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Variability and trait-specific accessions for grain yield and nutritional traits in germplasm of little millet (*Panicum sumatrense* Roth. Ex. Roem. & Schult.)

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Abstract

Little millet (*Panicum sumatrense* Roth. Ex. Roem. & Schult.), a member of the grass family *Poaceae*, is native to India. It is nutritionally superior to major cereals, grows well on marginal lands, and can withstand drought and waterlogging conditions. Two-hundred diverse little millet landraces were characterized to assess variability for agronomic and nutritional traits and identify promising accessions. Highly significant variability was found for all the agronomic and grain nutrient traits. Accessions of *robusta* were high yielding whereas those of *nana* were rich in grain nutrients. About 80% of the accessions showed consistent protein and zinc (Zn) contents whereas iron (Fe) and calcium (Ca) contents were less consistent (29.5 and 63.5%, respectively) over 2 yr. Promising trait-specific accessions were identified for greater seed weight (10 accessions), high grain yield (15), high biomass yield (15), and consistently high grain nutrients (30) over 2 yr (R^2 = .69–.74, $P \leq .0001$). A few accessions showed consistently high for two or more nutrients (IPmr 449 for Fe, Zn, Ca, and protein; IPmr 981 for Zn and protein). Five accessions (IPmr 855, 974, 877, 897, 767) were high yielding and also rich in Ca. Consumption of 100 g of little millet grains can potentially contribute to the recommended dietary allowance of up to 28% Fe, 37% Zn, and 27% protein. Multilocation evaluation of the promising accessions across different soil types, fertility levels, and climatic conditions would help to identify valuable accessions for direct release as a cultivar or use in little millet improvement.

1 | INTRODUCTION

Crop and dietary diversity by including climate-resilient and nutrient-rich underutilized crops can potentially contribute to sustainable development goals (SDGs) in overcoming malnutrition and hunger in a changing climate scenario (Vetriventhan & Upadhyaya, 2019). The widespread occurrence of malnutrition and changing consumer preferences toward healthy foods underline the importance of bringing back the neglected, underutilized, but traditionally important crops such as small millets into the food basket for food and nutritional security. Small millets are a

Abbreviations: DAS, days after sowing; DV, daily value; RDA, recommended daily allowance per 100 g; SNP, single nucleotide polymorphism.

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group of small-seeded cereal crops belonging to the grass family *Poaceae*. Small millets include finger millet [*Eleusine coracana* (L.) Gaertn.], foxtail millet [*Setaria italica* (L.) P. Beauv.], proso millet (*Panicum miliaceum L.*), barnyard millet [*Echinochloa crus-galli* (L.) P. Beauv. and *Echinochloa colona* (L.) Link], kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense* Roth. Ex. Roem. & Schult.), teff [*Eragrostis tef* (Zucc.) Trotter], fonio (*Digitaria exilis* Stapf and *D. iburua* Stapf.), Job's tears (*Coix lachrymal-jobi* L.), guinea millet [*Brachiaria deflexa* (Schumach.) C.E.Hubb. ex Robyns, = *Urochloa deflexa* (Schumach.) H.Scholz], and browntop millet [*Brachiaria ramosa* (L.) Stapf. = *Urochloa ramosa* (L.) T.Q. Nguyen]. Small millets are known for their climate-resilient features, including diverse adaptation, less water requirement, lesser affected by insect pests and diseases, and the minimum vulnerability to environmental stresses (Bandyopadhyay et al., 2017; Goron & Raizada, 2015; Saxena et al., 2018; Vetriventhan et al., 2020).

Little millet is one of the small millets, and a native crop of India (de Wet et al., 1983). Little millet is distributed in India, Pakistan, Sri Lanka, Nepal, Myanmar, Thailand, China, the Philippines, and Indonesia as a weed and/or wild plant, and it is cultivated as cereal crop India, and also in Nepal, Pakistan, Sri Lanka, eastern Indonesia, and Myanmar (Hiremath et al., 1990; ICRISAT & FAO, 1996) [https://uses.plantnet-project.org/en/Panicum_sumatrense_(PROSEA)]. Essentially all of the little millet production occurs in India, with a production of 0.12 Mt on 0.26 Mha as of 2018 (Bhat et al., 2018). Current production represents a substantial decline over historical levels, where small millets were planted in 7.56 Mha in the period 1951–1955, reducing to 1.86 Mha by 2011–2015 (http://www.aicrpsm.res.in/Reports.html). The decline is mainly due to a major shift in diet preferences from traditional millets to other major cereals (rice [*Oryza sativa*], wheat [*Triticum aestivum*], and maize [*Zea mays*]) and other commercial crops (Eliazer Nelson et al., 2019; Padulosi et al., 2015). Another reason for the decline could be difficulty in de-hulling (removal of husk from grains). However, there are now effective de-hulling and processing equipment available that enable easy processing (Padulosi et al., 2015). Currently, the demand for little millet and other small millets has increased, underlining the necessity of directing more research and development towards these crops for improving food and nutritional security.

Little millet can produce considerably higher grain yield even under limited water supply on marginal lands. It has several agronomic advantages including diverse adaptation with high water-use efficiency, salt and waterlogging tolerance, and is less prone to insect pests and diseases (Ganapathy, 2017; Kalaisekar et al., 2017; Matsuura et al., 2016; Upadhyaya et al., 2015). Besides these climate-resilient features, various studies conducted over a period of time on little millet show that these grains are a good source of energy, protein, fiber and minerals, and are particularly rich in iron (Fe = 1.26–9.3 mg per 100 g) and dietary fiber (7.7%) compared to rice (Fe = 0.65–1.02 mg per 100 g; dietary fiber = 2.8–4.4 g per 100 g), wheat (Fe = 1.77 mg per 100 g; dietary fiber = 11.2 g per 100 g; Longvah et al., 2017; Saleh et al., 2013), and it can easily substitute rice-based food recipes. Due to its high fiber and mineral content, little millet grains are increasingly being used as an ingredient in multigrain and gluten-free cereal products. Altogether, little millet can serve as a potential alternative and supplement crop for crops and dietary diversity to achieve food, feed, and nutritional security for sustainable agriculture and healthy lives.

Core Ideas

- Little millet is one of the small millets that belong to the *Poaceae*, or grass family.
- This crop is nutritionally superior to the major cereals but can grow well on marginal lands.
- Landrace accessions showed significant variation in both grain yield and nutritional traits.
- Promising germplasm for grain yield and nutrients can support little millet improvement.

Germplasm is the basic requirement to drive a robust breeding program. Globally, only a limited number of little millet accessions (about 3,000) have been conserved in genebanks compared to other major crops, and a majority of them are in India (Upadhyaya et al., 2015). The cultivated accessions of little millet consist of two races: *nana* and *robusta*, and four subraces: *laxa* and *erecta* in the race *nana*, and *laxa* and *compacta* in the race *robusta*, based on plant and panicle characteristics (de Wet et al., 1983). Accessions of the race *nana* produce plants with decumbent to almost prostrate culms that become erect at flowering, erect and open with strongly branched inflorescence (subrace *laxa*) or with the inflorescence branches sometimes clumped at the time of maturity (subrace *compacta*). Accessions of the race *robusta* produce erect culms with large, strongly branched, erect and open inflorescences (*laxa*) or compact and curved inflorescences (*compacta*; de Wet et al., 1983). The genebank at ICRISAT, India, conserves 473 landrace accessions, representing the races and their subraces of little millet (http://genebank.icrisat.org/). Since little millet is a highly self-pollinating crop and hybridization is a difficult task because of small floret size, a selection from the high yielding accessions can also be tested under multiple locations for their adaptation for their release as cultivars. In India, the majority (63%) of released varieties of little millet are through the selection from the existing landraces. Extensive evaluation of the conserved germplasm for grain yield and other important traits can potentially contribute to little millet improvement. However, there are only a few studies that have investigated variability in the germplasm of little millet for morpho-agronomic (Arunachalam et al., 2005; Nirmalakumari et al., 2010; Selvi, Nimalakumari, & Subramanian, 2015) and grain nutrient traits (Chandel et al., 2014; Selvi, Nirmalakumari, & Senthil, 2015), and using a few landraces or limited to a particular locality. In this study, 200 little millet landraces were evaluated to assess variability for agronomic and grain nutritional traits and to identify promising accessions for direct release as a cultivar or use in crop improvement programs to develop high-yielding and nutritionally dense cultivars.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental details

The materials for this study consisted of 200 little millet landrace accessions. In our previous study, we have developed a core collection of little millet using the morpho-agronomic traits based cluster analysis, and from each cluster, about 10% of accessions were selected to constitute a core collection of 56 accessions (Upadhyaya et al., 2014). In this study, all the 56 accessions of the core collection were included, and the additional accessions were randomly chosen from the cluster information that was used to constitute core collection. These 200 accessions represent the diversity of the entire collection of 473 little millet accessions conserved at the ICRISAT genebank. Country and race-wise number of accessions used in this study are presented in Supplemental Table S1. The 200 accessions represent 52% (65 accessions) and 40% (135 accessions) of the entire accessions of the race *robusta* (126 accessions) and nana (334 accessions), respectively conserved at ICRISAT genebank. Country-wise, the majority of the collection site were from India (196), and a few were from Myanmar (2), Sri Lanka (1), and Syria (1). The 196 accessions from India originated (collection site) from 13 states, mostly Andhra Pradesh (26%), Maharashtra (25.5%), and Odisha (16.5%), whereas accessions from other states were represented by <10%. Figure 1 shows the collection sites of little millet accessions (103) with known geographical coordinates. The final set of 200 accessions represent both the races (67.5% *nana* and remaining were *robusta*) and subraces (*erecta* and *laxa* of race *nana* and *compacta* and *laxa* of race *robusta*; Supplemental Table S1).

The field experiments were conducted on red soils (alfisols) during the rainy season in 2015 and 2016 at ICRISAT, Hyderabad, Telangana, India (17˚ 30′ N latitude, 78˚ 15′ E longitude, altitude 545 m above msl), following the α-design in two replications. Accessions were sown in the third week of July in both years. Each accession occupied a single row of 4-m-length ridge (plot) with a spacing of 60 cm between ridges and plant-to-plant spacing of approximately 10 cm resulting in about 40 plants per accession. Fertilizers were applied at the rate of 20 kg N_2 ha^{-1} and 50 kg P_2O_5 ha^{-1} as basal dose and 45 kg N_2 ha^{-1} as a top dressing. Irrigation, hand weeding, and plant protection measures were provided as needed. The local climate of the study area is semi-arid with an average rainfall of 728 mm in 2015 and 1,238 mm in 2016; 73% of rains were received during the cropping period (July to Oct.) in 2015 and 83% in 2016. The maximum temperature varied from 26.4 to 40.8 ˚C in 2015 and from 24.6 to 38.8 ˚C in 2016, and the minimum temperature varied from 16.4 to 26.6 ˚C in 2015 and from 13.6 to 25.0 ˚C in 2016 during the crop period.

2.2 | Data collection

2.2.1 | Morpho-agronomic traits

The data on four qualitative and 15 agronomic traits were recorded following the descriptors of *Panicum sumatrense* (IBPGR, 1985). The data on all qualitative traits (plant pigmentation: pigmented and green; growth habit: erect, erect geniculate, and decumbent; inflorescence shape: arched lax, contracted arched, contracted stiff, diffuse open, elliptic compact, open lax, and open stiff; seed color: cinnamon brown, dark brown, dark olive green, grey-brown, light olive green, and straw) were recorded on a plot basis. The agronomic traits, namely days to 50% flowering, days to maturity, grain yield, and straw yield were recorded on a plot basis, whereas plant height, basal tillers number, culm thickness, flag leaf blade length, flag leaf blade width, flag leaf sheath length, inflorescence length, inflorescence lowest primary branch length, and inflorescence primary axis nodes number were recorded on five randomly selected plants in a plot. The 100-seed weight of each accession was estimated from the bulked seeds of each accession. Grain yield and straw yield per plot were estimated only in 2016, because in 2015 the crop was damaged due to lodging because of rain at maturity. The measure of grain and straw (dry weight) yields per plot were converted into grain and straw yields kg ha^{-1}, respectively. Harvest index was estimated by dividing the grain yield kg ha^{-1} by the total biological yield (grain yield plus straw yield) of each accession.

2.2.2 | Estimating grain nutrient content

Grain samples of 200 accessions, replicated twice in both years, were harvested at maturity from each plot of 4-m length. Bulk grain samples (whole grain, unhusked) of each accession (10 g) were washed for a few seconds using distilled water and dried in a hot air oven for 2 h at 40 ˚C to remove dust and metal particles in the samples to estimate grain protein, Ca, Fe, and Zn content at the Charles Renard Analytical Laboratory, ICRISAT, India. Nitric acid–hydrogen peroxide digestion of grain samples was conducted and grain

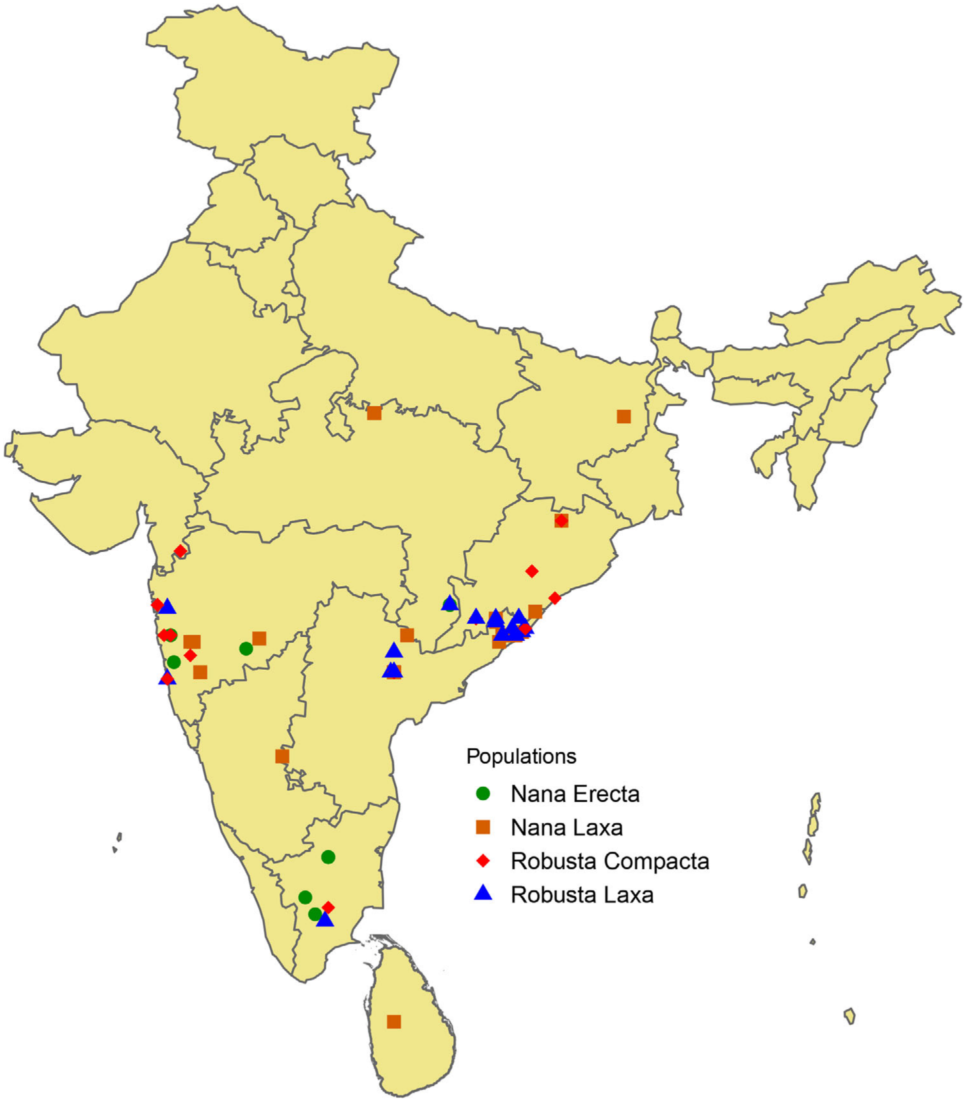

FIGURE 1 Geographical distribution of little millet accession (103) with known geographical coordinates

Ca, Fe, and Zn content in the digests were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Wheal et al., 2011). The Sulfuric acid–selenium digestion method was used to estimate total protein in which total nitrogen (N) was estimated in grain samples using Skalar Autoanalyzer, and protein percentage was calculated as N% times 6.25 conversion factor (Sahrawat et al., 2002).

2.3 | Statistical analyses

The data on 15 agronomic and four grain nutritional traits were analyzed for each rainy season individually and pooled of two rainy seasons following α-design using GenStat 17th edition (https://www.vsni.co.uk/). Homogeneity of error variance between years for all agronomic and grain nutrient traits was tested following Bartlett's test (Bartlett, 1937). Broad-sense heritability (h^2_b) was estimated for agronomic and grain nutrient traits, and traits were categorized as low (<.30), moderate (.30 to .60), and high (>.60). Means of the agronomic and grain nutrient traits were compared among races using the Newman–Keuls test (Keuls, 1952; Newman, 1939) using the R package "*agricolae*" (Felipe de Mendiburu, 2019). Correlation coefficients and the Shannon–Weaver diversity index (H′; Shannon & Weaver, 1949) were estimated using GenStat 17th edition (https://www.vsni.co.uk/). Gower's phenotypic distance matrix (Gower, 1971) was constructed using data on qualitative, agronomic, and grain nutrient traits of both the years together. We used high-quality single nucleotide polymorphism (SNP) markers data developed in our previous study on 165 accessions (Johnson et al., 2019) and estimated modified Roger's distance (MRD) matrix (Goodman & Stuber, 1983). Then, the phenotype and SNP-based distance matrices were merged by taking the average pair-wise distance of both matrices using the "*fuse*()" function in the R package "*analogue*" (Simpson & Oksanen, 2020). The base R function "*hclust*" was used for Ward.D2 hierarchical clustering (R Core Team, 2018), and the R package named "*dendextend*" was used for visualization of the dendrogram (Galili, 2015). The correlation between the phenotype and SNP-based distance was assessed by Mantel's test (Mantel, 1967). The promising trait-specific accessions for high grain yield, straw yield, greater seed weight, and grain nutrient-rich accessions were identified. The accessions were considered consistent for grain nutrients when the difference between 2 yr for a given trait was ≤ the average least significant difference (LSD) of both years (Vetriventhan & Upadhyaya, 2019). Further linear regression (R^2) between years were performed following '*lm*' function using '*stats*' package in R (R Core Team, 2018).

2.4 | Estimating percentage daily value

Percent Daily Value (DV) of nutrients from 100 g of little millet grain on a dry weight basis was calculated based on the amount of a particular nutrient present in little millet that contributes to Recommended Dietary Allowance (RDA) of the nutrients for an Indian adult male and female per 100 g of consumption (ICMR, 2010). The DV was calculated by using the formula below:

$$\%\text{DV} = (\text{amount of nutrient per 100 g of grain/RDA})100$$

The % DV for Fe is 17 mg d^{-1} for men and 21 mg d^{-1} for women; for Zn 12 mg d^{-1} for men and 10 mg d^{-1} for women; for Ca 600 mg d^{-1} for men and women; and for protein 60 g d^{-1} for men and 55 g d^{-1} for women (ICMR, 2010).

3 | RESULTS

3.1 | Analysis of variance and heritability

The analysis of variance following α-design showed that genotypes differed significantly for all the agronomic and grain nutrient traits (Table 1), indicating the presence of significant variability in little millet germplasm. Homogeneity of variance test (Bartlett, 1937) revealed that the error variances between 2 yr were heterogeneous for all the agronomic traits and grain nutritional traits, except days to maturity, inflorescence longest primary branch length, and protein content. Thus, individual year data were used separately to assess variability and to identify consistently performing promising trait-specific accessions. The $h^2{}_b$ estimates were high (>.60) for all the agronomic traits in both the years, except for basal tillers number and flag leaf blade width that showed moderate heritability in 2015. All four grain nutrients showed high $h^2{}_b$ in both the years, ranging from .88 for Zn to .94 for Ca in 2015, and .82 for Ca to .88 for Fe in 2016 (Table 1).

3.2 | Variability for qualitative traits

In the full set, the most predominant classes of qualitative traits were green plant pigmentation (89%), erect growth habit (56%), open lax (28%), and contracted arched (25%) inflorescence shapes, and cinnamon brown (29.5%) and straw (27.5%) seed colors (Table S2). However, considerable differences were observed among races and subraces for all the qualitative traits, except plant pigmentation. Green plant pigmentation was the more predominant class in both the races, their subraces, and in the full set. The subrace *erecta* of race *nana* was characterized by erect growth habit (86.2%), contracted stiff inflorescences (89.6%), with largely cinnamon brown (37.9%) and straw (27.6%) seed colors, whereas the subrace *laxa* was characterized by erect growth habit (43.3%), decumbent (28.3%) and erect geniculate (26.4%) growth habits, open lax (44.3%) and arched lax (33.0%) inflorescence shapes with five different seed colors in high frequencies (15 to 22.6%). Subraces of race *robusta* had higher frequencies of erect plants, contracted arched inflorescence shape, and cinnamon brown and straw-colored seeds. The H' index of the full set across qualitative traits was 0.51, and the race *nana* had a higher H' value (0.48) than *robusta* (0.38; Supplemental Table S3). The subraces of *laxa* in both the races had the highest H' value in comparison with the other two subraces across traits. The H' ranged from 0.15 (plant pigmentation) to 0.74 (inflorescence shape) in the entire set, whereas subrace *laxa* of both races had high H' values for inflorescence shape and seed color (Supplemental Table S3).

3.3 | Variability for agronomic traits

On average, little millet accessions matured 5-d later and 17-cm taller in 2015 (maturity in 85 d after sowing [DAS], plant height 145 cm) than in 2016 (maturity in 80 DAS, plant height 128 cm; Table 2). Races *nana* and *robusta* differed significantly from each other for all the agronomic traits in both years. Accessions of *robusta* matured late (116 DAS in 2015, 107 DAS in 2016) and produced tall plants (169 cm in 2015, 152 cm in 2016) with thick culm (7.3 mm in 2015, 7.9 mm in 2016) and produced high grain (1,523 kg ha^{-1}) and straw (dry weight, 8,972 kg ha^{-1}) yields compared to *nana* (Table 2). Accessions of *nana* were early maturing (85 DAS in 2015, 80 DAS in 2016) and produced short plants (134 cm in 2015, 116 cm in 2016) with thin culm (4.2 mm in 2016 and 4.8 mm in 2016) and low grain (1,229 kg ha^{-1}) and straw (4,276 kg ha^{-1}) yields, whereas average seed weight of *nana* (0.22 g) was significantly greater than *robusta* (0.19 g;

TABLE 1 Mean sum of square, least significant difference (LSD), coefficient of variation (CV%) and heritability in a broad sense (h^2_b) for agronomic traits and grain nutritional traits of little millet germplasm evaluated in the 2015 and 2016 rainy seasons, ICRSAT, Hyderabad, India

	2015				2016			
Trait	Genotype mean sum of square	$LSD_{.05}$	CV	h^2_b	Genotype mean sum of square	$LSD_{.05}$	CV	h^2_b
			%				%	
Days to 50% flowering, d	666.8**	6.3	5.4	.98	417.4**	6.5	5.7	.97
Days to maturity, d	666.7**	6.8	3.4	.98	575.8**	9.0	5.1	.96
Plant height, cm	1,481.6**	15.1	5.2	.96	1,381.7**	14.3	5.6	.96
Basal tillers number	7.6**	3.6	18.3	.58	15.6**	4.1	20	.72
Culm thickness, mm	7.2**	1.1	10.2	.96	6.9**	1.2	10.3	.94
Flag leaf blade length, mm	1,782.9**	46.9	8.4	.70	2,549.0**	40.2	6.7	.84
Flag leaf blade width, mm	2.2**	2.0	9.3	.57	16.6**	2.4	11.8	.91
Flag leaf sheath length, mm	296.6**	16.6	7.4	.78	450.9**	17.5	8.3	.83
Inflorescence length, mm	1,874.1**	48.8	8.1	.68	3,591.3**	34.5	5.6	.91
Inflorescence lowest primary branch length, mm	1,500.1**	32.5	8.8	.83	1,728.4**	39.5	10.9	.77
Inflorescence primary axis nodes number	7.6**	2.6	9.4	.78	25.1*	3.2	9.7	.90
100-seed weight, g	0.003**	0.029	6.9	.91	0.003**	0.03	6.8	.93
Grain yield, kg ha^{-1}	NR[a]	NR	NR	NR	33,6447**	535	20.4	.71
Straw yield, kg ha^{-1}	NR	NR	NR	NR	2,216,6347**	2,850	24.5	.90
Harvest index	NR	NR	NR	NR	0.01**	0.1	22.7	.78
Iron, mg kg^{-1}	128.1**	7.2	10.6	.91	64.5**	5.7	9.2	.88
Zinc, mg kg^{-1}	25.0**	3.4	6.1	.88	26.7**	4.1	6.9	.84
Calcium, mg kg^{-1}	2511.2**	25.8	6.7	.94	1,111.0**	28.4	9	.82
Protein, %	9.4**	1.9	8.5	.91	5.1**	1.7	7.6	.86

[a]NR, not recorded.
*Significant at the .05 probability level.
**Significant at the .01 probability level.

Table 2). The subrace *compacta* and *laxa* of the race *robusta* differed significantly for plant height and inflorescence length in both years and for straw yield in 2016, whereas subraces of the race *nana* did not differ significantly from each other in both the years for all the agronomic traits (Supplemental Table S4). Subrace *laxa* of race *robusta* produced tall plants, long inflorescences, and high straw yield compared to subrace *compacta*. The *H'* varied from 0.45 (days to 50% flowering) to 0.64 (flag leaf blade width) in 2015, and from 0.51 (days to 50% flowering) to 0.63 (inflorescence length) in 2016 (Supplemental Table S3); races and subraces also had a similar range.

3.4 | Variability for grain nutrient content

Little millet accessions showed large variability for all the four grain nutrients as evidenced from the estimates of range (Fe = 17.6–58.0 mg kg^{-1} in 2015, 18.4–47.6 mg kg^{-1} in 2016; Zn = 19.4–36.9 mg kg^{-1} in 2015, 22.0–39.5 mg kg^{-1} in 2016; Ca = 105.7–389.7 mg kg^{-1} in 2015, 92.1–194.1 mg kg^{-1} in 2016; and protein = 6.0–15.6% in 2015, 6.4–14.6 in 2016) in the full set. Similar mean grain nutrient contents were observed in both the years for Fe (33.0 mg kg^{-1} in 2015, 30.7 mg kg^{-1} in 2016), Zn (28.5 mg kg^{-1} in 2015, 29.6 mg kg^{-1} in 2016), and protein (11.1% in 2015, 11.5% in 2016), whereas Ca content in 2015 was higher (189.6 mg kg^{-1}) than in 2016 (144.8 mg kg^{-1}). The frequency distribution and performance of each accession in individual years are presented in Figure 2. Among the races, *nana* and its subraces had significantly high Fe, Zn, and protein than the race *robusta* and its subraces in both years, whereas for Ca content, both races and their subraces did not differ significantly in both the years (Table 2). Subraces within each race did not differ significantly from each other for all the four-grain nutrient content (Supplemental Table S4). Shannon diversity analysis revealed high diversity in the little millet accessions, in the full set, and each race and subrace (Supplemental Table S3).

TABLE 2 Mean and range of agronomic and grain nutritional traits of little millet germplasm evaluated in the 2015 and 2016 rainy seasons, ICRSAT, Hyderabad, India

	2015						2016					
	Mean			Range			Mean			Range		
Trait	*Nana*	*Robusta*	Full set	*Nana*	*Robusta*	Full set	*Nana*	*Robusta*	Full set	*Nana*	*Robusta*	Full set
Days to 50% flowering, d	50b	81a	60	38–94	40–142	38–142	51b	74a	58	37–81	41–124	37–124
Days to maturity, d	85b	116a	95	73–129	75–177	73–177	80b	107a	89	62–119	71–157	62–157
Plant height, cm	134b	169a	145	93–191	89–200	89–200	116b	152a	128	78–173	86–184	78–184
Basal tillers number	10a	9b	10	6–17	6–14	6–17.0	12a	8b	10	6–17.0	4–13.0	4–17.0
Culm thickness, mm	4.2b	7.3a	5.2	2.4–9.0	2.1–10.6	2.1–10.6	4.8b	7.9a	6.0	3.1–8.9	3.7–10.5	3.1–10.5
Flag leaf blade length, mm	286a	255b	276	211–343	198–332	198–343	285b	327a	299	219–380	239–396	219–396
Flag leaf blade width, mm	10b	11a	11	8–16	8–13	8–16	9b	13a	10	6–14	7–17	6–17
Flag leaf sheath length, mm	114a	106b	111	87–140	76–138	76–140	103b	113a	106	77–133	71–160	71–160
Inflorescence length, mm	304a	290b	299	182–382	199–345	182–382	298b	340a	311	208–410	226–428	208–428
Inflorescence lowest primary branch length, mm	193a	160b	183	140–247	112–206	112–247	178b	191a	182	112–252	115–272	112–272
Inflorescence primary axis nodes number	13b	14a	14	9–19	11–21	9–21.0	15b	20a	16	11–24	13–26	11–26
100–seed weight, g	0.22a	0.19b	0.22	0.15–0.30	0.15–0.26	0.15–0.30	0.23a	0.16b	0.22	0.15–0.32	0.14–0.32	0.14–0.32
Grain yield, kg ha^{-1}	NR[#]	NR	NR	NR	NR	NR	1229b	1523a	1324	491–2377	541–2475	491–2475
Straw yield, kg ha^{-1}	NR	NR	NR	NR	NR	NR	4276b	8972a	5802	1871–12494	2600–16486	1871–16486
Harvest index	NR	NR	NR	NR	NR	NR	0.23a	0.15b	0.20	0.06–0.36	0.03–0.32	0.03–0.36
Iron, mg kg^{-1}	35.9a	27.5b	33.0	21.0–58.0	17.6–54.9	17.6–58.0	32.8a	26.3b	30.7	21.9–47.6	18.4–40.6	18.4–47.6
Zinc, mg kg^{-1}	29.6a	26.0b	28.5	22.2–36.9	19.4–34.6	19.4–36.9	31.1a	26.6b	29.6	22.6–37.7	22.0–39.5	22.0–39.5
Calcium, mg kg^{-1}	191.3a	185.9a	189.6	105.7–389.7	128.2–259.4	105.7–389.7	142.8a	148.7a	144.8	93.2–194.1	92.1–191.5	92.1–194.1
Protein, %	12.1a	9.2b	11.1	7.9–15.6	6.0–14.9	6.0–15.6	12.2a	9.9b	11.5	8.8–14.6	6.4–13.5	6.4–14.6

Note: Means not sharing a common letter are significantly different at the .05 probability according to the Newman–Keuls test (Newman, 1939; Keuls, 1952).
#NR, not recorded.

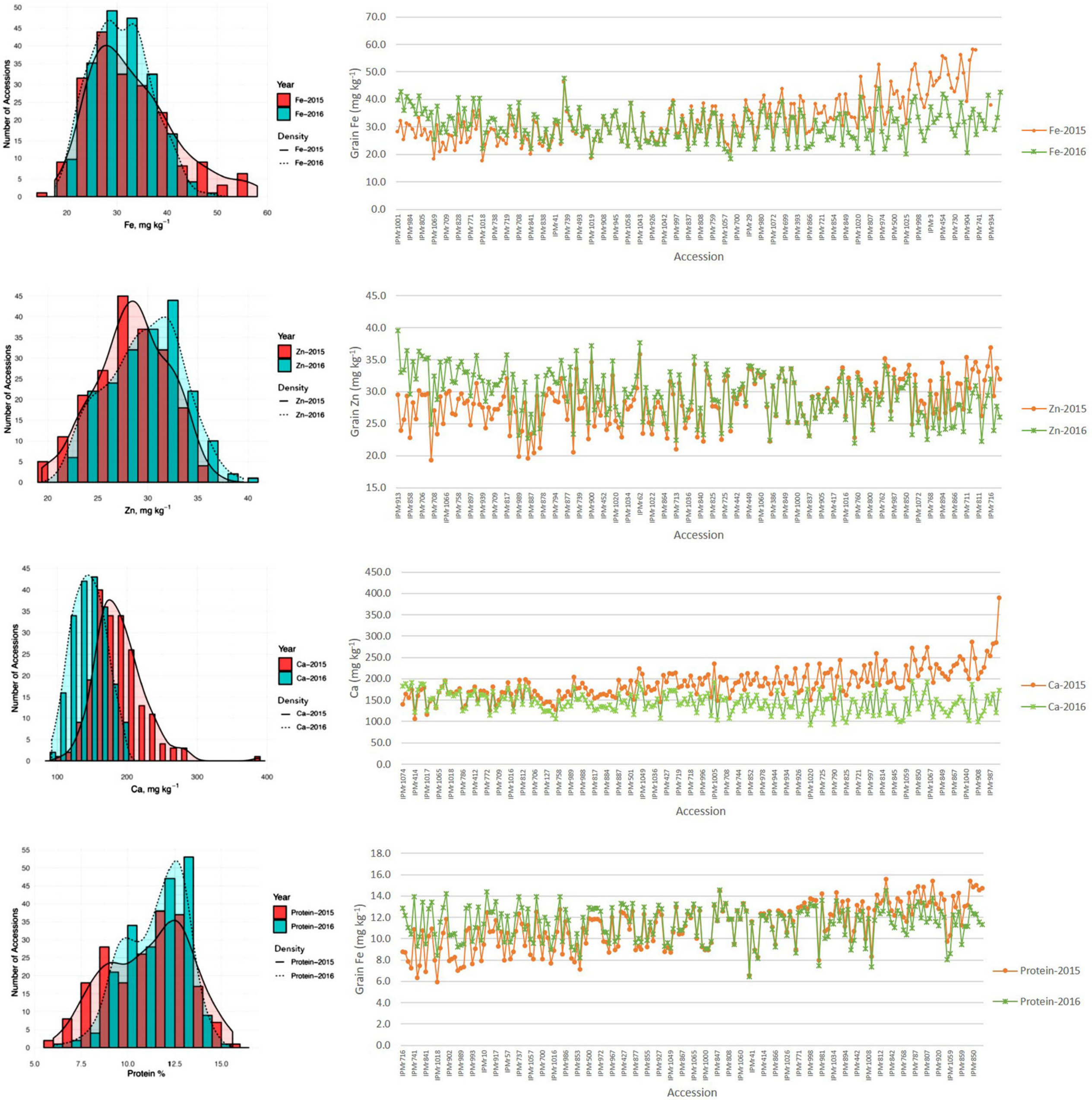

FIGURE 2 Histogram and line graph of grain Fe (mg kg^{-1}), Zn (mg kg^{-11}), Ca (mg kg^{-1}), and protein (%) in little millet accessions evaluated during 2015 and 2016 rainy seasons at ICRISAT, Hyderabad, India

Note: Only a few accessions were visible in the line graph and the remaining were not visible

3.5 | Correlation coefficients

Grain yield (kg kg^{-1}) was estimated only during 2016, whereas in 2015, the crop was damaged due to rain resulting in lodging and shattering. The bulk seeds harvested were used for grain nutrient analysis. Grain yield showed significant positive correlations with most agronomic traits, whereas basal tillers number showed a significantly negative correlation with grain yield (Table 3). Grain yield showed significant negative correlations with grain Fe, Zn and protein, while nonsignificant with Ca (Table 3; Supplemental Figure S1). Correlation between grain Fe and Zn were significantly positive (Table 3; Supplemental Figure S1). Calcium, Fe, Zn, and protein were significantly and positively correlated in 2015, whereas Ca in 2016 showed nonsignificant correlations with Fe and Zn and negatively significant correlation with protein. Correlation coefficients between years were highly significant and positive for all the nutrient traits (Fe = .594,

TABLE 3 Correlations of agronomic and grain nutrient traits with grain yield of little millet germplasm evaluated in the 2015 and 2016 rainy seasons, ICRSAT, Hyderabad, India

	2015				2016				
Trait[a]	Fe, mg kg^{-1}	Zn, mg kg^{-1}	Ca, mg kg^{-1}	Protein, %	Fe, mg kg^{-1}	Zn, mg kg^{-1}	Ca, mg kg^{-1}	Protein, %	Grain yield, kg ha^{-1}
Days to 50% flowering, d	−.575**	−.516**	−.085	−.757**	−.676**	−.573**	.119	−.728**	.407**
Days to maturity, d	−.575**	−.516**	−.085	−.757**	−.671**	−.571**	.119	−.727**	.439**
Plant height, cm	−.584**	−.422**	−.198**	−.702**	−.631**	−.583**	.102	−.708**	.511**
Basal tillers number	.214**	.099	−.088	.215**	.583**	.556**	−.268**	.697**	−.259**
Culm thickness, mm	−.610**	−.536**	−.102	−.780**	−.640**	−.626**	.207**	−.761**	.473**
Flag leaf blade length, mm	.306**	.464**	−.112	.476**	−.522**	−.574**	.089	−.634**	.448**
Flag leaf blade width, mm	−.274**	−.187**	−.032	−.364**	−.668**	−.669**	.159*	−.764**	.468**
Flag leaf sheath length, mm	.019	.218**	−.211**	.133*	−.397**	−.324**	−.008	−.385**	.129
Inflorescence length, mm	−.118	.074	−.177*	−.022	−.543**	−.510**	.103	−.562**	.381**
Inflorescence lowest primary branch length, mm	.199**	.317**	−.164*	.371**	−.416**	−.339**	−.050	−.363**	.224**
Inflorescence primary axis nodes number	−.402**	−.425**	−.154*	−.455**	−.645**	−.680**	.241**	−.778**	.469**
100-seed weight, g	.224**	.371**	−.187**	.422**	.408**	.452**	−.292**	.502**	−.094
Straw yield, kg ha^{-1}	NR[b]	NR	NR	NR	−.474**	−.443**	.123	−.597**	.504**
Harvest index	NR	NR	NR	NR	.357**	.320**	−.090	.402**	.088
Iron, mg kg^{-1}		.580**	.240**	.689**		.708**	−.024	.700**	−.319**
Zinc, mg kg^{-1}			0.173*	.785**			−.055	.789**	−.454**
Calcium, mg kg^{-1}				.217**				−.132*	−.047
Protein, %									−.464**

[a]The correlations were estimated using traits of respective seasons. Straw and grain yields (kg ha^{-1}) were estimated only in 2016, whereas other traits were recorded in both the seasons.
[b]NR, not recorded.
*Significant at the .05 probability level;
**Significant at the .01 probability level.

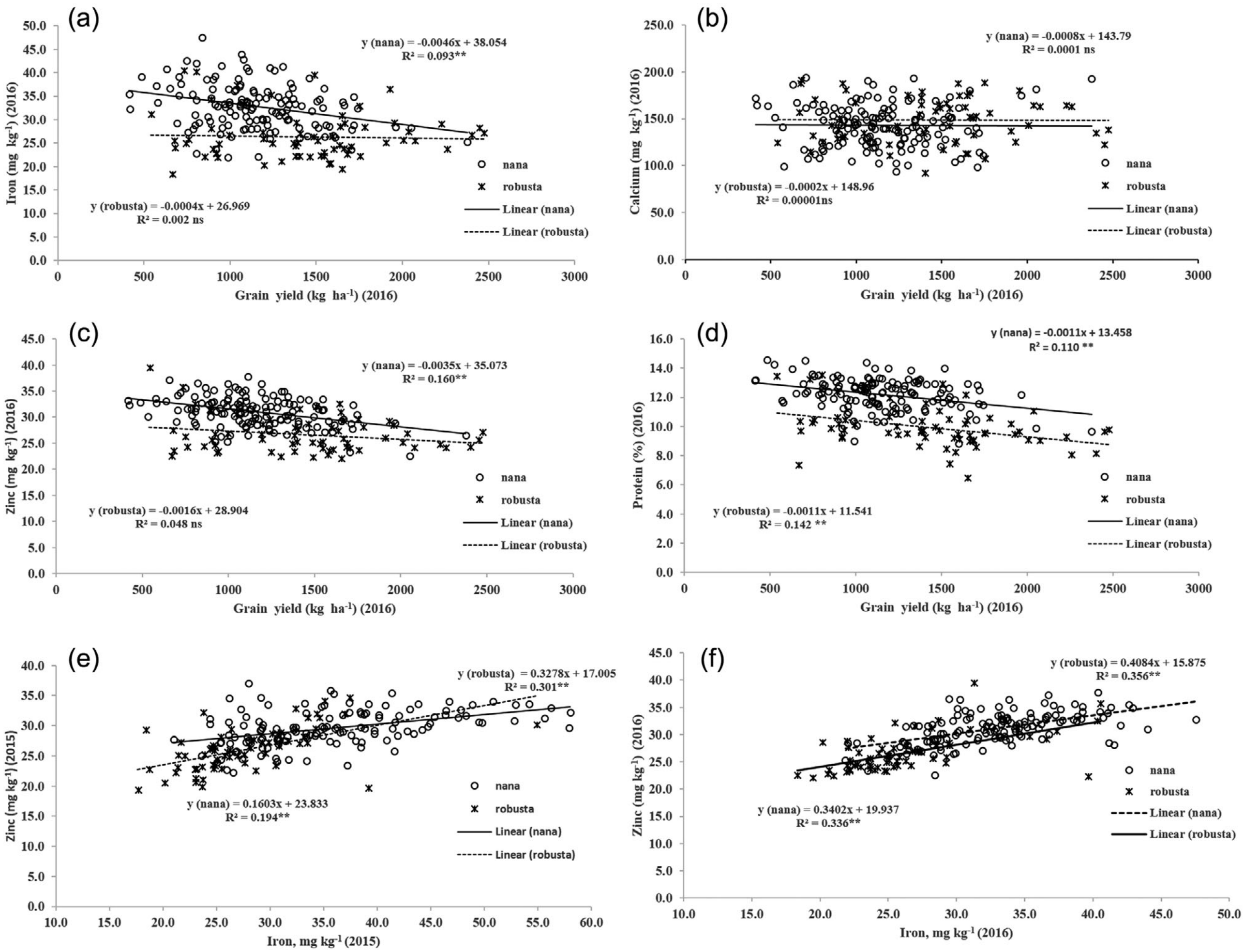

FIGURE 3 Relationship between grain yield with grain nutrients (a–d), and between iron and zinc (e, f) of little millet germplasm, evaluated during 2015 and 2016 rainy seasons at ICRISAT, Hyderabad, India

$P \leq .0001$; Zn = .696, $P \leq .0001$; Ca = .421, $P \leq .0001$; and protein = .767, $P \leq .0001$).

Correlations among agronomic and grain nutrient traits within each race revealed that the most significant associations in *nana* were not significant in *robusta*, and vice versa for a few traits (Supplemental Table S5). The important traits, namely days to 50% flowering, days to maturity, inflorescence length, and inflorescence lowest primary branch length were significantly positively correlated with grain yield in the race *nana*, whereas they showed nonsignificant associations in the race *robusta*. Race-wise, the relationship between grain yield and grain nutrients, and between Fe and Zn contents of little millet germplasm are presented in Figure 3. Calcium content in both the races showed nonsignificant association with grain yield in the entire set as well as in the race *nana* and *robusta*; grain yield in the race *robusta* showed nonsignificant correlation with Fe and Zn contents, but it was significantly associated in accessions of race *nana*. Protein content in both races showed a negative correlation with grain yield (Figure 3; Supplemental Table S5).

3.6 | Genetic distance and population structure

Average phenotype-based Gower's distance among accessions in the full set ($n = 200$) was 0.238 and varied from 0.061 (between IPmr 996 and IPmr 1058, both belong to the race *robusta* subrace *compacta*) to 0.511 (between IPmr 889 that belongs to the race *robusta* subrace *laxa* and IPmr 718 that belongs to the race *nana* subrace *laxa*; Table 4). The SNP-based distance matrix was estimated for 165 accessions for which SNP data is available (Johnson et al., 2019). The SNP-based distance varied from 0.201 (between IPmr 1002 and IPmr 993) to 0.664 (IPmr 1016 and IPmr 713), both the pairs belong to the race *robusta*, but different subraces. The least diverse pair of accessions, IPmr 1002 and IPmr 993, were collected from Odisha, whereas the highly diverse pair was collected from Maharashtra (IPmr 1016) and Telangana (IPmr 713). The combined distance considering both the phenotypic and SNP-based distances range from 0.157 (between IPmr 984 and IPmr 758) to 0.531 (between IPmr 841 and IPmr 449).

TABLE 4 Phenotypic, genotypic (SNP) and combined (both phenotypic and SNP data) distance within and between races and/or subraces of little millet germplasm

Race and subrace	Phenotypic distance (n = 200)			Genotypic (SNP) distance (n = 165)			Combined distance (both phenotypic and SNP data; n = 165)		
	Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range
Entire set	0.238	0.082	0.061-0.511	0.513	0.057	0.201-0.664	0.376	0.058	0.157-0.531
Within race and subrace									
nana	0.190	0.060	0.067-0.424	0.495	0.042	0.203-0.612	0.342	0.042	0.157-0.492
laxa	0.193	0.061	0.068-0.424	0.496	0.041	0.203-0.596	0.343	0.042	0.157-0.492
erecta	0.174	0.060	0.067-0.355	0.486	0.050	0.207-0.612	0.332	0.048	0.159-0.456
robusta	0.202	0.071	0.061- 0.441	0.506	0.083	0.201-0.664	0.355	0.065	0.166-0.513
laxa	0.183	0.052	0.084 −0.410	0.471	0.073	0.205-0.643	0.327	0.056	0.166-0.491
compacta	0.211	0.082	0.061-0.432	0.523	0.085	0.234-0.660	0.369	0.070	0.175-0.497
Between race and/or subrace									
nana and *robusta*	0.296	0.066	0.076-0.511	0.532	0.041	0.275-0.641	0.413	0.045	0.186-0.531
nana-laxa and *nana-erecta*	0.188	0.058	0.074-0.381	0.493	0.042	0.211-0.611	0.341	0.042	0.162-0.477
robusta-laxa and *robusta-compacta*	0.204	0.068	0.081-0.411	0.513	0.082	0.201-0.664	0.359	0.062	0.185-0.513
robusta-laxa and *nana-erecta*	0.298	0.062	0.119-0.468	0.529	0.033	0.397-0.641	0.412	0.033	0.301-0.502
robusta-laxa and *nana-laxa*	0.292	0.063	0.095-0.511	0.528	0.036	0.295-0.624	0.411	0.040	0.213-0.530
robusta-compacta and *nana-erecta*	0.298	0.072	0.076-0.429	0.532	0.043	0.275-0.632	0.412	0.050	0.186-0.513
robusta-compacta and *nana-laxa*	0.298	0.067	0.087-0.473	0.537	0.046	0.304-0.635	0.416	0.050	0.212-0.531

The least diverse pair of accessions, IPmr 984 and IPmr 758, both belong to the race *nana* and subrace *laxa*, collected from Maharashtra and Bihar, respectively; while the most diverse pair of accessions IPmr 841 and IPmr 449, belong to race *robusta* subrace *compacta* and race *nana* subrace *laxa*, respectively. Similar trends of within and among race distances were observed in all the three types of distance (phenotypic, SNP and combined), where the average distance between accessions of the races *nana* and *robusta* was higher than that of average distance among accessions within races (Table 4). The Mantel's test between the phenotypic and SNP-based matrices indicated a highly significant correlation ($r = .249$, $P \leq .0001$). Ward's clustering using combined distance matrices of both phenotypic and SNP-based data revealed two major clusters, C-I and C-II, that correspond to two races of little millet, *nana* and *robusta*, respectively (Figure 4). Subraces within each race did not show clear grouping. Clustering was also performed separately for phenotypic (Supplemental Figure S2) and SNP data (Supplemental Figure S3), and both revealed two major clusters that differentiated both the races.

3.7 | Promising germplasm resources

3.7.1 | Agronomic traits

Greater seed weight and high grain yield are important agronomic traits for little millet improvement. In the full set, 42 accessions had significantly, and consistently greater seed weight compared to the trial mean of 0.22 (+ LSD) in both years; however, the top 10 accessions (IPMr 807, 825, 983, 417, 808, 738, 741, 814, 1069, 1063) were selected after considering grain yield together with greater seed size (Table 5). Promising accessions for greater seed weight mostly belong to the race *nana* of subrace *laxa*, from the southern states of India (Andhra Pradesh, Tamil Nadu and Karnataka; Table 5). For grain yield, 15 accessions (IPMr 1036, 1040, 891, 862, 1042, 1035, 712, 855, 991, 1000, 974, 877, 875, 1006, 699) were identified that produced significantly higher grain yields (1,781 to 2,476 kg ha^{-1}) compared to the trial mean of 1,240 kg ha^{-1} (Table 5). High yielding accessions mostly belonged to race *robusta* (12 accessions) and matured in 88 to 124 DAS and were mostly from the states of Odisha (5) and Andhra Pradesh (5), whereas the remaining were from Telangana (2), Maharashtra (1), and Chhattisgarh (1).

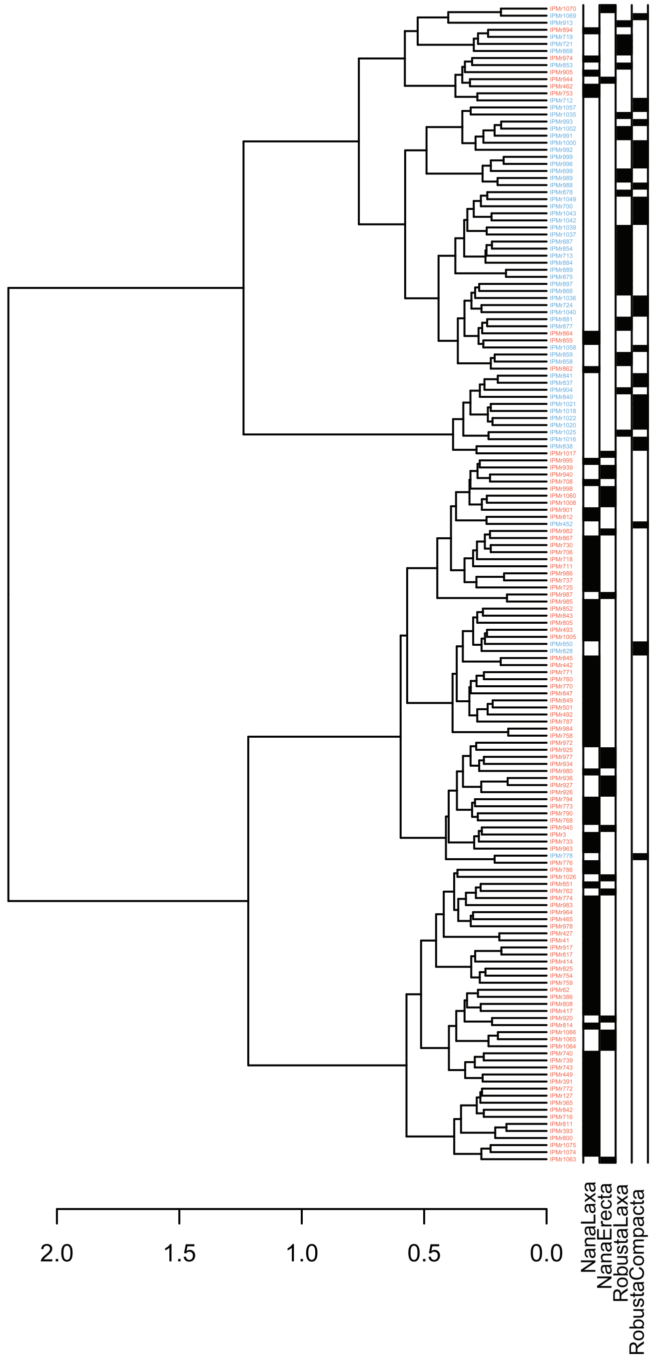

FIGURE 4 The hierarchical clustering of little millet accessions using combined distance matrix, obtained from both phenotype and single nucleotide polymorphism (SNP)-based distances, following Ward's method. Accessions name color code: blue, *robusta*; red, *nana*. The black color in the bars adjacent to the dendrogram represents the subrace of the corresponding accession

3.7.2 | Grain nutrient content

Considerable differences were observed between years for grain nutrient content indicating the influence of genotype, year, and their interactions. Thus, many accessions that had significantly high grain nutrient in one year did not show similar performance during another year. Therefore, it is important to identify consistently nutrient-rich accessions in both years (Table 6). The accessions were considered consistent when the difference between years for a given trait was ≤ the

TABLE 5 Little millet germplasm accessions identified for greater seed weight and high grain yield and biomass yield based on evaluation in the 2015 and 2016 rainy seasons, ICRSAT, Hyderabad, India

Accession	Origin (India)	Race	Subrace	Days to maturity		Plant height		100-seed weight		Grain yield	Straw yield	Iron		Zinc		Calcium		Protein	
				2015	2016	2015	2016	2015	2016	2016	2016	2015	2016	2015	2016	2015	2016	2015	2016
				d		cm		g		kg ha^{-1}		mg kg^{-1}						%	
IPmr 807	Andhra Pradesh	*Nana*	*Laxa*	91	88	182	153	0.30*	0.31*	1,718	6,457	36.7	28.8	31.8	27.8	189.8	134.8	13.1*	11.5
IPmr 825	Andhra Pradesh	*Nana*	*Laxa*	94	89	151	130	0.28*	0.26*	1,709	5,840	21.0	27.8	27.7	28.7	162.2	98.5	11.3	12.8
IPmr 983	Maharashtra	*Nana*	*Laxa*	91	89	165	136	0.29*	0.28*	1,703	4,362	26.5	23.5	22.2	23.3	224.5*	141.0	9.8	10.4
IPmr 417	Karnataka	*Nana*	*Laxa*	84	86	137	118	0.25*	0.29*	1,613	3,647	36.8	26.2	28.9	28.5	154.7	107.4	14.7*	11.3
IPmr 808	Andhra Pradesh	*Nana*	*Laxa*	85	86	147	125	0.30*	0.31*	1,579	4,374	30.8	28.6	27.5	29.2	148.2	103.6	11.8	11.8
IPmr 738	Andhra Pradesh	*Nana*	*Laxa*	90	86	157	147	0.27*	0.27*	1,541	2,917	28.9	32.6	30.7	31.3	192.0	153.2	12.2	11.9
IPmr 741	Maharashtra	*Nana*	*Laxa*	82	80	137	116	0.27*	0.29*	1,520	3,965	–	34.4	26.6	31.6	158.2	128.0	10.8	14.0*
IPmr 814	Andhra Pradesh	*Nana*	*Laxa*	91	89	126	128	0.27*	0.27*	1,513	4,564	40.2*	28.3	31.3	28.0	221.0*	149.7	13.2*	11.6
IPmr 1069	Tamil Nadu	*Robusta*	*Compacta*	129	123	177	167	0.25*	0.25*	1,509	13,820*	18.3	25.5	29.3	32.1	151.5	116.7	10.5	12.9
IPmr 1063	Tamil Nadu	*Nana*	*Erecta*	99	91	173	158	0.29*	0.28*	1,450	6,470	33.3	26.5	28.8	27.9	204.4	148.0	10.5	11.7
IPmr 1036	Odisha	*Robusta*	*Compacta*	119	107	183	171	0.18	0.17	2,476*	14,174*	26.2	27.1	25.9	27.1	176.3	137.8	8.9	9.8
IPmr 1040	Odisha	*Robusta*	*Compacta*	120	108	181	163	0.19	0.17	2,451*	13,716*	25.0	28.3	24.3	25.6	219.2*	122.0	8.0	9.7
IPmr 891	Andhra Pradesh	*Robusta*	*Laxa*	118	108	191	160	0.20	0.19	2,404*	13,448*	23.0	26.7	21.2	24.3	164.0	134.9	8.3	8.1
IPmr 862	Andhra Pradesh	*Nana*	*Laxa*	122	113	179	143	0.20	0.21	2,378*	8,793*	31.7	25.2	24.4	26.4	273.8*	193.2	9.2	9.6
IPmr 1042	Odisha	*Robusta*	*Compacta*	120	107	176	166	0.18	0.18	2,260*	9,376*	24.6	23.7	26.7	24.2	213.0	163.5	9.7	8.1
IPmr 1035	Odisha	*Robusta*	*Laxa*	124	122	171	146	0.20	0.18	2,229*	8,536	27.6	29.2	23.8	24.8	170.4	164.9	8.8	9.3
IPmr 712	Telangana	*Robusta*	*Compacta*	95	92	176	155	0.20	0.19	2,077*	5,413	25.7	25.4	25.0	24.1	172.0	163.4	10.0	9.0
IPmr 855	Andhra Pradesh	*Nana*	*Laxa*	116	103	164	159	0.19	0.18	2,052*	11,145*	27.4	28.4	24.5	22.6	198.5	181.9*	9.2	9.9
IPmr 991	Odisha	*Robusta*	*Laxa*	124	114	171	156	0.19	0.18	2,036*	9,892*	21.4	27.5	25.1	26.8	165.4	164.5	7.8	11.1
IPmr 1000	Odisha	*Robusta*	*Compacta*	123	112	179	151	0.20	0.20	2,008*	9,386*	23.9	25.7	25.2	25.2	180.9	143.8	8.9	9.1
IPmr 974	Maharashtra	*Nana*	*Laxa*	92	88	142	124	0.19	0.17	1,964*	4,742	37.2	28.4	23.4	28.8	160.9	175.0*	8.7	12.2
IPmr 877	Andhra Pradesh	*Robusta*	*Laxa*	120	109	174	160	0.18	0.17	1,956*	12,091*	25.9	29.5	25.7	28.5	181.1	180.4	8.9	9.6

(Continues)

TABLE 5 (Continued)

Accession	Origin (India)	Race	Subrace	Days to maturity		Plant height		100-seed weight		Grain yield	Straw yield	Iron		Zinc		Calcium		Protein	
				2015	2016	2015	2016	2015	2016	2016	2016	2015	2016	2015	2016	2015	2016	2015	2016
IPmr 875	Andhra Pradesh	*Robusta*	*Laxa*	119	105	167	158	0.22	0.22	1,930*	10234*	31.7	36.6*	27.5	29.2	145.7	124.8	9.5	9.5
IPmr 1006	Chhattisgarh	*Robusta*	*Laxa*	117	108	162	151	0.19	0.20	1,907*	9,066*	36.9	25.0	32.0*	26.0	190.8	136.5	10.9	10.2
IPmr 699	Telangana	*Robusta*	*Laxa*	112	98	175	164	0.20	0.21	1,781*	14,571*	33.1	28.5	28.1	28.1	169.3	156.7	9.7	10.6
IPmr 913	Tamil Nadu	*Robusta*	*Laxa*	177	157	199	152	0.21	0.22	539	16,433*	34.2	31.3	29.5	39.5*	173.7	124.3	10.8	13.5*
IPmr 1037	Odisha	*Robusta*	*Laxa*	112	106	189	175	0.19	0.18	1,651	14,953*	37.4	31.0	34.6*	30.9	234.8*	175.4*	11.6	10.4
IPmr 997	Odisha	*Robusta*	*Compacta*	121	113	183	169	0.17	0.17	1,487	13,945*	27.6	26.3	25.2	25.1	235.6*	165.3	8.6	9.7
IPmr 897	Andhra Pradesh	*Robusta*	*Laxa*	118	102	190	168	0.18	0.18	1,665	13,870*	26.1	23.8	24.8	29.3	191.3	177.7*	9.2	9.4
IPmr 700	Telangana	*Robusta*	*Compacta*	122	111	174	165	0.20	0.19	1,191	13,283*	30.2	27.2	28.6	26.8	186.5	122.5	8.1	9.6
IPmr 1057	Odisha	*Robusta*	*Compacta*	134	126	186	182	0.20	0.18	1,464	12922*	24.4	22.0	26.3	28.8	203.8	158.9	8.5	10.0
IPmr 993	Odisha	*Robusta*	*Compacta*	124	114	159	157	0.22	0.17	1,395	12,917*	24.6	25.1	23.5	25.3	163.0	134.0	7.6	9.7
IPmr 866	Andhra Pradesh	*Robusta*	*Laxa*	116	109	200	167	0.20	0.20	921	12,569**	28.2	22.7	27.4	24.6	189.4	130.4	9.4	9.2
IPmr 902	Maharashtra	*Nana*	*Laxa*	129	119	163	136	0.15	0.14	1,164	12,442*	25.4	22.1	24.3	28.5	223.1*	161.6	7.9	10.3
IPmr 881	Andhra Pradesh	*Robusta*	*Laxa*	122	113	170	160	0.20	0.18	996	12,396*	29.8	26.8	27.3	26.9	247.4*	167.8	9.3	10.1
Trial Mean				95	89	145	128	0.22	0.22	1,240	5,713	33.0	30.7	28.5	29.6	189.6	144.8	11.1	11.5
Trial range				73–177	62–157	89–200	78–184	0.15–0.30	0.14–0.32	412–2,476	1,848–1,6433	17.6–58.0	18.4–47.6	19.4–36.9	22.0–39.5	105.7–389.7	92.1–194.1	6.0–15.6	6.4–14.6
$LSD_{.05}$				6.8	9.0	15.1	14.3	0.029	0.03	535	2,850	7.2	5.7	3.4	4.1	25.75	28.4	1.87	1.7
CV%				3.4	5.1	5.2	5.6	6.91	6.80	20.4	24.5	10.6	9.2	6.07	6.9	6.7	9.0	8.46	7.6

*Significant at the .05 probability level.

TABLE 6 Grain nutrients dense little millet accessions identified based on evaluation in the 2015 and 2016 rainy seasons, ICRISAT, Hyderabad, India

Accession	Origin (India)	Race	Subrace	Days to maturity		Iron		Zinc		Calcium		Protein		Grain yield
				2016	2016	2015	2016	2015	2016	2015	2016	2015	2016	2016
				d		mg kg^{-1}						%		kg ha^{-1}
IPmr 855	Andhra Pradesh	Nana	Laxa	116	103	27.4	28.4	24.5	22.6	**198.5**	**181.9***	9.2	9.9	2,052*
IPmr 974	Maharashtra	Nana	Laxa	92	88	37.2	28.4	23.4	28.8	**160.9**	**175.0***	8.7	12.2	1,964*
IPmr 877	Andhra Pradesh	Robusta	Laxa	120	109	25.9	29.5	25.7	28.5	**181.1**	**180.4***	8.9	9.6	1,956*
IPmr 897	Andhra Pradesh	Robusta	Laxa	118	102	26.1	23.8	24.8	29.3	**191.3**	**177.7***	9.2	9.4	1,665
IPmr 767	Andhra Pradesh	Nana	Laxa	80	80	46.4*	36.7*	32.4*	33.4	**191.5**	**173.8***	13.8*	12.6	1,546
IPmr 964	Maharashtra	Nana	Laxa	79	77	**41.4***	**37.8***	29.5	32.5	189.5	141.8	11.0	13.1	1,352
IPmr 901	Andhra Pradesh	Nana	Laxa	75	72	47.8*	34.2	**32.6***	**34.8***	226.5*	172.3	13.4*	12.3	1,322
IPmr 740	Maharashtra	Nana	Laxa	82	79	47.5*	30.8	31.3	32.7	196.4	117.7	**15.4***	**13.8***	1,322
IPmr 1065	Tamil Nadu	Nana	Erecta	83	72	33.3	41.3*	**32.1***	**34.9***	168.9	169.5	13.0	13.2	1,307
IPmr 414	Karnataka	Nana	Laxa	97	87	35.9	40.5*	**35.2***	**34.1***	105.7	121.8	12.3	12.2	1,257
IPmr 998	Odisha	Nana	Erecta	74	63	45.3*	33.5	31.4	30.4	214.6	153.2	**13.8***	**13.3***	1,152
IPmr 945	Maharashtra	Nana	Erecta	88	80	35.5	35.8	31.2	32.2	212.5	151.3	**13.2***	**13.3***	1,137
IPmr 62	Bihar	Nana	Laxa	90	86	35.6	40.4*	**35.8***	**37.7***	193.6	119.2	12.8	14.0*	1,111
IPmr 977	Maharashtra	Nana	Erecta	76	71	52.9*	41.1*	**33.5***	**33.9***	285.8*	187.1*	15.4*	13.0	1,098
IPmr 985	Maharashtra	Nana	Laxa	76	72	38.4	34.3	32.0	30.6	**155.8**	**175.2***	**14.9***	**13.4***	1,070
IPmr 817	Andhra Pradesh	Nana	Laxa	93	91	58.0*	36.5*	**32.1***	**35.8***	154.8	126.0	11.8	12.8	1,036
IPmr 844	Odisha	Nana	Laxa	83	85	39.2	33.9	**33.6***	**36.4***	196.7	150.4	13.5*	12.7	997
IPmr 838	Gujarat	Robusta	Compacta	109	91	23.0	24.7	20.6	23.4	**196.7**	**181.1***	7.3	10.4	939
IPmr 1021	Maharashtra	Robusta	Compacta	116	109	21.3	22.9	23.1	26.7	**174.4**	**188.2***	6.3	9.3	923
IPmr 1008	Chhattisgarh	Nana	Erecta	76	73	35.0	30.6	**33.3***	**34.3***	231.7*	162.2	12.9	11.9	873
IPmr 725	Telangana	Nana	Laxa	87	64	**46.3 ***	**47.6***	31.7	32.7	181.7	121.0	12.6	12.4	841
IPmr 452	Odisha	Robusta	Compacta	79	72	54.9*	40.3	30.2	32.5	172.6	124.9	**14.3***	**13.5***	806
IPmr 980	Maharashtra	Nana	Laxa	76	70	38.9	35.6	32.1*	31.6	252.4*	157.9	**14.9***	**13.5***	783
IPmr 981	Maharashtra	Nana	Erecta	75	66	NR	42.7	**34.3***	**35.5***	244.1*	165.2	**14.2***	**13.6***	749
IPmr 449	Odisha	Nana	Laxa	81	71	**43.8***	**39.3***	**33.6***	**34.1***	**194.9**	**194.1***	**15.6***	**14.5***	705
IPmr 992	Odisha	Robusta	Compacta	122	116	24.8	24.0	25.9	23.5	**174.6**	**191.5***	9.0	9.7	681
IPmr 840	Maharashtra	Robusta	Compacta	110	107	24.8	25.4	26.4	27.5	**164.8**	**187.9***	8.1	10.4	677

(Continues)

TABLE 6 (Continued)

Accession	Origin (India)	Race	Subrace	Days to maturity		Iron		Zinc		Calcium		Protein		Grain yield
				2016	2016	2015	2016	2015	2016	2015	2016	2015	2016	2016
IPmr 900	Andhra Pradesh	Nana	Laxa	75	72	29.1	36.8*	**34.6***	**37.2***	223.4*	167.5	14.0*	12.3	654
IPmr 737	Madhya Pradesh	Nana	Laxa	75	65	48.2*	40.8*	31.6	33.1	**178.2**	**186.6***	12.3	13.9*	632
IPmr 718	Telangana	Nana	Laxa	73	67	50.8*	39.1*	33.9*	32.8	207.2	164.3	**14.5***	**14.6***	484
Trail mean				95	89	33.0	30.7	28.5	29.6	189.6	144.8	11.1	11.5	1,240
Trial range				73–177	62–157	17.6–58.0	18.4–47.6	19.4–36.9	22.0–39.5	105.7–389.7	92.1–194.1	6.0–15.6	6.4–14.6	412–2,476
$LSD_{.05}$				6.8	9.0	7.2	5.7	3.4	4.1	25.75	28.4	1.8	1.7	535

Note: Accessions with nutrient value in bold are consistent in both the seasons for a given nutrient.
*Significant at the .05 probability level.

average LSD of both years (Vetriventhan & Upadhyaya, 2019). For Fe, differences of up to 38 mg kg^{-1} were found between years, and 127 accessions were found consistent (R^2 = .69, $P \leq .0001$) with differences between the years < .5 mg kg^{-1}, and three accessions (IPmr 964, 725, 449) were found to be consistently significantly high in both the years. For Zn, differences of up to 10 mg kg^{-1} were found, and 159 accessions were found to be consistent (R^2 = .74, $P \leq .0001$) with differences between the years ≤3.8 mg kg^{-1}, including 11 accessions with significantly higher Zn content (IPmr 901, 1065, 414, 62, 977, 817, 844, 1008, 981, 449, 900; Table 6). For Ca, differences up to 120 mg kg^{-1} between years were observed and 59 of them were consistent (R^2 = .70, $P \leq .0001$) with ≤27.1 mg kg^{-1} differences between years; none of the accession was found to be significantly high in both the years. Therefore, those accessions significantly high in Ca content in 2016 (as grain nutrient content in 2016 were significantly lower than in 2015) and consistent with 2015 were identified as promising for calcium rich accessions. This includes 12 accessions (IPmr 855, 974, 877, 897, 767, 985, 838, 1021, 449, 992, 840, 737). Protein content in both the years had differences of up to 4%, and 163 accessions had ≤1.8% differences between years which were considered consistent (R^2 = .73, $P \leq .0001$), and nine of them (IPmr 740, 998, 945, 985, 452, 980, 981, 449, 718) were found to have significantly and consistently higher protein content (13.2 to 15.6%) in both years (Table 6). Altogether, 30 accessions were identified for grain nutrient content, including three, 11, 12, and nine accessions for Fe, Zn, Ca, and protein content, respectively, a few of them were consistent for two or more nutrients, and grain yield ranged from 484 to 2,052 kg ha^{-1} (Table 6).

3.7.3 | High biomass

Little millet accessions, particularly those belonging to the race *robusta*, produced high biomass in general. In total, 42 accessions produced significantly high straw yield (8,640 to 16,433 kg ha^{-1}) and produced grain yields of 539 to 2,476 kg ha^{-1}, of which the top 15 accessions (IPMr 913, 1037, 699, 1036, 997, 897, 1069, 1040, 891, 700, 1057, 993, 866, 902, 881) that yielded 12,396 to 16,433 kg ha^{-1} straw (dry weight) were identified as high biomass yielding (Table 5). These high biomass yielding accessions matured in 112 to 134 DAS in 2015 and 98 to 126 DAS in 2016, except IPmr 913 which took 177 DAS in 2015 and 157 DAS in 2016. Figure 5 shows high grain and biomass yielding accessions.

IPmr 1037 (plant height 175 cm, rainy 2016) IPmr 1040 (plant height 163 cm, rainy 2016)

FIGURE 5 Little millet germplasm (left) IPmr 1037 (high biomass) and (right) IPmr 1040 (high biomass and grain yield)

3.8 | Percent daily value (DV) of little millet consumption

The selected 30 accessions as promising for grain nutrient contents were used for estimating %DV contribution per 100 g grain. The % DV of 30 accessions varied from 10.5 to 28.0% for Fe, 18.3 to 36.8% for Zn, 13.0 to 27.4% for protein, whereas the % DV for Ca was very low with an average of 3%. Accessions with consistently high Fe content contribute up to 27.6% DV, accessions with consistently high Zn content contribute up to 36.8%, and those with consistently high protein content contribute up to 27.4% of RDA (Supplemental Table S6).

4 | DISCUSSION

Characterization and evaluation of germplasm open the doors for their effective utilization in crop improvement. Little millet germplasm accessions used in this study were highly diverse for all the agronomic and grain nutritional traits and also showed high heritability. The two races of little millet differed significantly for all the agronomic and grain nutrient content. Subraces within each of the races did not differ significantly for agronomic traits (except subrace *compact* and *laxa* of the race *robusta* that differed significantly for plant height and inflorescence length), and grain nutrient content, whereas they showed variation for qualitative traits particularly inflorescence shape, based on which these races and subraces were formed (de Wet et al., 1983). Therefore, correlation analysis considering racial structure can provide interesting information. For example, grain and straw yields showed a highly significant and positive correlation with days to maturity in the full set; however, that relationship was not similar when we assessed them individually race-wise. An increase in maturity duration resulted in increased straw yield in both races, but an increase in maturity did not result in high grain yield in the race *robusta*, whereas increased grain yield was observed with increasing maturity in *nana*. This is because accessions of the race *nana* were mainly early maturing with short plants, thin culm, and produced low grain and straw yields, whereas accessions of *robusta* were late maturing and produced tall plants with thick culm, high grain, and straw yields. Early maturing accessions (race *nana*) could be utilized for late sowing during the late onset of monsoon whereas late-maturing accessions (race *robusta*) could be utilized for growing during timely monsoon to obtain considerably high grain and straw yields.

Diversity assessment either using morpho-agronomic traits or genomic data can provide useful information about the structure of the population. However, combining both phenotypic and genomic data can complement each other to reveal a better understanding of crop diversity. Here we assessed pairwise distance among little millet accessions separately using phenotypic data and SNP data and combined of both the data. Little millet germplasm was considerably diverse with an average phenotypic, SNP-based and combined pairwise distance of 0.238, 0.513, and 0.376, respectively and the race *robusta* was slightly more diverse than *nana*. The Ward's clustering of both phenotypic and SNP data and combination of both the data revealed two major groups that correspond well with two races of little millet, whereas subraces within each race did not show clear grouping, indicating that little millet diversity is structured by race.

Dietary deficiency of micronutrients, particularly Fe and Zn, and also macronutrients such as Ca and protein, have been reported to be a grave public health problem (Beto, 2015; Govindaraj et al., 2019; Minocha et al., 2017). Millets, including pearl millet (*Pennisetum glaucum*) and small millets, are reported to have considerably high grain nutrients compared to major cereals (Saleh et al., 2013). In little millet, there are only a few studies on grain nutrient traits estimation, using a few landraces or limited to a particular locality (Chandel et al., 2014; Selvi, Nirmalakumari, & Senthil, 2015). (Chandel et al., 2014) reported Fe (32.20–35.1 mg kg^{-1}), Zn (30.30–33.00 mg kg^{-1}) and protein (7.96–10.66%) content in four genotypes of little millet. In this study, grain nutrient content assessed using whole grains showed a large variation for grain Fe, Zn, Ca, and protein content. In our previous study on little millet, dehulling resulted in a slight but nonsignificant increase in Fe, Zn, Ca, and protein contents when compared to the whole grain (Vetriventhan, unpublished data, 2021). Dehulling requires considerable time and manpower, and it causes variation in nutrient loss among test entries. Therefore, whole grains could be utilized for screening the large number of germplasm. The race *nana* was found to have high grain Fe, Zn, and protein contents, whereas Ca content was similar in both the races. Our previous studies on grain nutrient assessment of proso millet germplasm indicated that the race *ovatum* of proso millet was found to have considerably higher grain nutrients (Fe, Zn, Ca, and protein) than other races (Vetriventhan & Upadhyaya, 2018), whereas no significant differences were observed for grain nutrients among three races of kodo millet germplasm (Vetriventhan & Upadhyaya, 2019). Average Fe (33.0 mg kg^{-1} in 2015, 30.7 mg kg^{-1} in 2016), Zn (28.5 mg kg^{-1} in 2015, 29.6 mg kg^{-1} in 2016), and protein (11.1% in 2015; 11.5% in 2016) contents in little millet accessions studied here were greater than in finger millet germplasm (Fe 29.3 mg kg^{-1}, Zn 19.9 mg kg^{-1}, protein 7.3%; Upadhyaya et al., 2011a), and Ca content in little millet (189.6 mg kg^{-1} in 2015, 144.8 mg kg^{-1} in 2016) was comparable to that in proso millet (165 mg kg^{-1}; Vetriventhan & Upadhyaya, 2018), foxtail millet (146 mg kg^{-1}; Upadhyaya et al., 2011b) and kodo millet (213 mg kg^{-1} in 2015, 189 mg kg^{-1} in 2016; Vetriventhan & Upadhyaya, 2019) germplasm. Average Fe and Zn contents in little millet germplasm in this study were greater than those of the final target content established by HarvestPlus for rice (Fe 13 mg kg^{-1}, Zn 24 mg kg^{-1}) and comparable with the average Fe and Zn content at the baseline for wheat, maize, and sorghum (*Sorghum bicolor*; Garcia-Oliveira et al., 2018). Consumption of little millet could potentially contribute up to 28% Fe, 36.8% Zn, and 27.4% protein of RDA, which is higher than rice (Fe 10.6% DV, Zn 16.8% DV, protein 13.4% DV), wheat (Fe 20.6% DV, Zn 8.8% DV, protein 21.1% DV), and maize (Fe 15.9% DV, Zn 22.7% DV, protein 16.7% DV; Longvah et al., 2017; Saleh et al., 2013). Moreover, depending on the amount of little millet consumed daily basis, it can even meet with 100% DV of these important and major nutrients (Anitha et al., 2019). A previous study has shown that little millet can be eaten like rice and can easily replace the staple (Anitha et al., 2019); therefore, it is easy to achieve adequate consumption in staple form. The high amount of major nutrients in little millet has the potential to meet the RDA of these major nutrients. This needs to be studied further based on these high nutrient little millet accessions. These studies highlight the importance of little millet in terms of grain nutrients and demonstrate that this crop and other millets can be a good supplement for crop and dietary diversity.

The genotype and genotype × environment (locations and sites) interactions significantly influence grain nutrients content. High genotype × environment (sites) interactions have been reported for grain nutrient content in several crops, including in maize, pearl millet, sorghum (Hariprasanna et al., 2012; Oikeh et al., 2004; Phuke et al., 2017; Pucher et al., 2014; Singhal et al., 2018), and small millets (Vetriventhan & Upadhyaya, 2018, 2019). Thus, high-grain, nutrient-rich accessions in one year may not display the same performance in another year, therefore, identifying consistently high-grain nutrient-rich accessions across years and locations is essential. In this study, 60, 63.5, 79.5, and 81.5% of the total accessions were found to be consistent between years for Ca, Fe, Zn, and protein contents, respectively, indicating the relative contribution of the genotype, year and genotype × year interactions on the expression of the traits. Grain Fe and Zn contents showed significant positive correlation with each other in both the years, as in other small millets such as proso millet (Vetriventhan & Upadhyaya, 2018) and finger millet (Upadhyaya et al., 2011a), as well as in sorghum (Phuke et al., 2017; Upadhyaya et al., 2016), and pearl millet (Kanatti et al., 2014). In the full set, grain yield was negatively correlated with Fe, Zn, and protein, and had a nonsignificant correlation with Ca, indicating that the simultaneous improvement of Fe, Zn, protein and grain yield in little millet is difficult, whereas Ca content could be improved together with high grain yield. Interestingly, when correlations were estimated for each race individually, grain Fe and Zn in the race *robusta* showed nonsignificant correlation with grain yield, but showed significantly negative correlation in the race *nana*. This significance in *nana* is not due to differences in the number of accessions of two races, as *nana* has more accessions than *robusta*. Both races are morphologically different in terms of maturity, stature, the thickness of stem, and yield potential. Correlation between Ca content and grain yield in both the races was negligible, indicating that it would be possible to enhance Ca content in both the races and Fe and Zn content in the race *robusta*, together with high grain yield in little millet.

Identification of promising trait-specific accessions for the traits of interest is essential to enhance their use in breeding programs and for release through selection from germplasm if they are found to be superior. Little millet is mostly cultivated in India, where until 2020, only 32 varieties were released, of which 20 were based on the selection from existing landraces, and the remaining were developed through recombination breeding (9) and mutation breeding (3; http://www.aicrpsm.res.in/; AICSMIP, 2014). This is because of difficulty in hybridization and low breeding focus. However, emasculation and crossing techniques are reported in little millet (Nandini et al., 2019) which can provide support in recombination-based breeding. In this study, 15 accessions were identified as promising for higher grain (IPmr 1036, 1040, 891, 862, 1042, 1035, 712, 855, 991, 1000, 974, 877, 875, 1006, 699; grain yield 1,781 to 2,476 kg ha^{-1}). For grain nutrients, based on consistent performance across 2 yr, 30 accessions were identified as promising for high grain nutrients including three for Fe, 11 for Zn, 12 for Ca, and nine for protein, and a few of them were consistently high for two or more nutrients (IPmr 449 for Fe, Zn, Ca, and protein; IPmr 981 for Zn and protein). Five accessions (IPmr 855, 974, 877, 897, 767) yielded over 1,500 kg ha^{-1} and had consistently high Ca in both years. IPmr 767 and IPmr 977 had high Fe, Zn, Ca, and protein, early maturity (<80 d), and produced over 1,000 kg ha^{-1}. Promising accessions identified for higher grain yield were mostly belonged to the race *robusta* (12) and originated in Odisha (5), and Andhra Pradesh (5), while those identified for greater seed weight as well as rich in grain nutrients belong to the race *nana*. The germplasm identified in this study could be tested in little millet growing regions in India and similar ecologies in other countries that could result in the release of selection from germplasm as varieties.

Many crops including undomesticated plants have the potential to become important feedstock, with substantial prospects for offsetting greenhouse gas emissions arising from the use of fossil fuels. However, only a few crops supply the bulk of biofuel and bioenergy production globally (Long et al., 2015). Little millet is a C_4 crop, similar to switchgrass (*Panicum virgatum*); both belong to the grass genus *Panicum*, with high photosynthetic efficiency and high biomass yield potential even with limited water supply. In the current study, 15 accessions that yielded about 12 to 16 t ha^{-1} straw (dry weight) were identified as high biomass-yielding (14 belong to *robusta*, and 1 to *nana*), indicating high potential of utilizing little millet for dual purposes (as food and bioenergy feedstock crop).

5 | CONCLUSION

Crop and dietary diversity are essential for sustainable agriculture and healthy lives. Little millet together with other small millets has huge potential to contribute to achieving crop and dietary diversity. Little millet is a good choice particularly in a changing climate scenario because of its diverse adaptation, less water requirement, being less affected by biotic and abiotic stresses, and having high nutrient density. It can be grown as a sole crop or as an intercrop with other crops, particularly legumes. It can be grown as a multi-purpose crop—for food and fodder—and it has the potential to use as a bioenergy feedstock crop. But little millet continues to remain an under-researched and under-utilized crop even among small millets. To increase the popularity of this low-priority crop, the promising high yielding and nutrient-dense accessions identified in this study could be used as potential starting materials for little millet improvement including direct release as cultivar following national protocols. The scope of the little millet evaluations needs to be expanded to include multiple sites, ideally with different soil types and fertility, to make a better estimate for the heritability of grain yield and nutrient composition. Further, assessing little millet as a potential bioenergy feedstock, fodder quality profiling, and genomic investigation can promote little millet as a multi-purpose, climate-resilient, nutrient-rich crop in a climate change scenario. Researchers can obtain seed samples of little millet accessions from the ICRISAT genebank (http://genebank.icrisat.org/) following a standard material transfer agreement.

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AUTHOR CONTRIBUTIONS

Vetriventhan Mani: Conceptualization; Formal analysis; Investigation; Writing-original draft; Writing-review & editing. Hari D Upadhyaya: Conceptualization; Writing-review & editing. Vania CR Azevedo: Writing-review & editing. Victor Allan Jayapal: Formal analysis; Writing-review & editing. S Anitha: Formal analysis; Writing-review & editing.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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