ICRISAT, Patancheru conserves 849 proso millet germplasm accessions originating from 30 countries. They were characterized from 1977 onwards at ICRISAT, Patancheru located at (17°53' N, 78°27' E, 545 m asl), as and when new germplasm entries were received at the ICRISAT genebank. Sowings were done during second fortnight of July in each year, on red soils (Alfisols), following augmented block design, along with controls. Each accession was planted in a single row of 4 m length, 60 cm between rows, with plant-to-plant spacing of 10 cm and at uniform depth. Diammonium phosphate was applied at 100 kg ha⁻¹ as a basal dose to supply nitrogen and phosphorus. In addition, 100 kg ha⁻¹ of urea was applied as top dressing. The precision fields at the ICRISAT center have uniform fertility and a gentle slope of 0.5%. All cultural practices and data recordings were the same for all years of evaluation. The average annual rainfall at this location is about 890 mm (averaged from 1977 to 2018), which normally occurs during June to September.

2.2. Data Collection

Data on various qualitative traits, namely, growth habit (erect, decumbent, erect geniculate, and prostrate), culm branching (low, medium, and high), sheath pubescence (sparse, medium, and dense), ligule pubescence (sparse, medium, and dense), leaf pubescence (sparse, medium, and dense),

inflorescence shape (arched sparse, arched dense, diffuse sparse, diffuse dense, elliptic sparse, elliptic dense, globose sparse, and globose dense), seed color (white, light red, straw, dark red, dark green, olive green, dark olive green, light brown, brown, dark brown, black), and apiculus color (straw and purple); and quantitative traits, namely, days to 50% flowering, plant height (cm), basal tillers number, flag leaf blade length (mm), flag leaf blade width (mm), flag leaf sheath length (mm), peduncle length (mm), panicle exertion (mm), inflorescence length (mm), and inflorescence primary branches number were recorded following descriptors for *Panicum miliaceum* [17].

2.3. Data Analyses

Data collected in the individual years were analyzed independently and stored in the database (also available in the ICRISAT genebank webpage <http://genebank.icrisat.org/>) and used in this study. The range and means were calculated for all traits, for each race, and for each country of origin. The mean performances of races for each quantitative trait were compared using Newman–Keuls test [18,19], and the homogeneity of variances was tested using Levene’s procedure [20] using the R packages “*agricolae*” [21] and “*car*” [22]. Principal component analysis (PCA) based on 10 quantitative traits was performed to determine the relative importance of different traits in capturing the variation in proso millet collection, and the Shannon–Weaver diversity index (H') [23] was used as a measure of the phenotypic diversity of eight qualitative and ten quantitative traits, using GenStat 17th Edition (<https://www.vsni.co.uk/>). Gower’s dissimilarity matrix [24] was constructed using both qualitative and quantitative traits using the R package “*cluster*” [25]. The Gower’s dissimilarity matrix was then used to identify most diverse pairs of accessions, and accessions were clustered following the neighbor-joining method [26] using the software DARwin 6.0.14 [27].

3. Results

3.1. Racial and Geographical Distribution

Accessions of proso millet can be classified into five races: *miliaceum*, *patentissimum*, *contractum*, *compactum*, and *ovatum*, based on panicle morphology and shape [1]. The 849 accessions of proso millet conserved in the ICRISAT genebank were classified into five races, namely, *miliaceum* (63.5%), *compactum* (11.5%), *contractum* (10.8%), *ovatum* (5.7%), and *patentissimum* (7.4%) (Table 1). The majority of proso millet accessions conserved in the ICRISAT genebank originated from Asia (37.1%) and Europe (18.5%). At the country level, 14% of the entire collection was from the Russian Federation, while about 9% each were from India and Republic of Korea, 4–6% were from Syria (4.1%), Pakistan (4.8%), and Turkey (5.8%), and the remaining countries represented <2% (Table 1).

Table 1. Proso millet germplasm conserved at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) genebank, Patancheru, India.

| Country | Race | | | | | Unclassified | Total |
|--|------------------|-------------------|------------------|---------------|----------------------|--------------|-------|
| | <i>compactum</i> | <i>contractum</i> | <i>miliaceum</i> | <i>ovatum</i> | <i>patentissimum</i> | | |
| Russian Federation | 28 | 13 | 64 | 5 | 11 | | 121 |
| India | | 3 | 57 | | 8 | 8 | 76 |
| Republic of Korea | 2 | 16 | 52 | | 3 | | 73 |
| Turkey | 8 | 6 | 30 | 3 | 2 | | 49 |
| Pakistan | 1 | 1 | 31 | | 8 | | 41 |
| Syria | 1 | 10 | 17 | 2 | 4 | 1 | 35 |
| Afghanistan | 5 | | 11 | | | | 16 |
| Mexico | | 4 | 3 | 2 | 4 | | 13 |
| Germany | 1 | 2 | 9 | | | | 12 |
| Hungary | 1 | 1 | 6 | | 2 | | 10 |
| Iran | 1 | | 8 | | | | 9 |
| Nepal | | 4 | | | 2 | | 6 |
| Ukraine | 1 | | 3 | | | | 4 |
| United Kingdom | | 1 | 2 | | 1 | | 4 |
| Australia | | | 2 | | | | 2 |
| Bangladesh | | | 2 | | | | 2 |
| China | 1 | | 1 | | | | 2 |
| Iraq | 2 | | | | | | 2 |
| Kazakhstan | | 1 | | 1 | | | 2 |
| Sri Lanka | | | 2 | | | | 2 |
| Argentina | | | 1 | | | | 1 |
| Canada | | | 1 | | | | 1 |
| Japan | 1 | | | | | | 1 |
| Kenya | | | | | 1 | | 1 |
| Kyrgyzstan | | | | | 1 | | 1 |
| Lebanon | | | 1 | | | | 1 |
| Malawi | | | 1 | | | | 1 |
| Romania | | | 1 | | | | 1 |
| Spain | | 1 | | | | | 1 |
| Socialist Federal Republic of Yugoslavia | | | | | 1 | | 1 |
| Unknown origin | 45 | 29 | 234 | 35 | 15 | | 358 |
| Total | 98 | 92 | 539 | 48 | 63 | 9 | 849 |

3.2. Racial and Geographical Diversity

3.2.1. Qualitative Traits

The frequency distributions of different phenotypic classes of the eight qualitative traits showed considerable variation (Table 2). In the entire set, decumbent growth habit (74.6%), high culm branching (45.0%), medium sheath pubescence (40.6%), sparse and medium ligule pubescence (45.5% and 39.9%, respectively), sparse leaf pubescence (51.1%), diffuse sparse and diffuse dense inflorescence shape (30.2% and 27.7%, respectively), light brown-colored seed (42.1%), and straw apiculus color (63.2%) were the predominant classes of qualitative traits. Among the races, the frequencies of qualitative trait classes varied, particularly for culm branching, sheath pubescence, and inflorescence shape. High culm branching was the most common trait in all races except *patentissimum*, where low (41.3%) and medium (34.9%) branching were the more prevalent, while *contractum* had all the three classes of culm branching—medium (39.6%), low (34.1%), and high (27.5%), in high frequency. Accessions of the race *compactum* and *miliaceum* had all three classes of sheath pubescence in high frequency (26.5–38.8% in *compactum*; 26.3–42.7% in *miliaceum*), while *contractum* had medium sheath pubescence (46.2%), *ovatum* showed sparse (43.8%) sheath pubescence, and *patentissimum* showed dense (39.7%) and sparse (36.5%) pubescence. For inflorescence shape, arched dense inflorescence in *contractum* (80.2%), elliptic dense (40.9%) and elliptic sparse (48.0%) inflorescence in *compactum*, diffuse sparse (43.4%) and diffuse dense (41.2%) inflorescence in *miliaceum*, globose dense (54.2%) and globose sparse (35.4%) inflorescence in *ovatum*, and arched sparse (68.3%) inflorescence in *patentissimum* were in high proportion. Light-brown seed was in higher proportion in all five races, except in *ovatum* where both straw (45.8%) and light-brown (27.1%) colored seeds were more prevalent. Race *miliaceum* had all eleven classes of seed color (Table 2). Regarding other traits, a large portion of accessions in all five races had decumbent growth habit (58.2–89.6%), sparse ligule pubescence (40.6–58.3%), and sparse leaf pubescence (45.8–64.6%), and straw apiculus color (53.8–89.6%). The H' revealed that in the entire set, inflorescence shape had the highest diversity (0.767) while seed color had the highest H' in each race (0.582 in *ovatum* to 0.709 in *compactum*). Figure 1 shows variability in the proso millet germplasm accessions for seed color. The lowest H' was observed for apiculus color in the entire set (0.286) (Table 3).

3.2.2. Quantitative Traits

Considerable variability was observed in the entire collection of proso millet germplasm conserved at the ICRISAT genebank: days to 50% flowering varied from 26 to 50 days after sowing (DAS), plant height varied from 20 to 133 cm and inflorescence length varied from 22 to 400 mm. Figure S2 shows frequency (histogram) of all the ten quantitative traits. On average, accessions of the race *ovatum* flowered at 33 DAS and produced short plants (46 cm) and short inflorescence (108 mm), whereas *miliaceum* flowered at 35 DAS and produced tall plants (62 cm) and long inflorescences (209 mm) with high number of inflorescence primary branches (17); however, a wide range of variation existed within each race (Table 4). At the country level, early flowering and shortest inflorescence accessions were from Russian Federation (average 31 DAS), and late flowering were from India (average 39 DAS), the shortest plants (average 34 cm) were from Mexico, and the tallest plants (average 93 cm) and longest inflorescences (average 307 mm) were from Nepal (data not shown). The H' revealed that the trait inflorescence primary branch number had the highest diversity (0.649) in the entire set as well as in the races *miliaceum* (0.649) and *patentissimum* (0.586), and in Asia (0.631) and Europe (0.651), while basal tillers number in *compactum* (0.643), flag leaf blade length in *contractum* (0.624), plant height in *ovatum* (0.625), and days to 50% flowering (0.544) and flag leaf sheath length (0.544) in the Americas had the highest H' value. However, all the quantitative traits showed high diversity in the entire set (0.576–0.649) as well as in each race (0.315–0.649) and region of origin (0.276–0.651) (Table 3).

Table 2. Frequency of phenotypic classes of each qualitative trait in the five races and in the entire set of proso millet germplasm conserved at the ICRISAT genebank, Patancheru, India.

| Trait | Class | Race | | | | | Entire Set |
|---------------------|------------------|------------------|-------------------|------------------|---------------|----------------------|------------|
| | | <i>compactum</i> | <i>contractum</i> | <i>miliaceum</i> | <i>ovatum</i> | <i>patentissimum</i> | |
| Growth habit | Decumbent | 85 (86.7) † | 53 (58.2) | 407 (75.5) | 43 (89.6) | 38 (60.3) | 627 (74.6) |
| | Erect | 11 (11.2) | 24 (26.4) | 93 (17.3) | 3 (6.3) | 18 (28.6) | 149 (17.8) |
| | Erect geniculate | 2 (2.0) | 14 (15.4) | 39 (7.2) | 2 (4.2) | 7 (11.1) | 64 (7.6) |
| Culm branching | High | 48 (49.0) | 25 (27.5) | 269 (49.9) | 21 (43.8) | 15 (23.8) | 379 (45.0) |
| | Low | 14 (14.3) | 31 (34.1) | 93 (17.3) | 1 (2.1) | 26 (41.3) | 165 (19.6) |
| | Medium | 36 (36.7) | 36 (39.6) | 177 (32.8) | 26 (54.2) | 22 (34.9) | 297 (35.4) |
| Sheath pubescence | Dense | 26 (26.5) | 23 (25.3) | 167 (31.0) | 7 (14.6) | 25 (39.7) | 249 (29.5) |
| | Medium | 34 (34.7) | 42 (46.2) | 230 (42.7) | 20 (21.7) | 15 (23.8) | 341 (40.6) |
| | Sparse | 38 (38.8) | 27 (29.7) | 142 (26.3) | 21 (43.8) | 23 (36.5) | 251 (29.9) |
| Ligule pubescence | Dense | 17 (17.3) | 8 (9.9) | 85 (15.8) | 4 (8.3) | 9 (14.3) | 124 (14.6) |
| | Medium | 30 (30.6) | 34 (37.4) | 235 (43.6) | 16 (33.3) | 20 (31.7) | 335 (39.9) |
| | Sparse | 51 (52.0) | 50 (54.9) | 219 (40.6) | 28 (58.3) | 34 (54.0) | 382 (45.5) |
| Leaf pubescence | Dense | 17 (17.3) | 8 (8.8) | 79 (14.7) | 4 (8.3) | 6 (9.5) | 114 (13.6) |
| | Medium | 26 (26.5) | 28 (30.8) | 213 (39.5) | 13 (21.1) | 17 (27.0) | 297 (35.4) |
| | Sparse | 55 (56.1) | 56 (61.5) | 247 (45.8) | 31 (64.6) | 40 (63.5) | 430 (51.1) |
| Inflorescence shape | Arched dense | 3 (3.1) | 73 (80.2) | 47 (8.7) | - | 1 (1.6) | 124 (14.8) |
| | Arched sparse | 2 (2.0) | 5 (5.5) | 13 (2.4) | - | 43 (68.3) | 63 (7.5) |
| | Diffuse dense | 1 (1.0) | 2 (2.2) | 222 (41.2) | - | 8 (12.7) | 233 (27.7) |
| | Diffuse sparse | 4 (4.1) | 3 (3.3) | 234 (43.4) | 2 (4.2) | 11 (17.5) | 254 (30.2) |
| | Elliptic dense | 40 (40.9) | 2 (2.2) | 16 (3.0) | 1 (2.1) | - | 59 (7.0) |
| | Elliptic sparse | 47 (48.0) | 7 (7.7) | 6 (1.1) | 2 (4.2) | - | 62 (7.4) |
| | Globose dense | 1 (1.0) | - | - | 26 (54.2) | - | 27 (3.2) |
| | Globose sparse | - | - | 1 (0.2) | 17 (35.4) | - | 18 (2.1) |
| Seed color | Black | - | 1 (1.1) | 2 (0.4) | - | - | 3 (0.4) |
| | Brown | - | - | 4 (0.7) | - | - | 4 (0.5) |
| | Dark brown | 3 (3.1) | 1 (1.1) | 8 (1.5) | - | - | 12 (1.4) |
| | Dark green | - | 1 (1.1) | 4 (0.7) | - | - | 5 (0.6) |
| | Dark olive green | 1 (1.0) | - | 39 (7.2) | - | 3 (4.8) | 43 (5.1) |
| | Dark red | 12 (12.2) | 2 (2.2) | 8 (1.5) | 2 (4.2) | - | 24 (2.9) |
| | Light brown | 35 (35.7) | 28 (30.8) | 255 (47.3) | 13 (27.1) | 23 (36.5) | 354 (42.1) |
| | Light red | 9 (9.2) | 7 (7.8) | 31 (5.8) | 5 (10.4) | 2 (3.2) | 55 (6.5) |
| | Olive green | - | 2 (2.2) | 13 (2.4) | - | 4 (6.3) | 19 (2.3) |

Table 2. Cont.

| Trait | Class | Race | | | | | Entire Set |
|----------------|--------|------------------|-------------------|------------------|---------------|----------------------|------------|
| | | <i>compactum</i> | <i>contractum</i> | <i>miliaceum</i> | <i>ovatum</i> | <i>patentissimum</i> | |
| Apiculus color | Straw | 17 (17.3) | 28 (30.8) | 93 (17.3) | 22 (45.8) | 20 (31.7) | 180 (21.4) |
| | White | 21 (21) | 22 (24.2) | 82 (15.2) | 6 (12.5) | 11 (17.5) | 142 (16.9) |
| | Purple | 24 (24.5) | 19 (20.9) | 249 (46.2) | 5 (10.4) | 12 (19.0) | 309 (36.8) |
| | Straw | 74 (75.5) | 73 (80.2) | 290 (53.8) | 43 (89.6) | 51 (81.0) | 531 (63.2) |

[†] values within parentheses indicate the percentage of accessions in each phenotypic classes of each qualitative trait.

Table 3. Shannon–Weaver diversity indices (H') of eight qualitative and ten quantitative traits in the entire set, five races and regions of proso millet germplasm conserved at the ICRISAT genebank, Patancheru, India.

| Trait | Entire | Race | | | | | Region | | |
|---------------------------------------|--------|------------------|-------------------|------------------|---------------|----------------------|----------|-------|--------|
| | | <i>compactum</i> | <i>contractum</i> | <i>miliaceum</i> | <i>ovatum</i> | <i>patentissimum</i> | Americas | Asia | Europe |
| Qualitative traits | | | | | | | | | |
| Growth habit | 0.313 | 0.195 | 0.415 | 0.306 | 0.176 | 0.394 | 0.171 | 0.436 | 0.293 |
| Culm branching | 0.455 | 0.432 | 0.472 | 0.441 | 0.336 | 0.467 | 0.374 | 0.436 | 0.460 |
| Sheath pubescence | 0.472 | 0.472 | 0.462 | 0.468 | 0.437 | 0.467 | 0.413 | 0.430 | 0.320 |
| Ligule pubescence | 0.437 | 0.437 | 0.396 | 0.443 | 0.386 | 0.424 | 0.371 | 0.458 | 0.293 |
| Leaf pubescence | 0.426 | 0.426 | 0.381 | 0.437 | 0.366 | 0.376 | 0.171 | 0.454 | 0.272 |
| Inflorescence shape | 0.767 | 0.490 | 0.354 | 0.520 | 0.454 | 0.388 | 0.647 | 0.728 | 0.732 |
| Seed color | 0.716 | 0.709 | 0.685 | 0.698 | 0.582 | 0.637 | 0.508 | 0.737 | 0.830 |
| Apiculus color | 0.286 | 0.242 | 0.221 | 0.300 | 0.145 | 0.212 | 0.171 | 0.294 | 0.252 |
| Mean | 0.484 | 0.425 | 0.423 | 0.451 | 0.360 | 0.420 | 0.353 | 0.497 | 0.431 |
| Quantitative traits | | | | | | | | | |
| Days to 50% flowering | 0.585 | 0.568 | 0.588 | 0.572 | 0.315 | 0.484 | 0.544 | 0.594 | 0.478 |
| Plant height (cm) | 0.576 | 0.567 | 0.607 | 0.602 | 0.625 | 0.565 | 0.461 | 0.621 | 0.600 |
| Basal tillers number | 0.593 | 0.643 | 0.603 | 0.604 | 0.566 | 0.562 | 0.276 | 0.540 | 0.579 |
| Flag leaf blade length (mm) | 0.618 | 0.629 | 0.624 | 0.600 | 0.568 | 0.519 | 0.541 | 0.622 | 0.609 |
| Flag leaf blade width (mm) | 0.609 | 0.420 | 0.558 | 0.558 | 0.506 | 0.556 | 0.471 | 0.605 | 0.531 |
| Flag leaf sheath length (mm) | 0.635 | 0.508 | 0.591 | 0.626 | 0.565 | 0.556 | 0.544 | 0.585 | 0.569 |
| Peduncle length (mm) | 0.622 | 0.614 | 0.584 | 0.625 | 0.620 | 0.579 | 0.509 | 0.604 | 0.592 |
| Panicle exertion (mm) | 0.598 | 0.571 | 0.584 | 0.595 | 0.526 | 0.584 | 0.471 | 0.600 | 0.604 |
| Inflorescence length (mm) | 0.628 | 0.491 | 0.561 | 0.626 | 0.598 | 0.561 | 0.471 | 0.602 | 0.598 |
| Inflorescence primary branches number | 0.649 | 0.598 | 0.604 | 0.649 | 0.601 | 0.586 | 0.403 | 0.631 | 0.651 |
| Mean | 0.611 | 0.561 | 0.590 | 0.606 | 0.549 | 0.555 | 0.469 | 0.600 | 0.581 |

Table 4. Mean and range of proso millet germplasm conserved at the ICRISAT genebank for agronomic traits.

| Trait | Mean [§] | | | | | | Range | | | | | |
|---------|-------------------|---------------|---------------|--------------|--------------|-----------------------------|--------|---------|--------|---------|---------|-----------------------------|
| | CP # | CT | ML | OV | PA | Entire set (849 Accessions) | CP | CT | ML | OV | PA | Entire set (849 Accessions) |
| DF † | 33 c (2.5) † | 34 ab (3.0) | 35 a (3.5) | 33 c (1.8) | 34 ab (4.5) | 34 (3.4) | 29–40 | 28–44 | 26–49 | 30–40 | 30–50 | 26–50 |
| PLHT | 52 b (12.9) | 59 a (1.9) | 62 a (18.1) | 46 c (7.5) | 58 a (16.8) | 59 (17.6) | 25–110 | 25–118 | 23–113 | 25–60 | 20–120 | 20–133 |
| BTN | 3 b (1.2) | 3 b (1.3) | 4 a (1.5) | 4 a (1.2) | 3 b (1.3) | 4 (1.4) | 1–7 | 1–8 | 1–9 | 2–7 | 1–9 | 1–9 |
| FLBL | 212 ab (48.9) | 214 ab (59.1) | 229 a (54.2) | 205 b (33.0) | 205 b (47.4) | 222 (53.5) | 90–320 | 80–380 | 90–370 | 140–280 | 130–340 | 80–380 |
| FLBW | 21 b (5.5) | 19 c (6.1) | 19 c (5.8) | 24 a (5.1) | 16 d (5.2) | 19 (6.0) | 9–30 | 6–30 | 7–30 | 14–30 | 6–30 | 6–30 |
| FLSL | 76 b (16.4) | 81 a (17.6) | 84 a (16.5) | 70 c (11.1) | 82 a (13.6) | 82 (16.6) | 50–170 | 40–140 | 30–140 | 55–100 | 50–120 | 30–170 |
| PEDL | 191 ab (71.8) | 197 ab (67.6) | 175 bc (58.8) | 164 c (49.7) | 206 a (78.4) | 181 (63.6) | 50–370 | 60–400 | 15–380 | 55–270 | 70–400 | 15–400 |
| PANEX | 116 ab (68.8) | 117 ab (59.4) | 91 c (53.6) | 99 bc (54.6) | 124 a (78.0) | 100 (59.8) | 0–270 | 20–300 | 0–280 | 0–300 | 0–320 | 0–320 |
| INFL | 145 b (41.9) | 191 a (51.2) | 209 a (53.5) | 108 c (16.6) | 198 a (50.9) | 193 (58.2) | 80–370 | 110–400 | 22–350 | 70–140 | 120–380 | 22–400 |
| INF-PBN | 16 ab (3.5) | 15 ab (4.8) | 17 a (4.1) | 15 ab (3.6) | 14 b (3.4) | 16 (4.2) | 8–24 | 5–26 | 5–29 | 7–25 | 7–23 | 5–29 |

† DF: days to 50% flowering; PLHT: plant height (cm); BTN: basal tillers number; FLBL: flag leaf blade length (mm); FLBW: flag leaf blade width (mm); FLSL: flag leaf sheath length (mm); PEDL: peduncle length (mm); PANEX: panicle exertion (mm); INFL: inflorescence length (mm); INF-PBN: inflorescence primary branches number. # CP: *compactum*; CT: *contractum*; ML: *miliaceum*; OV: *ovatum*; PA: *patentissimum*. [§] Means of races were tested following the Newman-Keuls test [18,19]. Means followed by the same letters are not significant at $p \leq 0.05$ and means followed by different letters are significant at $p \leq 0.05$. † value within parentheses indicate standard deviation.



Figure 1. Variability in the proso millet germplasm accessions for seed color.

Gower's phenotypic distance constructed based on 18 traits including 8 qualitative traits and 10 quantitative traits using 841 accessions revealed an average distance of 0.261, varying from 0.010 between IPm 2011 and IPm 2012 to 0.591 between IPm 370 and IPm 2806, and the top ten pairs of the most diverse accessions were identified (Table 5). On average, *miliaceum* had the highest among accessions within race pairwise distance (0.254) while the lowest was seen in *ovatum* (0.192). The races *miliaceum* and *ovatum* showed the highest divergence with each other (0.275), while the lowest divergence was observed between *compactum* and *ovatum* (0.229) (Table 6). Clustering of accessions based on Gower's distance matrix following the neighbor-joining method revealed three major groups of accessions: Cluster I (C-I) largely contained accessions from Asia, Cluster II (C-II) represented accessions from Europe, and Cluster III (C-III) represented accessions from both Asia and Europe (Figure 2). Accessions of *miliaceum*, *contractum*, and *patentissimum* were found in all three clusters, while those of *ovatum* and *contractum* were found mostly in C-II and C-III. Principal component analysis revealed the first three principal components (PCs) as important, explaining about 73.32% of the total variance. Plant height, flag leaf blade length, flag leaf sheath length, and inflorescence length contributed largely to PC1, which explained about 37.19% of the total variance (Table 7).

Table 5. Most diverse pairs of accessions identified in the entire set using Gower's distance.

| Top Ten Pairs of Accessions | Gower's Phenotypic Distance |
|-----------------------------|-----------------------------|
| IPm 370 and IPm 2806 | 0.591 |
| IPm 381 and IPm 2734 | 0.586 |
| IPm 381 and IPm 2747 | 0.581 |
| IPm 460 and IPm 2747 | 0.579 |
| IPm 370 and IPm 2734 | 0.578 |
| IPm 2748 and IPm 381 | 0.578 |
| IPm 381 and IPm 2806 | 0.575 |
| IPm 2748 and IPm 370 | 0.575 |
| IPm 381 and IPm 2723 | 0.574 |
| IPm 362 and IPm 2734 | 0.572 |

Table 6. Average phenotypic distance among races.

| Race | <i>compactum</i> | <i>contractum</i> | <i>miliaceum</i> | <i>ovatum</i> |
|----------------------|------------------|-------------------|------------------|---------------|
| <i>contractum</i> | 0.269 | | | |
| <i>miliaceum</i> | 0.264 | 0.266 | | |
| <i>ovatum</i> | 0.229 | 0.274 | 0.275 | |
| <i>patentissimum</i> | 0.266 | 0.254 | 0.267 | 0.274 |

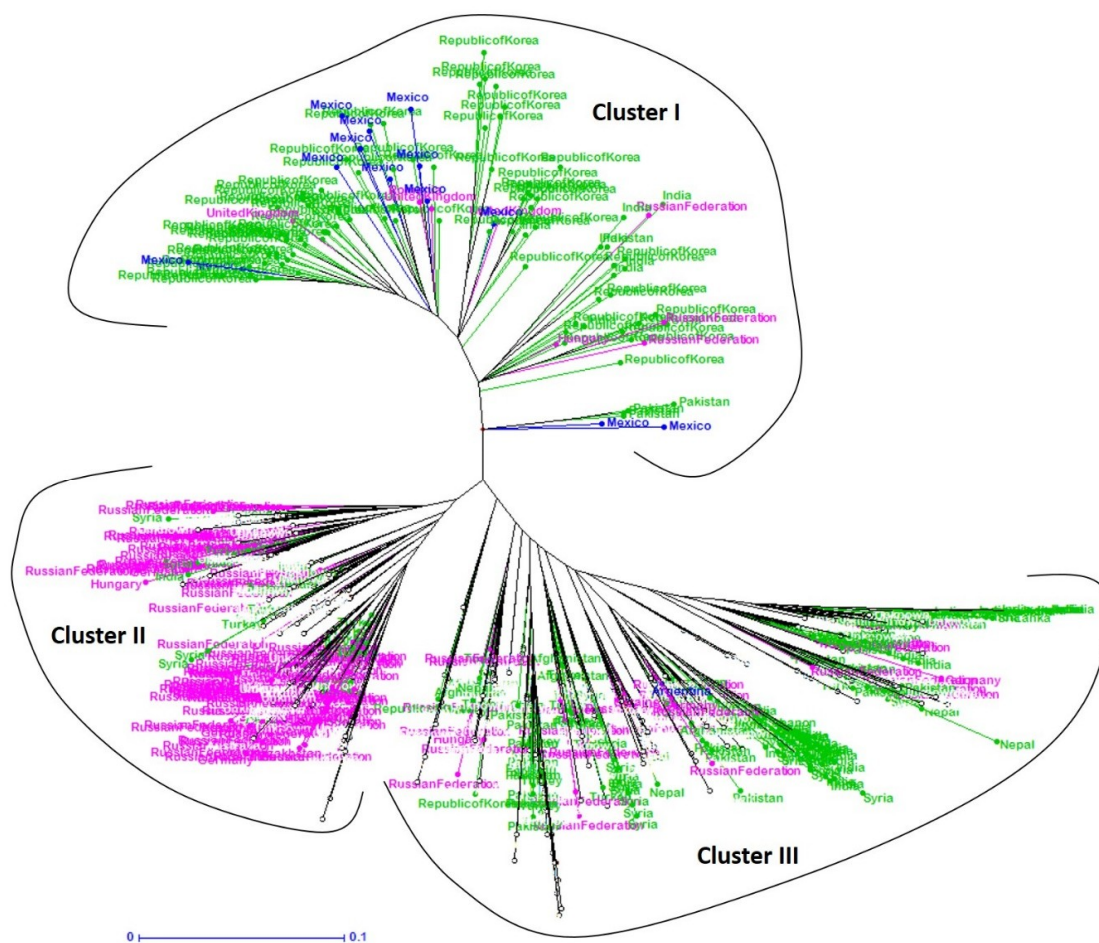


Figure 2. Neighbor-joining clustering of proso millet accessions based on morpho-agronomic traits. Color code: pink—Europe; green—Asia; blue—the Americas.

Table 7. Principal component analysis of proso millet germplasm conserved at the ICRISAT genebank, India.

| Principal Component (PC) | PC1 | PC2 | PC3 |
|---------------------------------------|--------|-------|--------|
| Eigenvalue | 3.72 | 2.33 | 1.28 |
| Percent variance | 37.19% | 23.3% | 12.83% |
| Trait | | | |
| Days to 50% flowering | 0.06 | −0.41 | 0.50 |
| Plant height (cm) | 0.46 | 0.00 | 0.28 |
| Basal tillers number | 0.15 | −0.23 | −0.26 |
| Flag leaf blade length (mm) | 0.44 | −0.13 | −0.18 |
| Flag leaf blade width (mm) | 0.28 | 0.16 | −0.56 |
| Flag leaf sheath length (mm) | 0.40 | 0.05 | 0.04 |
| Peduncle length (mm) | 0.19 | 0.58 | 0.18 |
| Panicle exertion (mm) | 0.08 | 0.60 | 0.20 |
| Inflorescence length (mm) | 0.42 | −0.15 | 0.32 |
| Inflorescence primary branches number | 0.34 | −0.13 | −0.27 |

3.2.3. Trait-Specific Sources

In the entire collection, 12 accessions flowered in <30 days (26 to 29 DAS), eight of them from the Russian Federation (IPm 2577, IPm 2601, IPm 2526, IPm 2510, IPm 2527, IPm 2774, IPm 2509, IPm 2521), one from Syria (IPm 2903), and three with unknown origin (IPm 2273, IPm 2035, IPm 2007); six accessions had inflorescence length of over 350 mm (IPm 2661, IPm 2660, IPm 2095, IPm 2198, IPm

2107, IPm 2197); and four accessions (IPm 2198, IPm 2100, IPm 2197, IPm 2200) were over 125 cm tall (125–133 cm); 8 accessions (IPm 2535, IPm 370, IPm 381, IPm 79, IPm 121, IPm 2140, IPm 2122, IPm 38) had a maximum seed length of 3.5–4.3 mm.

3.2.4. Trait Associations

Correlations among agronomic traits were estimated in the entire set and in each race (Table 8). In the entire set, days to 50% flowering was significantly and positively correlated with plant height, basal tillers number, flag leaf blade length, and inflorescence length, while it was significantly negatively correlated with flag leaf blade width, peduncle length, and panicle exertion. Peduncle length and panicle exertion in the entire set and in each race showed significant negative correlations with days to 50% flowering (except panicle exertion in *ovatum*, which showed positive correlation with days to 50% flowering). Days to 50% flowering in the entire set and in *miliaceum* showed significantly positive correlation with plant height, while *ovatum* showed negatively significant correlation. Similarly, inflorescence length in the entire set showed significant positive correlation with days to 50% flowering, plant height, basal tillers number, flag leaf blade length, flag leaf blade width, flag leaf sheath length, peduncle length, and inflorescence primary branches number; however, the magnitude of correlations differed within each race. For instance, inflorescence length showed significant positive correlations: with peduncle length and panicle exertion in *ovatum*; with days to 50% flowering, plant height, flag leaf blade length, flag leaf sheath length, and inflorescence primary branches number in *patentissimum*; with all traits in *miliaceum*; and with plant height, basal tillers, flag leaf blade length, flag leaf blade width, and flag leaf sheath length in *contractum* and *compactum*. This signifies that while estimating correlation, we must also consider existing racial characteristics/groups because correlations depend on the nature of the population.

Table 8. Correlation coefficient among quantitative traits of the entire set and each race of proso millet germplasm conserved at the ICRISAT genebank, Patancheru, India.

| | DF # | PLHT | BTN | FLBL | FLBW | FLSL | PEDL | PANEX | INFL |
|----------------------|-----------|----------|-----------|----------|----------|------|------|-------|------|
| PLHT | | | | | | | | | |
| Entire | 0.259 ** | | | | | | | | |
| <i>compactum</i> | −0.126 | | | | | | | | |
| <i>contractum</i> | 0.008 | | | | | | | | |
| <i>miliaceum</i> | 0.296 ** | | | | | | | | |
| <i>ovatum</i> | −0.566 ** | | | | | | | | |
| <i>patentissimum</i> | 0.187 | | | | | | | | |
| BTN | | | | | | | | | |
| Entire | 0.092 ** | 0.183 ** | | | | | | | |
| <i>compactum</i> | 0.065 | 0.200 * | | | | | | | |
| <i>contractum</i> | 0.006 | 0.121 | | | | | | | |
| <i>miliaceum</i> | 0.078 | 0.214 ** | | | | | | | |
| <i>ovatum</i> | 0.094 | −0.024 | | | | | | | |
| <i>patentissimum</i> | 0.211 | −0.02 | | | | | | | |
| FLBL | | | | | | | | | |
| Entire | 0.117 ** | 0.656 ** | 0.338 ** | | | | | | |
| <i>compactum</i> | 0.101 | 0.479 ** | 0.358 ** | | | | | | |
| <i>contractum</i> | −0.001 | 0.634 ** | 0.371 ** | | | | | | |
| <i>miliaceum</i> | 0.074 | 0.686 ** | 0.341 ** | | | | | | |
| <i>ovatum</i> | −0.104 | 0.078 | 0.286 * | | | | | | |
| <i>patentissimum</i> | 0.377 ** | 0.693 ** | 0.135 | | | | | | |
| FLBW | | | | | | | | | |
| Entire | −0.265 ** | 0.267 ** | 0.118 ** | 0.524 ** | | | | | |
| <i>compactum</i> | −0.136 | 0.446 ** | 0.289 ** | 0.593 ** | | | | | |
| <i>contractum</i> | −0.164 | 0.515 ** | 0.287 ** | 0.709 ** | | | | | |
| <i>miliaceum</i> | −0.273 ** | 0.295 ** | 0.088 * | 0.556 ** | | | | | |
| <i>ovatum</i> | −0.383 ** | 0.350 * | 0.353 * | 0.539 ** | | | | | |
| <i>patentissimum</i> | −0.147 | 0.514 ** | −0.347 ** | 0.373 ** | | | | | |
| FLSL | | | | | | | | | |
| Entire | 0.037 | 0.597 ** | 0.101 ** | 0.551 ** | 0.410 ** | | | | |
| <i>compactum</i> | −0.171 | 0.417 ** | 0.051 | 0.423 ** | 0.227 * | | | | |
| <i>contractum</i> | 0.023 | 0.728 ** | 0.295 ** | 0.637 ** | 0.530 ** | | | | |
| <i>miliaceum</i> | −0.006 | 0.574 ** | 0.079 | 0.547 ** | 0.544 ** | | | | |
| <i>ovatum</i> | −0.146 | 0.249 | 0.205 | 0.453 ** | 0.332 * | | | | |
| <i>patentissimum</i> | 0.107 | 0.601 ** | −0.082 | 0.548 ** | 0.444 ** | | | | |

Table 8. Cont.

| | DF # | PLHT | BTN | FLBL | FLBW | FLSL | PEDL | PANEX | INFL |
|----------------------|-----------|----------|-----------|----------|----------|----------|-----------|-----------|----------|
| PEDL | | | | | | | | | |
| Entire | −0.354 ** | 0.380 ** | −0.149 ** | 0.089 ** | 0.264 ** | 0.341 ** | | | |
| <i>compactum</i> | −0.561 ** | 0.592 ** | −0.105 | −0.011 | 0.279 ** | 0.219 * | | | |
| <i>contractum</i> | −0.349 ** | 0.519 ** | 0.044 | 0.270 ** | 0.314 ** | 0.448 ** | | | |
| <i>miliaceum</i> | −0.268 ** | 0.381 ** | −0.137 ** | 0.122 ** | 0.297 ** | 0.405 ** | | | |
| <i>ovatum</i> | −0.524 ** | 0.825 ** | −0.285 * | 0.028 | 0.076 | 0.277 | | | |
| <i>patentissimum</i> | −0.666 ** | 0.297 * | −0.241 | 0.019 | 0.447 ** | 0.214 | | | |
| PANEX | | | | | | | | | |
| Entire | −0.368 ** | 0.225 ** | −0.193 ** | −0.071 * | 0.153 ** | 0.087 * | 0.937 ** | | |
| <i>compactum</i> | −0.552 ** | 0.500 ** | −0.138 | −0.104 | 0.234 * | 0.075 | 0.983 ** | | |
| <i>contractum</i> | −0.394 ** | 0.346 ** | −0.064 | 0.059 | 0.154 | 0.184 | 0.941 ** | | |
| <i>miliaceum</i> | −0.285 ** | 0.238 ** | −0.175 ** | −0.043 | 0.152 ** | 0.141 ** | 0.951 ** | | |
| <i>ovatum</i> | 0.006 | 0.476 ** | −0.284 * | −0.142 | −0.178 | −0.101 | 0.672 ** | | |
| <i>patentissimum</i> | −0.644 ** | 0.219 | −0.241 | −0.047 | 0.357 ** | 0.044 | 0.920 ** | | |
| INFL | | | | | | | | | |
| Entire | 0.327 ** | 0.800 ** | 0.209 ** | 0.638 ** | 0.105 ** | 0.602 ** | 0.148 ** | −0.021 | |
| <i>compactum</i> | 0.086 | 0.709 ** | 0.270 ** | 0.568 ** | 0.264 ** | 0.500 ** | 0.143 | 0.025 | |
| <i>contractum</i> | 0.19 | 0.746 ** | 0.244 * | 0.686 ** | 0.429 ** | 0.712 ** | 0.14 | −0.076 | |
| <i>miliaceum</i> | 0.276 ** | 0.838 ** | 0.237 ** | 0.696 ** | 0.252 ** | 0.582 ** | 0.244 ** | 0.086 * | |
| <i>ovatum</i> | 0.192 | 0.257 | −0.247 | 0.003 | −0.166 | 0.014 | 0.313 * | 0.309 * | |
| <i>patentissimum</i> | 0.442 ** | 0.654 ** | 0.157 | 0.710 ** | 0.189 | 0.542 ** | −0.113 | −0.192 | |
| INF_PBN | | | | | | | | | |
| Entire | 0.022 | 0.496 ** | 0.185 ** | 0.554 ** | 0.390 ** | 0.350 ** | 0.003 | −0.115 ** | 0.445 ** |
| <i>compactum</i> | 0.235 * | 0.002 | 0.133 | 0.218 * | 0.077 | −0.022 | −0.399 ** | −0.431 ** | 0.264 ** |
| <i>contractum</i> | −0.047 | 0.588 ** | 0.192 | 0.689 ** | 0.566 ** | 0.456 ** | 0.114 | −0.066 | 0.544 ** |
| <i>miliaceum</i> | −0.035 | 0.534 ** | 0.210 ** | 0.578 ** | 0.440 ** | 0.381 ** | 0.092* | −0.022 | 0.486 * |
| <i>ovatum</i> | −0.061 | −0.008 | −0.031 | 0.25 | 0.342 * | 0.157 | −0.08 | −0.231 | 0.219 |
| <i>patentissimum</i> | 0.104 | 0.601 ** | −0.073 | 0.427 ** | 0.471 ** | 0.297 * | 0.062 | −0.003 | 0.384 ** |

DF: days to 50% flowering; PLHT: plant height (cm); BTN: basal tillers number; FLBL: flag leaf blade length (mm); FLBW: flag leaf blade width (mm); FLSL: flag leaf sheath length (mm); PEDL: peduncle length (mm); PANEX: panicle exertion (mm); INFL: inflorescence length (mm); INF-PBN: inflorescence primary branches number. * Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$.

4. Discussion

Germplasm without sufficient characterization and evaluation data—particularly in case of low-research-priority crops like proso millet, limiting the use of germplasm in breeding programs due to extremely low funding for research and development compared to other major crops. This study provides a broad overview of variability in the global proso millet germplasm conserved at the ICRISAT genebank for morpho-agronomic traits, and investigated racial and geographical diversity. The majority of accessions in the ICRISAT proso millet collection belonged to *miliaceum* (63.5%), while the other races represented <11%, indicating the low representation of other races in the entire set. de Wet [1] classified proso millet germplasm into five races based on panicle morphology and shape. Besides these characteristics, accessions also showed variations in different morpho-agronomic traits among the races. Frequencies of qualitative traits varied among the races, particularly for culm branching, sheath pubescence, and inflorescence shape. Most accessions in the *ovatum* flowered earlier, produced short plants, and short inflorescence, while *miliaceum* flowered late, produced tall plants, and long inflorescence. Accessions within *miliaceum* also had the highest phenotypic distance (0.254), while the lowest distance was seen in *ovatum* (0.192). Races *miliaceum* and *ovatum* highly diverged with each other (0.275), while low divergence was observed between *compactum* and *ovatum* (0.229).

In the ICRISAT genebank proso millet collection, the majority of accessions originated from Asia (37.1%) and Europe (18.5%), indicating these two regions as major centers of diversity for proso millet. The highest diversity (H') was found in accessions from Asia (0.497) compared to those from Europe (0.431). Neighbor-joining clustering of accessions revealed three major clusters, representing those from Asia in C-I and Europe in C-II, with C-III representing those from both regions. The results from this study support the independent domestication of proso millet in Central Asia and Eastern Europe, or indicate that they might also have originated from a domestication within China and then spread westward across the Eurasian steppe [5]. Early flowering and shortest inflorescence accessions were largely from the Russian Federation (average 31 DAS), and late flowering accessions were from India (average 39 DAS), the shortest plants (average 34 cm) were from Mexico, and the tallest plants (average 93 cm) and longest inflorescences (average 307 mm) were from Nepal. These sets of accessions could be selectively explored for the identification of useful germplasms for respective traits.

The trait-specific sources identified in this study for early maturity, tall plants, long inflorescences, and greater seed size from the entire set of proso millet germplasms conserved at the ICRISAT genebank, and those that were identified in our previous study [12] were as follows: 18 accessions for high grain yield (IPm 9, IPm 2784, IPm 2621, IPm 2802, IPm 390, IPm 361, IPm 2824, IPm 2783, IPm 366, IPm 2620, IPm 2660, IPm 2685, IPm 2158, IPm 388, IPm 2700, IPm 384, IPm 1545, IPm 2661; grain yield 1601–2334 kg ha⁻¹), 8 accessions with greater seed weight (IPm 362, IPm 2826, IPm 381, IPm 2575, IPm 2273, IPm 2769, IPm 2780, IPm 2037; 100-seed weight 0.60–0.66g); 2 accessions that produced high grain yield with greater seed size (IPm 2, IPm 2661); 12 accessions for high grain Fe (63.3–73.2 mg kg⁻¹); 27 for Zn (40.6–46.7 mg kg⁻¹), 56 for Ca (185.5–241.2 mg kg⁻¹), and 27 for protein (16%–19%) including IPm 2069, IPm 2076, and IPm 2537 rich in grain Fe, Zn, Ca, and protein contents [12] could potentially support the breeding of high-yielding nutrient-dense cultivars with broad genetic base in proso millet. Besides these sources, the top ten pairs of the most diverse accessions were identified that could be utilized to broaden the genetic base of proso millet cultivars.

The ICRISAT genebank supplied over 6900 seed samples of proso millet to researchers in 39 countries for use in proso millet improvement. Proso millet researchers can obtain seed samples from the ICRISAT genebank (<http://genebank.icrisat.org/>) for research purposes via a Standard Material Transfer Agreement.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0472/9/5/112/s1>, Figure S1: Five races of proso millet (*miliaceum*, *patentissimum*, *contractum*, *compactum*, *ovatum*) based on panicle morphology and shape, Figure S2: Frequency (histogram) of ten quantitative traits of proso millet germplasm conserved at the ICRISAT genebank, Patancheru, India.

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