

## Nutritional Diversity of Foxtail Millet (*Setaria italica*) From North Maharashtra

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### Abstract

This comprehensive study evaluates the nutritional variability in *Setaria italica* (foxtail millet) across 15 geographically distinct locations of North Maharashtra, a region recognized for its agro-biodiversity and tribal agricultural heritage. The investigation was carried out to quantify and compare the levels of key macronutrients: protein, carbohydrates, fat, and ash content, which are vital indicators of the grain's nutritional quality and food value. Standardized biochemical methods were employed—Kjeldahl method for protein analysis, Anthrone method for carbohydrate estimation, Soxhlet extraction for fat quantification, and dry ashing in a muffle furnace for determining total ash content. Each accession was represented by data from five randomly selected plants to ensure statistical reliability and minimize sampling bias. The results demonstrated substantial inter-accessional variation, with protein content ranging from 8.5 to 13.5 g/100g, carbohydrates from 61.6 to 70.65 g/100g, fat content from 2.9 to 3.75 g/100g, and ash content from 2.7 to 3.05 g/100g. Among all accessions, the sample from Dhadgaon emerged as the most nutritionally superior, showing peak values in both protein and carbohydrate content, alongside consistently high fat and ash levels. This accession may be considered a potential candidate for biofortification, functional food formulation, and climate-resilient crop development. The nutritional diversity observed among the accessions highlights the genetic richness and adaptive variation inherent in North Maharashtra's foxtail millet germplasm. These findings emphasize the importance of conserving and promoting region-specific landraces for their role in addressing nutritional security, especially in marginal and rain fed agro-ecosystems. The study also contributes baseline data for breeding programs, value chain development, and policy-level interventions aimed at mainstreaming millets in public distribution and nutrition-sensitive agriculture.

**Keywords:** Foxtail millet; *Setaria italica*; Nutritional diversity; Macronutrient profiling; Agro-biodiversity; Nutrition-sensitive agriculture; Tribal cropping systems

### INTRODUCTION

Foxtail millet (*Setaria italica*), one of the earliest domesticated millet species, is gaining prominence in contemporary nutrition and agriculture due to its adaptability, resilience to drought, and exceptional nutritional value. Indigenous to Asia and widely cultivated in semi-arid regions, this crop has historically supported subsistence farming in marginal

environments. In recent years, the significance of millets like foxtail millet has been revisited for their potential in combating malnutrition and ensuring food security, especially in light of changing climate patterns and the need for sustainable crops (Saleh et al., 2013).

The North Maharashtra region, encompassing districts such as Dhule, Nandurbar, and Jalgaon, offers a distinct agro-climatic zone conducive to millet cultivation. However, despite the region's potential, there exists a gap in the systematic assessment of the nutritional diversity present within local germplasm of *Setaria italica*. Evaluating and documenting this diversity is essential for identifying nutrient-rich genotypes and promoting them in health-focused agricultural systems.

This study aims to assess the nutritional variability of foxtail millet accessions from North Maharashtra by examining nine qualitative and quantitative traits, including protein, carbohydrates, fats, ash content, total phenolic compounds, and selected micronutrients. Observations were recorded from five randomly selected plants per accession, providing a comprehensive understanding of intra-regional nutritional diversity and paving the way for future breeding programs focused on health and nutrition.

#### Nutritional Characterization

Observations were meticulously recorded on five randomly selected plants from each foxtail millet (*Setaria italica*) accession collected from the North Maharashtra region. A total of nine nutritional characters, encompassing both qualitative and quantitative traits, were evaluated. These included macronutrients such as protein, carbohydrates, fats, and ash content, along with total phenolic compounds known for their antioxidant properties. Additionally, essential micronutrients were assessed to understand the mineral diversity among accessions. This comprehensive profiling provides a foundational insight into the nutritional variability present within the local genetic pool of foxtail millet, which is crucial for its promotion as a functional food and for use in biofortification and crop improvement programs.

#### Methodology

##### Estimation of Total Protein Content (g/100g)

The total protein content in foxtail millet samples was estimated using the Kjeldahl method, a widely accepted standard for determining nitrogen content, which is then converted into protein. The procedure followed is as described below:

**Sample Preparation:** A finely powdered sample of 100 mg from each foxtail millet accession was weighed accurately and transferred into a Kjeldahl digestion tube.

##### Digestion Process

To the digestion tube, 3–4 g of a catalyst mixture composed in a 5:1 ratio of potassium sulfate ( $K_2SO_4$ ) to copper sulfate ( $CuSO_4$ ) was added. Then, 10 ml of concentrated sulfuric acid ( $H_2SO_4$ ) was carefully introduced. The digestion tube was placed in a digestion unit, and the temperature was gradually raised to  $420^\circ C$ . The digestion was continued for 1 to  $1\frac{1}{2}$  hours until the mixture turned clear, indicating complete digestion. After cooling, the digested mixture was made ready for distillation.

### Distillation and Titration

The distillation was carried out using PELICAN EQUIPMENTS with the addition of boric acid and a small quantity of potassium permanganate ( $\text{KMnO}_4$ ) to assist in the conversion of ammonium to ammonia gas. The sample tube was loaded on the distillation unit, where it was processed for 9 minutes. Released ammonia was absorbed in a boric acid solution. The borate–ammonia complex was then titrated against 0.1 N hydrochloric acid (HCl) until a clear endpoint was observed.

Calculation of Nitrogen Content:

The nitrogen content in the sample was calculated using the following equation:

$$\text{Nitrogen (N)} = \frac{14.01 \times 0.1N \times (TV - BV) \times 100}{W}$$

Where:

14.01 = molecular weight of nitrogen (g/mol) 0.1N = normality of the HCl used

TV = titer value (volume of HCl used in titration) BV = blank value (volume of HCl for blank sample)

W = weight of the sample in grams Conversion to Protein Content:

The total nitrogen (%) was then converted to protein content (%) using the general protein conversion factor of 6.25, as:

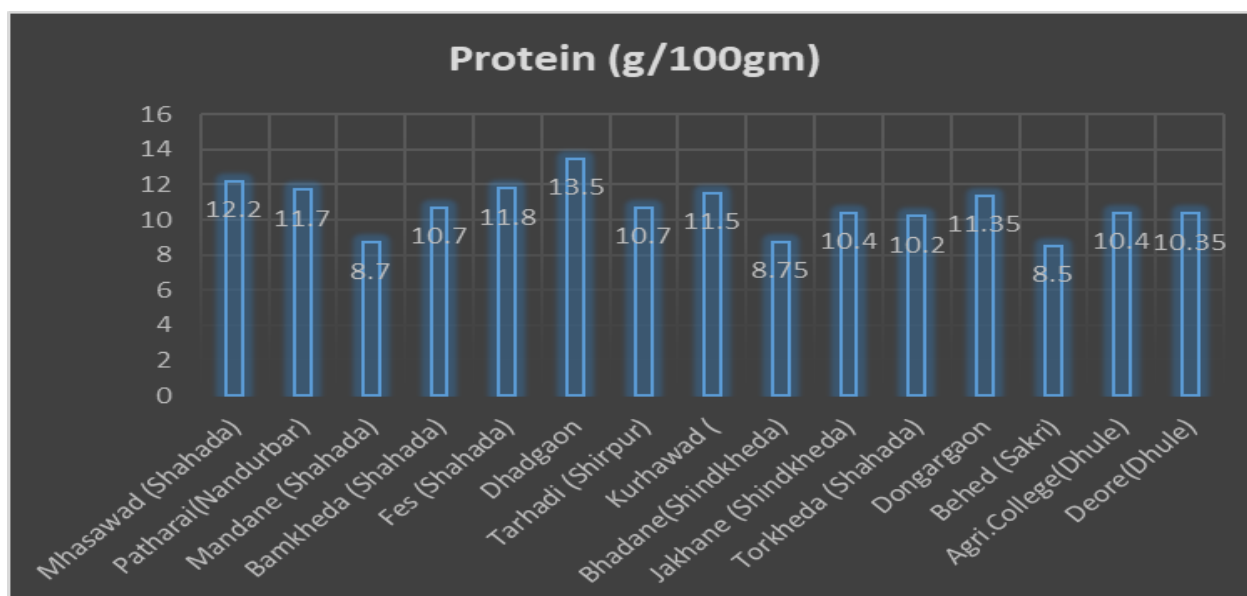
Protein (%) = Nitrogen (%)  $\times$  6.25

This method allowed precise quantification of total protein present in the foxtail millet samples, enabling the assessment of varietal nutritional variability across accessions collected from North Maharashtra.

Sr. No.	Sample Location	Protein Content (g/100gm)
1	Mhasawad (Shahada)	12.2
2	Patharai(Nandurbar)	11.7
3	Mandane (Shahada)	8.7
4	Bamkheda (Shahada)	10.7
5	Fes (Shahada)	11.8
6	Dhadgaon	13.5
7	Tarhadi (Shirpur)	10.7
8	Kurhawad	11.5
9	Bhadane(Shindkheda)	8.75
10	Jakhane (Shindkheda)	10.4
11	Torkheda (Shahada)	10.2
12	Dongargaon	11.35
13	Behed (Sakri)	8.5
14	Agri.College(Dhule)	10.4
15	Deore(Dhule)	10.35

TABLE 1:Protein Content Analysis of Foxtail Millet Accessions from North Maharashtra

The protein content among 15 foxtail millet accessions collected from various agro-climatic zones in North Maharashtra showed notable variability, ranging from 8.5 g/100g to 13.5 g/100g. This variation reflects the genetic and environmental influences on the nutritional composition of *Setaria italica*. The highest protein content was recorded in the accession from Dhadgaon at 13.5 g/100g, indicating its strong potential for use in biofortified millet breeding programs or nutritional enhancement. Other high-protein accessions included Mhasawad (Shahada) (12.2 g/100g), Fes (Shahada) (11.8 g/100g), Patharai (Nandurbar) (11.7 g/100g), and Kurhawad (11.5 g/100g), all above the regional mean value of approximately 10.72 g/100g. Accessions from Dongargaon, Agri. College (Dhule), and Deore (Dhule) showed moderate protein levels between 10.35 – 11.35 g/100g. The lowest protein content was observed in Behed (Sakri) at 8.5 g/100g, followed closely by Mandane (Shahada) (8.7 g/100g) and Bhadane (Shindkheda) (8.75 g/100g), suggesting comparatively lower nutritional potential. This pattern suggests that Shahada and Dhule regions have both high- and low-protein variants, implying diverse germplasm within a localized area. The presence of such variability is highly advantageous for selective breeding, conservation programs, and developing location-specific nutrition-sensitive agriculture strategies. The standard deviation of 1.38 further confirms moderate variability in protein content among these accessions. Such variability may be attributed to differences in genetics, soil fertility, local climate, and cultivation practices.



#### Total Carbohydrate Content (g/100gm)

The total carbohydrate content in foxtail millet samples was determined using the Anthrone method, a colorimetric technique based on the reaction of carbohydrates with anthrone reagent under acidic conditions to produce a green-colored complex. The intensity of the green color is proportional to the carbohydrate concentration and is measured spectrophotometrically.

### Sample Hydrolysis

A 100 mg powdered sample was taken in a test tube and hydrolyzed by heating with 5 ml of 2.5 N hydrochloric acid (HCl) in a boiling water bath for 3 hours. This process ensured the breakdown of polysaccharides into simple sugars. After hydrolysis, the mixture was allowed to cool to room temperature, and the total volume was adjusted to 100 ml with distilled water.

### Centrifugation and Sample Preparation

The hydrolyzed solution was centrifuged to remove particulate matter. From the supernatant, an aliquot was taken, and the volume in all test tubes (including standards and sample) was made up to 1 ml using distilled water.

### Color Development with Anthrone Reagent

To each tube, 4 ml of freshly prepared Anthrone reagent (typically 0.2% anthrone in concentrated sulfuric acid) was added. The tubes were then heated in a boiling water bath for 8 minutes to allow the reaction to occur. After heating, the tubes were rapidly cooled under running tap water or in an ice bath to stabilize the color.

### Spectrophotometric Measurement

The absorbance of the resulting green-colored solution was measured at 630 nm using a UV-Vis spectrophotometer. A standard curve of glucose was prepared to compare and quantify the carbohydrate content in the test samples.

**Calculation:** The amount of carbohydrate present in the sample was calculated using the formula:

$$\text{Carbohydrate content (mg/100mg sample)} = \frac{\text{mg of glucose from standard curve}}{\text{Volume of test sample (ml)}} \times 100$$

This result was then converted to grams per 100 grams of sample (g/100g) for reporting the carbohydrate content.

Sr. No.	Sample Location	Carbohydrate Content (g/100gm)
1	Mhasawad (Shahada)	69
2	Patharai(Nandurbar)	65.4
3	Mandane (Shahada)	61.9
4	Bamkheda (Shahada)	64.75
5	Fes (Shahada)	67.8
6	Dhadgaon	70.65
7	Tarhadi (Shirpur)	70.05
8	Kurhawad	66.6
9	Bhadane(Shindkheda)	61.6
10	Jakhane (Shindkheda)	66.7
11	Torkheda (Shahada)	65.45
12	Dongargaon	69.95
13	Behed (Sakri)	63.9
14	Agri.College(Dhule)	68.95
15	Deore(Dhule)	68.05

TABLE 2: Total Carbohydrate Content (g/100gm)

The analysis of total carbohydrate content across 15 foxtail millet accessions from different regions of North Maharashtra revealed a substantial nutritional range, with values varying from 61.6 g/100g to 70.65 g/100g. The highest carbohydrate content was recorded in the Dhadgaon sample, with 70.65 g/100g, followed closely by Tarhadi (Shirpur) (70.05 g/100g) and Dongargaon (69.95 g/100g), suggesting that accessions from these locations could serve as promising candidates for energy-rich millet cultivation. Mhasawad (Shahada) and Agri. College (Dhule) also exhibited high values, with 69.0 g/100g and 68.95 g/100g respectively. Moderate carbohydrate content, ranging between 64–68 g/100g, was observed in samples from Fes (Shahada), Deore (Dhule), Kurhawad, Jakhane (Shindkheda), Torkheda (Shahada), Patharai (Nandurbar), and Bamkheda (Shahada), all of which lie close to the calculated regional mean of ~66.4 g/100g. The lowest carbohydrate content was found in Bhadane (Shindkheda) at 61.6 g/100g, Mandane (Shahada) at 61.9 g/100g, and Behed (Sakri) at 63.9 g/100g, indicating relatively lower energy-yielding potential among these accessions. This variability is likely due to genotypic differences, soil properties, local climatic conditions, and agronomic practices. Such intra-regional variation offers scope for targeted selection and value-added breeding focused on energy-dense millet varieties for nutrition-sensitive agriculture. The data supports the view that foxtail millet from North Maharashtra presents good potential as a carbohydrate-rich staple, particularly useful in dietary interventions aimed at combating energy deficiency.

#### Total Fat Content (g/100gm)

The total fat content of foxtail millet samples was estimated using the Soxhlet extraction method, following the AOAC (Association of Official Analytical Chemists) standard protocol. This gravimetric method isolates crude fat using a non-polar organic solvent such as petroleum ether.

#### Sample Preparation

Accurately weighed 100 mg of finely ground foxtail millet sample was transferred into a porous thimble specially designed for use in a Soxhlet apparatus.

#### Fat Extraction Process

The thimble containing the sample was placed into the main chamber of the Soxhlet extractor. Petroleum ether (boiling point 40°C–60°C) was used as the solvent, filled into the solvent flask below the extraction unit. The solvent was heated to maintain a temperature range of 65°C to 70°C, allowing it to evaporate and repeatedly condense, dripping onto the sample and extracting fats over a period of 60 minutes.

#### Post-Extraction Drying and Weighing

After completion of the extraction process, the ether was evaporated, and the remaining lipid extract was collected in the previously weighed fat collection flask. The flask was then dried and cooled to room temperature in a desiccator to eliminate residual solvent.

#### Calculation of Fat Content

The total fat content was determined using the following formula:

Where:

W1 = Weight of the empty collection flask (g) W2 = Weight of the flask + ether extract (g)



W3 = Initial weight of the sample (g)

This method provides an accurate measurement of the total extractable fat in the sample, which is important for understanding the energy contribution and oil content of different foxtail millet accessions.

Sr. No.	Sample Location	Fat Content (g/100gm)
1	Mhasawad (Shahada)	3.75
2	Patharai(Nandurbar)	3.6
3	Mandane (Shahada)	3
4	Bamkheda (Shahada)	3.1
5	Fes (Shahada)	3.1
6	Dhadgaon	3.65
7	Tarhadi (Shirpur)	3.25
8	Kurhawad	3.55
9	Bhadane(Shindkheda)	2.9
10	Jakhane (Shindkheda)	3.3
11	Torkheda (Shahada)	3
12	Dongargaon	3.2
13	Behed (Sakri)	3.45
14	Agri.College(Dhule)	3.4
15	Deore(Dhule)	3.05

TABLE 3: Total Fat Content (g/100gm)

The fat content among 15 foxtail millet samples collected from various locations in North Maharashtra exhibited a narrow but meaningful variation, ranging from 2.9 g/100g to 3.75 g/100g. This reflects the relatively low but nutritionally significant lipid profile typical of millet grains, which contributes to their energy density and shelf stability. The highest fat content was recorded in the sample from Mhasawad (Shahada) at 3.75 g/100g, suggesting a promising candidate for high-energy diet formulations. Dhadgaon (3.65 g/100g), Patharai (Nandurbar) (3.6 g/100g), and Kurhawad (3.55 g/100g) also exhibited relatively high fat levels, making them nutritionally rich in terms of lipid contribution. A moderate fat range between 3.2–3.4 g/100g was observed in accessions from Dongargaon, Jakhane (Shindkheda), Behed (Sakri), and Agri. College (Dhule). The lowest fat content was found in the sample from Bhadane (Shindkheda) at 2.9 g/100g, followed closely by Mandane (Shahada) and Torkheda (Shahada), both at 3.0 g/100g, indicating relatively leaner energy sources. The regional mean fat content was approximately 3.28 g/100g, with a small standard deviation, suggesting relatively stable lipid expression among genotypes, possibly due to conserved metabolic traits in lipid biosynthesis pathways across the region. These findings are relevant for dietary planning, especially for communities dependent on millet as a staple. The accessions with slightly higher fat content may be prioritized for nutritional supplementation programs, energy-dense millet flour development, or functional food innovations.

Total Ash Content (g/100gm)

The total ash content of foxtail millet samples was determined using the dry ashing method

by incineration in a muffle furnace, following the AACC (2000) Method No. 08-01. Ash is defined as the inorganic mineral residue remaining after the complete combustion of organic matter and is an essential parameter in the proximate composition analysis of foods.

### Sample Preparation

A known weight (typically 2–5 g) of finely ground and pre-dried foxtail millet flour was transferred into a clean, dry, and pre-weighed silica crucible.

### Incineration Process

The crucible containing the sample was placed in a muffle furnace and incinerated at a temperature of 550°C. The sample was left in the furnace until a greyish-white residue appeared, indicating complete combustion of organic material. This typically took several hours (4–6 hours), depending on the sample matrix.

### Cooling and Weighing

After ashing, the crucible was cooled in a desiccator to room temperature to prevent moisture absorption and was then weighed again.

### Calculation

The ash content was calculated using the following formula:

Where;

Weight of Ash = Final weight of crucible with ash – weight of empty crucible  
Weight of Sample = Initial weight of millet flour taken

This method quantifies the total mineral content (including calcium, potassium, phosphorus, etc.) present in the sample. Ash content serves as an indicator of the nutritional and processing quality of millet grains and may also relate to the residual presence of bran and outer layers.

Sr.No.	Sample Location	Ash Content (g/100gm)
1	Mhasawad (Shahada)	2.7
2	Patharai(Nandurbar)	2.85
3	Mandane (Shahada)	2.7
4	Bamkheda (Shahada)	2.9
5	Fes (Shahada)	3.05
6	Dhadgaon	2.95
7	Tarhadi (Shirpur)	2.7
8	Kurhawad	2.95
9	Bhadane(Shindkheda)	2.95
10	Jakhane (Shindkheda)	2.9
11	Torkheda (Shahada)	2.75
12	Dongargaon	2.9
13	Behed (Sakri)	3.05
14	Agri.College(Dhule)	2.95
15	Deore(Dhule)	2.95

TABLE 4: Total Ash Content (g/100gm)



The ash content among the 15 foxtail millet accessions from North Maharashtra ranged from

2.7 g/100g to 3.05 g/100g, indicating a relatively consistent but nutritionally significant mineral composition across different locations. Ash represents the total inorganic mineral matter in the sample, such as calcium, iron, potassium, phosphorus, and other trace elements. The highest ash content was recorded in accessions from Fes (Shahada) and Behed (Sakri), both at 3.05 g/100g, indicating these samples may be richer in essential minerals and trace elements. Slightly lower but still relatively high values were observed in accessions from Bamkheda (Shahada), Jakhane (Shindkheda), Dongargaon, Kurhawad, Agri. College (Dhule), Dhadgaon, Deore (Dhule), and Bhadane (Shindkheda) — all in the range of 2.9–2.95 g/100g. The lowest ash content, 2.7 g/100g, was found in Mhasawad (Shahada), Mandane (Shahada), and Tarhadi (Shirpur). These samples may have slightly lower mineral retention or bran content, depending on genotype or soil profile. Torkheda (Shahada) had a marginally higher value at 2.75 g/100g, still below the overall regional mean. The mean ash content across all samples was approximately 2.89 g/100g, with minimal variation (standard deviation  $\approx 0.11$ ), indicating a uniform distribution of total mineral content across accessions. This uniformity may be attributed to common agro-ecological conditions or shared processing methods. where high-ash lines may be preferred to improve the mineral profile of millet-based diets in rural and tribal populations.

## Result

The nutritional profiling of 15 foxtail millet accessions collected from diverse locations across North Maharashtra revealed significant inter-accessional variation in protein, carbohydrate, fat, and ash content. Protein Content ranged from 8.5 to 13.5 g/100g, with the highest recorded in the Dhadgaon sample (13.5 g/100g) and the lowest in Behed (Sakri) (8.5 g/100g). Other protein-rich accessions included Mhasawad (12.2 g/100g) and Fes (11.8 g/100g). Carbohydrate Content varied between 61.6 and 70.65 g/100g. Dhadgaon (70.65 g/100g), Tarhadi (70.05 g/100g), and Dongargaon (69.95 g/100g) were among the highest, indicating their potential as energy-rich grain sources. Fat Content ranged from 2.9 to 3.75 g/100g. The maximum fat content was observed in Mhasawad (3.75 g/100g), followed by Dhadgaon (3.65 g/100g) and Patharai (3.6 g/100g), suggesting their utility in high-energy diet formulations. Ash Content, representing total mineral matter, ranged from 2.7 to 3.05 g/100g. The highest values were observed in Fes (3.05 g/100g) and Behed (3.05 g/100g), indicating higher mineral retention. The Dhadgaon accession consistently exhibited superior nutritional values, with the highest protein and carbohydrate levels and above-average fat and ash content. On the contrary, Behed (Sakri) and Mandane (Shahada) were found to be at the lower end of the nutritional spectrum. These results highlight the presence of genetic and environmental diversity in the nutritional makeup of foxtail millet accessions from North Maharashtra. The data can be effectively utilized for selective breeding, nutritional enhancement programs, and promotion of location-specific millet varieties suited for health-conscious and food-insecure populations.

## Conclusion

The present study aimed at evaluating the nutritional diversity of *Setaria italica* (foxtail millet) accessions collected from different agro-ecological zones of North Maharashtra, focusing on key proximate components—protein, carbohydrates, fat, and ash content. The findings revealed substantial variation among the 15 accessions, indicating the presence of rich genetic and environmental diversity within the regional millet germplasm. Among all accessions, Dhadgaon consistently emerged as the most nutritionally superior variety, exhibiting the highest protein content (13.5 g/100g) and maximum carbohydrate content (70.65 g/100g), along with above-average fat and ash levels, making it an ideal candidate for high-energy dietary formulations, biofortification, and crop improvement programs. In contrast, accessions such as Behed (Sakri) and Mandane (Shahada) were found to have relatively lower nutritional indices, which may limit their dietary value but could still be important for conserving genetic diversity. The protein content ranged from 8.5 to 13.5g/100g, and carbohydrates from 61.6 to 70.65 g/100g, showing high variability and potential for selective breeding. Fat content showed relatively less variation (2.9 to 3.75 g/100g), which is typical of millet grains, yet significant for energy contribution. The ash content, ranging from 2.7 to 3.05 g/100g, reflects consistent mineral availability among accessions, with few exceptions. This study not only confirms the nutritional potential of foxtail millet as a climate-resilient and nutrient-dense crop but also identifies specific local accessions that can be promoted in nutrition-sensitive agriculture, tribal food security programs, and value-added food product development. The documented variation provides a valuable foundation for future genetic improvement, germplasm conservation, and functional food innovation based on regional diversity.

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