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RESEARCH ARTICLE

Crop Science

Seasonal variation in crop yield and reduction of phytic acid content through soaking and germination of kodo millet (*Paspalum scrobiculatum L.*) germplasm

C Suneetha and M Sukumar*
Centre for Food Technology, A.C. Tech, Anna University, Chennai 600025, Tamil Nadu, India.

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Abstract: Kodo millet (*Paspalum scrobiculatum* L.) is a nutrient-rich, gluten-free grain with significant potential for enhancing food security. However, its high phytic acid content limits mineral bioavailability, necessitating strategies for reduction. This study evaluated 75 kodo millet germplasms sourced from ICRISAT and cultivated under surface drip irrigation during the 2022-2023 Rabi (dry/winter season) and 2023-2024 Kharif (wet/monsoon season) at the University of Hyderabad. Two-way ANOVA was used to identify IPs 69 as the highest-yielding variety across both seasons, while IPs 240 (Kharif) and IPs 236 (Rabi) were the lowest yielding. Seasonal variations influenced overall productivity, with Rabi conditions favouring grain production. Phytic acid levels were analysed in husked and dehusked grains, showing significant genetic variation but minimal seasonal influence on post-harvest dehusking. To enhance mineral bioavailability, response surface methodology (RSM) was used to optimize soaking (20.63 h, 38.49 °C, pH 4.08) and germination (36.29 h, 27.29 °C, 65.18% moisture) conditions. Soaking alone resulted in a substantial reduction of phytic acid by 48.11%, while soaking followed by germination led to a total reduction of 53.97%, indicating that germination contributed only a marginal additional decrease beyond soaking. These findings demonstrate that soaking is the primary contributor to phytic acid degradation, with germination playing a supplementary role. This study highlights the importance of selecting high-yielding, nutritionally superior kodo millet varieties while optimizing post-harvest treatments to improve their dietary value. The findings underscore the role of genetic diversity, environmental conditions, and processing interventions in determining the grain's nutritional and agronomic potential. Future research should focus on integrating advanced breeding strategies and optimized processing methods to enhance the bioavailability of essential micronutrients, ensuring kodo millet's broader adoption in sustainable food systems.

Keywords: Kodo millet flour*,* micro nutrient bioavailability, millet germplasms, *Paspalum scrobiculatum* L., phytic acid

INTRODUCTION

India has been cultivating kodo millets for over 3,000 years. Kodo millet (*Paspalum scrobiculatum* L.) belongs to the family Poaceae, subfamily Panicoideae, and tribe Paniceae. It is a tetraploid species (2n = 4x = 40) believed to have originated in the Indian subcontinent. The germplasm used in this study consisted of 75 genetically diverse accessions sourced from different agro-ecological regions of India, maintained at the ICRISAT. These accessions reflect a broad spectrum of genetic variability in terms of agronomic traits, phenology, and adaptability. Previous molecular and morphological characterizations of *P. scrobiculatum* have shown significant intra-specific variation, which provides a strong foundation for crop improvement and stress resilience breeding efforts (Kandlakunta & Golla, 2017). Before the 1980s, millets were a staple food for the poor, but they were gradually replaced by wheat and rice (Ghosh et al*.,* 2024). Millets are nutritionally superior to major cereals, containing

* Corresponding author (sukumarcbt@gmail.com; https://orcid.org/0000-0003-0614-5473)

higher levels of dietary fibre, resistant starches, vitamins, essential amino acids, storage proteins, and other bioactive compounds, which classify them as nutraceuticals (Saleem et al., 2023a). People today are increasingly aware of the health benefits of millets, which have long been considered a nutritional powerhouse, particularly in Indian communities. Millets are valued for their versatility in various dishes and their climate resilience, making them a sustainable crop option. Their renewed popularity stems from their rich nutritional profile and potential to improve overall health. They have become a modern superfood, gaining popularity in health diets (Kumar et al., 2022).

Furthermore, due to their high dietary fibre content, millets help prevent constipation, support regular bowel movements, and lower the risk of gastrointestinal issues (Nithiyanantham et al., 2019) The thermal effect of the antioxidants of kodo is also very effective (Yadav et al., 2009; Yadav et al., 2022). Individuals with gluten intolerance or celiac disease may benefit from kodo millet, since it is gluten-free (Saleem et al., 2023b). Additionally, its low glycaemic index makes it beneficial for managing blood sugar levels, making it a preferred choice for individuals with diabetes or metabolic disorders (Gupta, 2023; Kanimozhi & Sukumar, 2023)

Despite their nutritional advantages, millets contain anti-nutrients such as tannins, polyphenols, and phytic acid (Geisen et al., 2021), which reduce mineral bioavailability. The most common anti-nutrients in millets are tannins, polyphenols, and particularly phytic acid or phytate which is myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. It is the main storage form of phosphorus, making up 1-5% of the weight in cereals, legumes, nuts, oilseeds, and similar foods (Salim et al., 2023). Phytic acid, stored as phosphorus in seeds, can negatively affect the diet by hindering the absorption of minerals like zinc, magnesium, copper, calcium, and iron (Schlemmer et al., 2009). The recommended

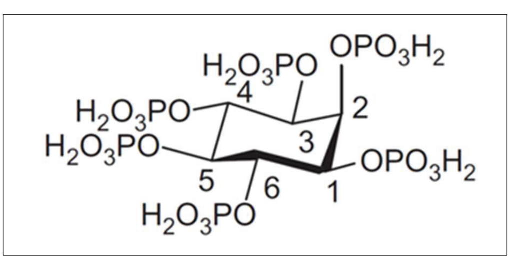

Figure 1: Structure of phytic acid in equatorial conformation. (Marolt & Kolar, 2021)

dietary allowances (RDAs) by the National Institute of Nutrition (NIN, 2020), for phosphorus in the human diet are as follows: ages 9–13 years: 1,250 mg/day. ; ages 14–18 years: 1,250 mg/day. ; ages 19 and older: 700 mg/day. Phytic acid has a chelating property that binds to minerals, making them unavailable. The removal of phytic acid enhances the bioavailability of various cations, thereby improving the nutritional value of the meal. Several methods have been developed to eliminate phytic acid from grains (Nout MJR, 1993; Sareen et al., 2024).

Anti-nutrients can be minimized using different processing techniques, such as soaking, germination or sprouting, cooking, malting, and fermenting the grains. Reducing phytic acid enhances mineral bioavailability (Sarkhel & Roy, 2022), thus improving the nutritional quality of millet-based diets. Studies have also associated high phytic acid levels with an increased risk of certain cancers. Several processing methods, including soaking, germination, fermentation, and cooking, help reduce phytic acid content (Sachdev et al., 2023). Among these, soaking and germination are widely preferred due to their ability to activate enzymes that degrade phytates (Ranjan et al., 2023). Optimizing these processes enhances the bioavailability of key nutrients, making kodo millet a more viable dietary option (Nobis et al., 2023).

This study optimized soaking and germination conditions to reduce phytic acid in kodo millet using response surface methodology (Dey et al., 2023a). Seventy-five germplasm varieties were cultivated and analysed for phytic acid content in grains with and without husk. The effects of soaking (time, temperature, and pH) and germination (time, temperature, and moisture) on phytic acid degradation were evaluated. Statistical analysis using ANOVA identified significant influencing factors, and RSM determined the optimal conditions for maximum phytic acid reduction, improving the nutritional potential of kodo millet (Gupta et al., 2015a; Mohana Priya et al., 2025).

MATERIALS AND METHODS

Source of raw material

A total of 75 different varieties of kodo millet (*Paspalum scrobiculatum* L.) germplasm core collection has been selected by the ICRISAT gene bank from various states of India such as IPs68, IPs69, IPs91, IPs105 from Bihar; IPs919 from Jharkhand; IPs593 from Gujarat; IPs699, IPs706, IPs709, IPs730, IPs741, IPs744, IPs764, IPs777, IPs782, IPs785, IPs793, IPs795, IPs803, IPs814, IPs828, IPs862, IPs870, IPs872, IPs883, IPs891, IPs908 from Maharashtra; IPs155, IPs159, IPs172, IPs176, IPs181, IPs207, IPs208, IPs236, IPs240, IPs245, IPs254, IPs429, IPs597, IPs599, IPs606, IPs614, IPs622, IPs627, IPs628, IPs630, IPs645, IPs648, IPs653, IPs654, IPs670 from Madhya Pradesh; IPs274, IPs275, IPs, IPs279, IPs280, IPs287, IPs292, IPs293, IPs669, IPs694, IPs695, IPs928 from Tamil Nadu; IPs318, IPs319, IPs329 from Orissa, IPs585 from unknown location; IPs344, IPs358, IPs368, IPs388, IPs415 from Uttar Pradesh; and IPs4, IPs5, IPs13 from West Bengal. The grains of these 75 kodo millet core germplasms, sourced from the ICRISAT gene bank, were meticulously cultivated in the fields of the University of Hyderabad. All of them represent traditional cultivars or landraces.

Chemicals required

The Megazyme Assay Kit was procured from Megazyme Ltd, Ireland. Ascorbic acid, Sulphuric acid, Ammonium molybdate, Trichloroacetic acid, Hydrochloric acid, Sodium hydroxide were purchased from HiMedia Private Limited, India.

Crop cultivation and harvesting of kodo millet

The study was conducted at the University of Hyderabad, Hyderabad, during the Rabi season (November 2022) and the Kharif season (July 2023). The University of Hyderabad is located in Gachibowli, Hyderabad, Telangana, at a latitude of 17°27′00″ North and a longitude of 78°20′45″ East, with an elevation of approximately 576 meters above mean sea level. All 75 genetically diverse genotypes of Kodo millet germplasms (Upadhyaya et al., 2014) were grown in a randomized block design (Ram et al., 2024) during two seasons from 14th November 2022 to April 25th 2023 in the Rabi season, and from 29th July 2023 to December 20th 2023 in the Kharif season. Millets can thrive in regions with as little as 350 mm of rainfall, making them resilient crops well-suited to withstand harsh climate conditions. The selected geographical land has a pH of 6.0 – 7.5, and receives direct sunlight for 6 – 8 h. Kodo millet is primarily cultivated in India's warm and arid regions. The minimum water requirement for maize is 400 mm, while sorghum requires 500 to 600 mm (Sukanya, 2022.) Kodo millet is typically planted at the start of the monsoon or early in the rainy season.

Seeds were sorted according to their accession numbers and sown in three replications of direct sowing, a common method for kodo millet cultivation. Each accession was planted in single rows measuring 3 meters in length, with a spacing of 30 cm by 10 cm. Observations were taken from five randomly chosen plants within each accession for qualitative and quantitative traits. Proper levelling of the land was done to avoid water stagnation and to ensure that there were no uneven areas. The crop was drip-irrigated to supply water at specific time intervals providing water directly to the roots. Seeds were sown in a randomized pattern that differed in each replication.

Pesticides were used to reduce shoot flies, which affect the yield of the crop. Germination, 50% flowering, days to anthesis, panicle emergence, time from seed set to maturity, senescence, and plant lodging were recorded. Fertilizers were used to enhance growth at 50% germination and 50% flowering stage (Gamulin et al., 2024) . The field was arranged with the accession number of each variety labelled with tags attached to iron rods, for each of the three replications in the field layout. Physiological properties of harvested seeds were estimated to evaluate the yield per plant along with seed weight, colour, and shape.

Estimation of phytic acid

The phytic acid content was estimated for the husked and dehusked grains of all millet varieties. Phytic acid content was measured using the K-PHYT Phytic Acid 05/19 (Phytate)/Total Phosphorus kit (Megazyme, Wicklow, Ireland) according to the manufacturer's instructions with minor modifications. In brief, 100 mg of seed flour was added to 2 mL of 0.66 M HCl and mixed by shaking at room temperature overnight. The resulting extract (1.5 mL) was centrifuged for 10 minutes at 16,000 g, and 0.5 mL of the supernatant was neutralized with 0.5 mL of 0.75 M NaOH. A 12.5 µL portion of the neutralized sample was used for the enzymatic dephosphorylation reaction and subsequent total phosphorus (Ptot) quantification. Simultaneously, another 12.5 µL of the neutralized sample was used to quantify inorganic phosphate (Pi). After enzymatic treatment, both total phosphorus and free phosphate were analysed in a spectrophotometer set at 655 nm. Standards were plotted following the manufacturer's instructions. For every batch of samples used in the colorimetric analysis of phosphorus, a corresponding phosphorus calibration curve was created simultaneously with the same batch of colour reagent. The assay specifically measures phosphorus released as "available phosphorus" from phytic acid, myo-inositol (phosphate)n, and monophosphate esters via phytase and alkaline phosphatase (Boncompagni et al., 2018). The experiments were conducted in triplicate for both seasons of each sample with husk and without husk (dehusked) for 75 varieties of the kodo millet germplasm.

Statistical analysis

The grain yield of 75 Kodo millet germplasms was evaluated across Rabi 2022–2023 and Kharif 2023 seasons using a two-way ANOVA performed using Microsoft Excel (Data Analysis ToolPak) to assess seasonal and varietal influences. The yield data were recorded for both husked and dehusked grains to determine differences in grain productivity and post-harvest characteristics. Based on the two-way ANOVA results, three germplasm varieties with the highest yield obtained during post-harvest (Mahajan et al., 2024), and phytic acid content were selected for further experiments (Singamsetti et al., 2022).

Processing of optimized grains

Kodo millet grains underwent soaking and germination treatments to evaluate phytic acid reduction. Soaking was performed at different times (6–24 h), temperature (25–45 °C), and pH levels (4–7), followed by germination under varying durations (12–48 h), temperature (20–40 °C), and moisture levels (35–75%). Phytic acid content was quantified using the K-PHYT Phytic Acid Kit (Megazyme, Ireland) after enzymatic dephosphorylation (Godrich et al., 2023). The experiment was conducted in triplicates, and data were statistically analysed to determine the impact of treatments on phytic acid degradation (Bhuvaneshwari et al., 2020) .

Response surface methodology (RSM)

The study employed a box–behnken design (BBD) under response surface methodology (RSM) to optimize phytic acid reduction in kodo millet through soaking and germination, using Design-Expert software version 13**.** Independent variables for soaking included time (h), temperature (°C), and pH, while for germination, they included time (h), temperature (°C), and moisture (%). The BBD experimental design generated multiple combinations of these factors with triplicate central points (Buenaño et al., 2024). Phytic acid content was quantified using the K-PHYT Phytic Acid Kit (Megazyme, Ireland) after enzymatic dephosphorylation. ANOVA was performed to determine the significance of variables, and the optimal conditions for maximum phytic acid degradation (%) were obtained through the desirability function method, which identifies the combination of factors that produces the most favorable overall response (Dey et al., 2023b).

RESULTS AND DISCUSSION

Effect of season on crop yield of various germplasm kodo millet grains

Grain yield is a multifaceted trait affected by numerous other component traits. This study on grain yield analysis

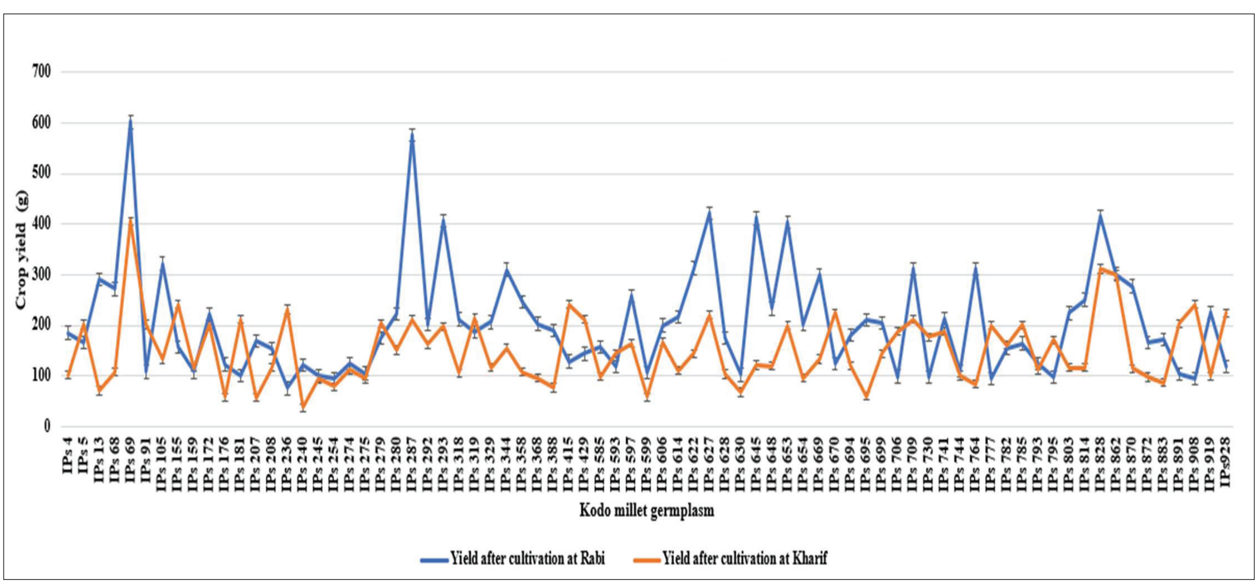

Figure 2: Comparison of the effect of different seasons on the yield of various kodo millet germplasm.

showed that the two different seasons significantly influence yield for each plant variety (Supplementary table 1). Yield per plant was calculated as the average value of three samples obtained from three replications (Figure 2). In this study the seasons and environment played a crucial role in crop yield. Previous studies have shown similar effects on hybrid maize varieties (Mohammed et al., 2016). Recent studies in other millets further substantiate our study. Comparable findings have been reported in pearl millet, where genotype-by-environment interactions had a strong influence not only on the yield but also on associated traits such as starch and mineral content. A recent G×E analysis highlighted the necessity of evaluating pearl millet across multiple seasons to identify stable and high-performing genotypes (Rouamba et al., 2024).

The above results provide insights into the relationships between yield in different seasons, which enhances the effectiveness for the development of elite traits. To ensure statistical reliability additional validation across larger datasets and environments is required (Silva et al., 2024; Preethi & Revadekar, 2013).

The yield distribution of 75 kodo millet germplasm during the Rabi and Kharif seasons is depicted in the box plots (Figure 3). Kharif yields were comparatively lower, with a median of approximately 151 g and an interquartile range of about 101–201 g. The minimum yield was 38.1 g (IPs240), while the maximum yield reached 405.2 g (IPs69). In contrast, the Rabi season yields were significantly higher, with a median of around 185 g and an interquartile range of 122–250 g. Outliers exceeding 575 g were observed in Rabi (e.g., IPs287, IPs69), highlighting the presence of elite high-yielding accessions. Other studies on kodo millet by (SR *et al.,* 2024) under the Kharif season found that treatments employing different organic amendments resulted in significant differences in yield among accessions, further supporting the presence of elite accessions with markedly higher yields under improved management and environmental conditions.

The higher medians, broader yield range, and superior outliers in Rabi indicate that this season provides more favourable conditions for millet cultivation compared to Kharif. These differences are likely attributed to optimal temperature, rainfall, and soil moisture availability during Rabi, which enhance crop growth and grain filling. Similar seasonal trends in yield advantage during Rabi have been reported in studies on small millet productivity, supporting the potential of Rabi cultivation for identifying germplasm with stable and superior yield performance.

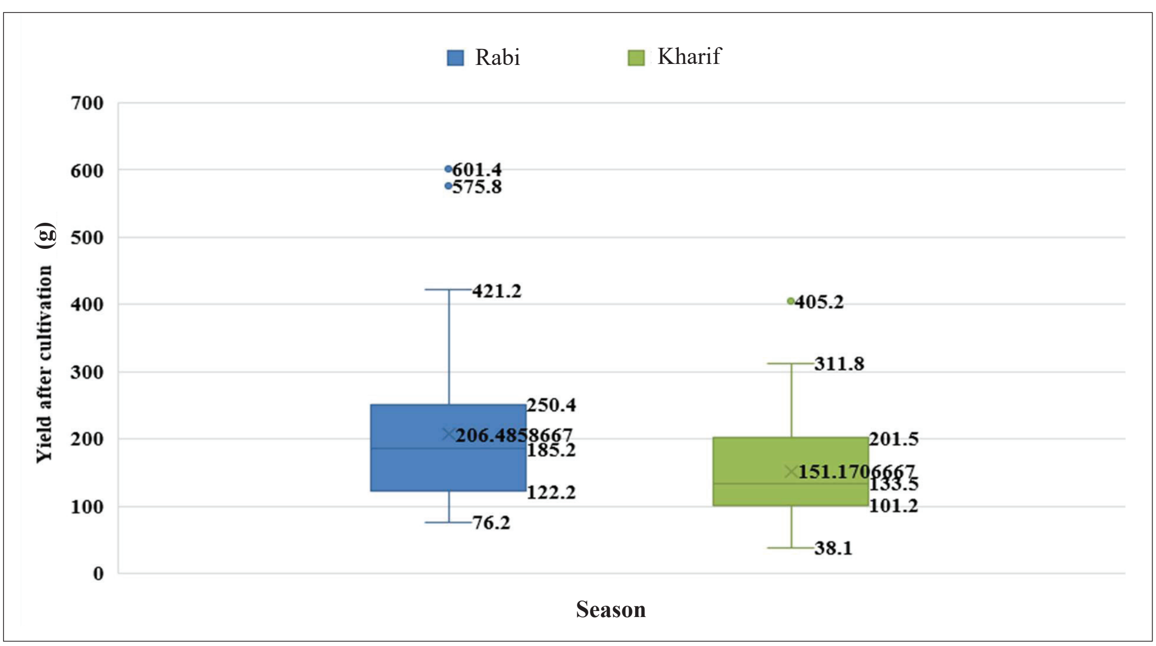

Figure 3: Box plot showing the yield distribution of kodo millet germplasm during Rabi and Kharif seasons.

Estimation and comparison of phytic acid analysis in husked and dehusked grains

Supplementary Table 2 shows the presence of phytic acid (PA) content in kodo millet grains grown during Rabi (R) – with husk (WH) & dehusked (DH) grains, and Kharif (K) – with husk (WH) & dehusked (DH) grains. The top five varieties with the highest PA content under Rabi-WH conditions were IPs 597 (1.0639 g), IPs 585 (0.9821 g), IPs 429 (0.9778 g), IPs 69 (0.9742 g), and IPs 208 (0.9726 g). Under Rabi-DH conditions, the highest PA content was recorded in IPs 597 (0.9913 g), followed by IPs 155 (0.8896 g), IPs 176 (0.8865 g), IPs 606 (0.8766 g), and IPs 319 (0.871 g). These current findings are consistent with previously reported results on dehusked grains of kodo millet (Singh et al., 2024a). The five highest phytic acid content varieties of Kharif-WH were IPs670s - 1.2695 g; IPs883 - 1.1949 g; IPs593 - 1.1834 g; IPs870 - 1.146 g; and IPs155 - 1.1256 g. The highest PA content germplasm varieties of Kharif-DH were IPs706 - 1.1174 g; IPs645 - 1.1057 g; IPs741 - 0.9907 g; IPs694 - 0.9127 g; and IPs329 - 0.8911 g. The highest PA content was shown in the germplasm variety IPs670-1.2695/100 g grown in Kharif-WH. The results indicated that the lowest values were recorded in Rabi-WH and were selected for further analysis. The ANOVA revealed significant differences in phytic acid content among kodo millet germplasms with husk across Rabi and Kharif seasons (Figure 4a). A strong genetic influence was observed (F = 5.96, P < 0.001), along with a significant seasonal effect (F = 8.17, P = 0.0055), indicating environmental conditions impact phytic acid accumulation. These findings highlight the potential for selecting low-phytic acid accessions and optimizing cultivation practices to enhance nutritional quality.

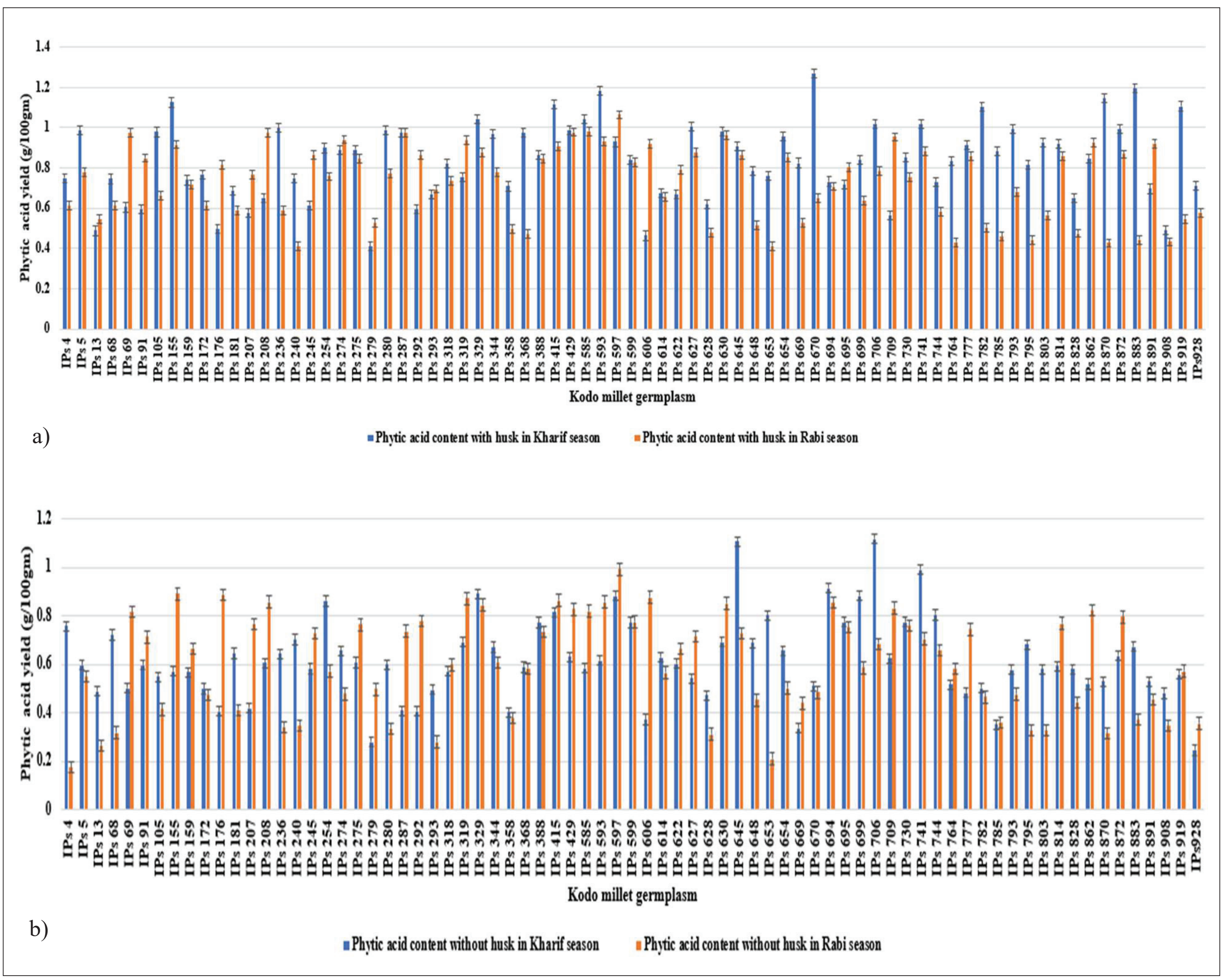

Figure 4: Comparison of phytic acid content of kodo millet grains in Kharif & Rabi season; a) with husk; b) dehusked

Significant differences were found for phytic acid assay analysis between the two seasons for the with-husk trait, as shown in Figure 4a. The five highest PA content varieties of R-WH husk and K-WH were considered to estimate the average PA content. From the results, the Kharif season has the PA content with an average mean value of 1.18 g/100 g, which is higher than the Rabi season with husk, with an average mean value of 0.99 g/100 g.

A two-way ANOVA on dehusked grains (Figure 4.b) confirmed genetic factors as the primary determinant of phytic acid content (F = 1312.53, P < 0.001), while seasonal variation had no significant impact (F = 1.266, P = 0.264). Consequently, seasonal variation no longer affects phytic acid content after husk removal (Tullu et al., 2001).

Table 1(a): Analysis of variance of phytic acid (g/100 g) in kodo millet grains with husk cultivated in different seasons

Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F- statistic	P-value	F critical value
Rows (Accession)	17.453	74	0.235	5.955	2.64E-13	1.469
Columns (season)	0.323	1	0.323	8.169	0.005	3.970

Table 1(b): Analysis of variance of phytic acid (g/100gm) in dehusked kodo millet grains cultivated in different seasons.

Source of variation	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F- statistic	P-value	F crit
Rows (Accession)	4002.858	75	53.37144	1312.531	0	1.465625
Columns (season)	0.051491	1	0.051491	1.266276	0.264055	3.968471

Although the two seasons exhibited distinct differences, the PA content in each accession was relatively similar, with minimal variation. The phytic acid content among the accessions in R-DH were: IPs 597 (1.0639 g/100 g) > IPs 585 (0.9821 g/100 g) > IPs 429 (0.9778 g/100 g) > IPs 69 (0.9742 g/100 g) > IPs 208 (0.9726 g/100 g). In the K-DH samples, the phytic acid content were: IPs 670s (1.2695 g/100 g), IPs 883 (1.1949 g/100 g), IPs 593 (1.1834 g/100 g), IPs 870 (1.146 g/100 g), and IPs 155 (1.1256 g/100 g). The results demonstrated that the lowest values, illustrated in Figures 4a. and 4b, were observed in Rabi-WH and selected for further analysis. Similar to our findings, Singh *et al.,* (2024b), reported that dehusked kodo millet grains contained significantly lower levels of phytic acid compared to whole grains, while processing consistently reduces its content and improves mineral bioavailability (Shobana *et al.,* 2013). These studies corroborate our results that Rabi with husked grains had lower PA levels than Kharif, but the seasonal effect diminished after husk removal. Our results showing seasonal variation in phytic acid content of kodo millet align with recent findings that phytate levels in cereals are significantly influenced by cropping system and harvest year (Korge *et al.,* 2025)

Effect of soaking intervals and germination time on phytic acid content reduction using RSM:

The response surface methodology (RSM) analysis, performed using Design-Expert software version 13, demonstrated that both soaking and germination significantly influenced phytic acid degradation in kodo millet (Sharma *et al.,* 2016).

Effect of soaking in kodo millet

The model F-value of 362.26 confirms statistical significance, with only a 0.01% probability of occurring due to noise. P-values < 0.0500 indicate significant model terms. The predicted R^2 (0.9804) aligns well with the adjusted R^2 (0.9960), with a difference < 0.2, confirming model reliability.

The adeq precision ratio of 59.716 indicates a strong signal-to-noise ratio (Table 4b) (Zhang & Bai, 2014). Analysis by RSM and the 3D response surface plot (Figure 5) show that soaking time, temperature, and pH significantly impact phytic acid degradation. The optimized conditions of 20.63 h soaking, 38.49 °C, and pH 4.08 resulted in 48.11% degradation, aligning with high-degradation regions in the 3D plot. Extended soaking (18–24 h) and elevated temperatures (~40–45 °C) enhance enzymatic activity and phytic acid diffusion. Additionally, acidic conditions (pH ~4) promote phytase activity, accelerating phytic acid breakdown *(Lu* et al., 2024).

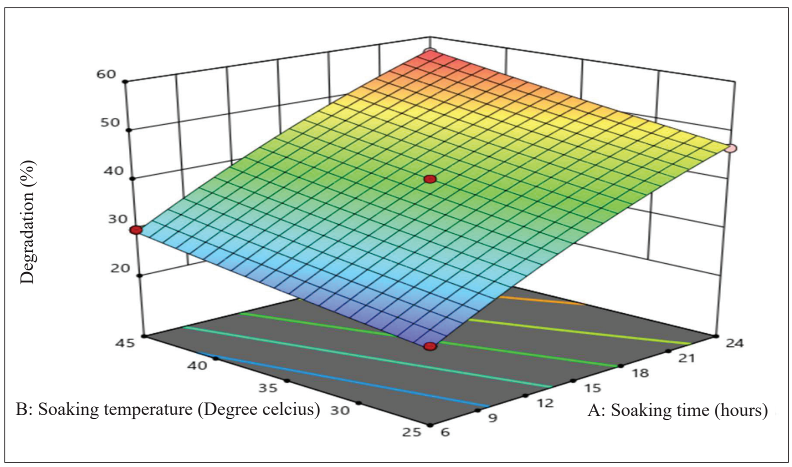

Figure 5: 3D surface plot of soaking conditions on phytic acid reduction.

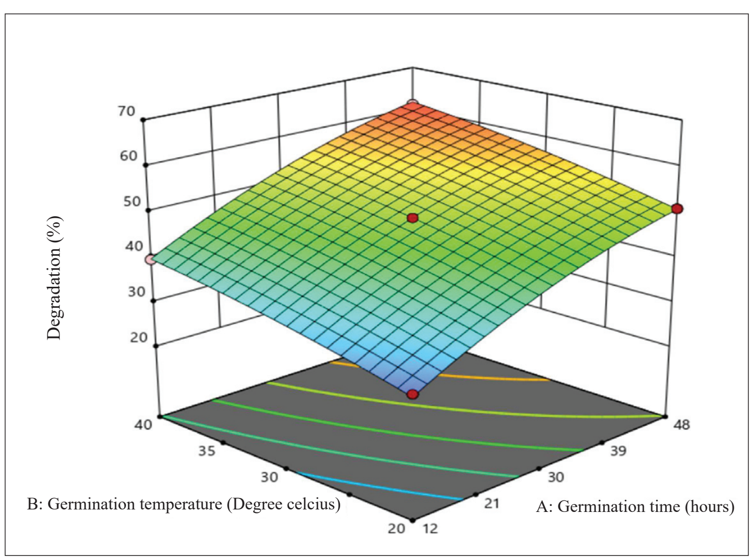

Figure 6): 3D surface plot of germination conditions on phytic acid reduction.

Effect of germination on kodo millet

The model F-value of 493.01 confirms statistical significance, with only a 0.01% probability of occurring due to noise. The P-value of < 0.0500 indicates significant model terms. The predicted R^2 (0.9856) closely aligns with the adjusted R^2 (0.9971), confirming model reliability. The adeq precision ratio of 69.522 indicates a strong signal (Gupta et al., 2015b).

An RSM analysis and the 3D response surface plot (Figure 6) show that germination time, temperature, and moisture significantly influence phytic acid degradation. The optimized conditions of 36.29 h for germination, at 27.29 °C, and 65.18% moisture achieved a 53.97% reduction in phytic acid, as determined by the box-behnken design (BBD). Extended germination (30–48 hours) and moderate temperatures (~27–40 °C) enhance phytase activity, improving mineral bioavailability. However, excessive moisture or prolonged germination may not yield further benefits (Maldonado-Alvarado et al., 2023).

These findings confirm that controlled germination effectively reduces phytic acid levels, enhancing the nutritional quality of plant-based foods.

Unlike other cereals and grains, millets can be germinated easily and have significantly more nutrients, boosting the bioavailability of micro nutrients (Harshitha et al., 2024). This, further increases the intake of vitamins, and other supplementary foods (Senthilnathan & Muthusamy, 2022).

As a result, the body can absorb minerals such as iron and zinc more efficiently, promoting improved digestion. According to a review, sprouted grains have a lower glycaemic index (Lestari et al., 2017), making them an excellent choice for individuals who need to manage their blood sugar levels regularly (Ikram et al., 2021). Parallel studies on germinated legumes contained more essential amino acids, which are the building blocks of protein that help repair muscles and strengthen your immune system (Semba et al., 2021). Sprouted grains are high in fibre, which helps maintain a healthy gut and encourages regular bowel movements. They can also reduce the risk of digestive problems, such as irritable bowel syndrome (IBS). The current study revealed that soaking and germination of kodo millets can decrease antinutrient phytic acid and increase micronutrient absorption.

CONCLUSION

This study highlights the potential of optimizing soaking and germination conditions to reduce the phytic acid content in kodo millet, thereby improving mineral bioavailability. The findings confirm that genetic factors dominate phytic acid levels, while seasonal variation has minimal influence after dehusking. Optimized soaking conditions (20.63 h, 38.49 °C, pH 4.08) alone accounted for a major reduction in phytic acid (48.11%), whereas soaking followed by germination (36.29 h, 27.29 °C, 65.18% moisture) resulted in a total reduction of 53.97%. This indicates that germination by itself contributes only a relatively small additional reduction beyond the effect achieved through soaking. These results provide valuable insights into improving the nutritional profile of kodo millet, making it a more accessible and functional food ingredient. Additionally, integrating these optimized techniques with modern food processing methods, such as puffing and popping, can enhance the commercial viability of kodo millet as a ready-to-eat product. The findings of this study can also aid in the pre-selection and release of elite cultivars with improved yield and nutritional properties, contributing to sustainable millet production and helping the way forward for future research on new genetic variations and a promising crop for food security.

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Supplementary Table 01: Estimates of crop yield of 75 Kodo millet accessions cultivated during the Rabi and Kharif seasons

Accession Identifier (Core germplasm samples)	Yield (Rabi season, g)	Yield (Kharif season, g)
IPs 4	185.2±0.01	101.4±0.23
IPs 5	166.2±0.07	202±0.01
IPs 13	291.5±0.17	71.5±0.74
IPs 68	271.6±0.09	107.6±0.17
0.IPs 69	601.4±0.02	405.2±0.14
IPs 91	108.4±0.12	201.5±0.20
IPs 105	321.4±0.05	133.5±0.19
IPs 155	157.6±0.05	241.2±0.05
IPs 159	108.8±0.04	117.2±0.85
IPs 172	222.1±0.02	201.5±0.41
IPs 176	122.78±0.18	58.2±0.73
IPs 181	100.2±0.12	211.5±0.12
IPs 207	169.4±0.26	57.2±0.33
IPs 208	154.2±0.08	117.2±0.61
IPs 236	76.2±0.06	231.5±0.09
IPs 240	122.2±0.43	38.1±0.06
IPs 245	101.6±0.65	94.6±0.71
IPs 254	95.28±0.01	79.5±0.69
IPs 274	125.2±0.02	112.5±0.12
IPs 275	105±0.81	94.2±0.35
IPs 279	175.36±0.43	204.2±0.27
IPs 280	222.8±0.06	151.2±0.38
IPs 287	575.8±0.03	211.4±0.01
IPs 292	201.44±0.18	162.4±0.11
IPs 293	407.25±0.43	197.5±0.75
IPs 318	211.87±0.08	105.4±0.85
IPs 319	187.2±0.03	214.2±0.30
IPs 329	206.3±0.05	116.4±0.27
IPs 344	309.8±0.30	155.4±0.01
IPs 358	247.1±0.43	107.2±0.01
IPs 368	202.66±0.01	95.4±0.14
IPs 388	190.5±0.28	77.1±0.76
IPs 415	129±0.21	241.2±0.30
IPs 429	144.2±0.22	211.5±0.08
IPs 585	157.5±0.45	98.5±0.25
IPs 593	119.1±0.32	144.5±0.86
IPs 597	258.2±0.12	164.2±0.19
IPs 599	106.4±0.01	58.4±0.06
IPs 606	199.8±0.32	166.9±0.44
IPs 614	217±0.62	111.2±0.33

Accession Identifier (Core germplasm samples)	Yield (Rabi season, g)	Yield (Kharif season, g)
IPs 622	313±0.75	144.5±0.71
IPs 627	421.2±0.11	221±0.47
IPs 628	175.5±0.17	103.8±0.22
IPs 630	102.4±0.26	68.4±0.09
IPs 645	411.2±0.35	121.5±0.22
IPs 648	233.1±0.81	119.4±0.20
IPs 653	404.2±0.22	200.1±0.01
IPs 654	201.2±0.89	96.4±0.22
IPs 669	300±0.18	133.5±0.17
IPs 670	124.5±0.80	224.7±0.73
IPs 694	181.2±0.85	119.4±0.19
IPs 695	211.3±0.75	59.7±0.08
IPs 699	205.1±0.83	144.5±0.05
IPs 706	99.4±0.62	188.3±0.85
IPs 709	311.4±0.01	211.5±0.89
IPs 730	97.2±0.42	177.6±0.01
IPs 741	214.1±0.47	188.2±0.07
IPs 744	109.2±0.27	101.2±0.72
IPs 764	311.4±0.30	84.5±0.83
IPs 777	94.7±0.04	199.5±0.21
IPs 782	154.8±0.09	161.5±0.19
IPs 785	164.7±0.11	201.2±0.11
IPs 793	124.2±0.06	111.4±0.05
IPs 795	97.2±0.27	171.4±0.73
IPs 803	224.1±0.05	116.5±0.57
IPs 814	250.4±0.42	116.5±0.60
IPs 828	414.2±0.16	311.8±0.23
IPs 862	301.2±0.08	299.4±0.15
IPs 870	277.1±0.72	114.5±0.09
IPs 872	166.7±0.04	97.8±0.33
IPs 883	172.6±0.69	87.5±0.41
IPs 891	103.4±0.23	203.4±0.20
IPs 908	94.8±0.03	241.3±0.52
IPs 919	224.8±0.20	100±0.64
IPs928	119.4±0.09	224.6±0.02

Supplementary Table 02: Estimation of phytic acid (g/100 g) in kodo millet grains with husk cultivated in the two seasons

Accession Identifier (Core germplasm samples)	Rabi season - kodo millet grains with husk - Phytic acid (g/100 g) Grains with Husk	Kharif season - kodo millet grains with husk - Phytic acid (g/100 g) Grains with Husk
IPs04	0.6120±0.003	0.7499±0.002
IPs05	0.7810±0.014	0.9881±0.002
IPs13	0.5450±0.012	0.4879±0.037
IPs 68	0.6121±0.002	0.7450±0.009
IPs69	0.9742±0.006	0.6042±0.011
IPs91	0.849±0.010	0.5950±0.058
IPs105	0.6637±0.012	0.9784±0.043
IPs155	0.9166±0.055	1.1256±0.007
IPs159	0.7157±0.014	0.7389±0.024
IPs172	0.6124±0.003	0.7668±0.012
IPs176	0.8131±0.023	0.4945±0.082
IPs181	0.5896±0.006	0.6869±0.022
IPs207	0.7652±0.010	0.5782±0.038
IPs208	0.9726±0.014	0.6504±0.052
IPs236	0.5865±0.014	0.9986±0.027
IPs240	0.4108±0.007	0.7495±0.031
IPs245	0.8667±0.006	0.6135±0.022
IPs254	0.7573±0.041	0.8983±0.016
IPs274	0.9409±0.011	0.8887±0.039
IPs275	0.8455±0.009	0.8908±0.035
IPs279	0.5271±0.011	0.4092±0.041
IPs280	0.7708±0.071	0.9872±0.035
IPs287	0.9767±0.071	0.9725±0.007
IPs292	0.8640±0.002	0.5932±0.012
IPs293	0.6957±0.003	0.6703±0.028
IPs318	0.7340±0.062	0.8220±0.007
IPs319	0.9361±0.019	0.7564±0.070
IPs329	0.8772±0.024	1.0439±0.086
IPs344	0.7778±0.006	0.9699±0.023
IPs358	0.4981±0.005	0.7087±0.018
IPs368	0.4710±0.021	0.9725±0.013
IPs388	0.8449±0.047	0.8644±0.006
IPs415	0.9066±0.041	1.1165±0.032
IPs429	0.9778±0.074	0.9876±0.026
IPs585	0.9821±0.011	1.0407±0.039
IPs593	0.9344±0.014	1.1834±0.002
IPs597	1.0639±0.051	0.9295±0.028
IPs599	0.8276±0.041	0.8386±0.034
IPs606	0.9191±0.096	0.4634±0.034
IPs614	0.6575±0.013	0.6726±0.049

Accession Identifier (Core germplasm samples)	Rabi season - kodo millet grains with husk - Phytic acid (g/100 g) Grains with Husk	Kharif season - kodo millet grains with husk - Phytic acid (g/100 g) Grains with Husk
IPs622	0.7913±0.057	0.6668±0.023
IPs627	0.8746±0.050	1.0078±0.036
IPs628	0.4766±0.002	0.6177±0.007
IPs630	0.9650±0.018	0.9813±0.038
IPs645	0.8639±0.007	0.9085±0.024
IPs648	0.5134±0.008	0.7842±0.047
IPs653	0.4113±0.013	0.7593±0.014
IPs654	0.8513±0.051	0.9557±0.056
IPs669	0.5271±0.054	0.8240±0.018
IPs670	0.6526±0.021	1.2695±0.017
IPs694	0.7082±0.024	0.7320±0.005
IPs695	0.8019±0.041	0.7171±0.020
IPs699	0.6387±0.008	0.8376±0.007
IPs706	0.7839±0.07	1.0166±0.008
IPs709	0.9534±0.01	0.5659±0.031
IPs730	0.7523±0.091	0.8501±0.084
IPs741	0.8847±0.024	1.0170±0.007
IPs744	0.5839±0.054	0.7293±0.067
IPs764	0.4318±0.047	0.8322±0.024
IPs777	0.8590±0.061	0.9119±0.011
IPs782	0.5049±0.038	1.1048±0.028
IPs785	0.4586±0.024	0.8824±0.032
IPs793	0.6807±0.081	0.9917±0.013
IPs795	0.4439±0.007	0.8140±0.004
IPs803	0.5644±0.004	0.9273±0.008
IPs814	0.8590±0.051	0.9209±0.002
IPs828	0.4752±0.011	0.6508±0.025
IPs862	0.9263±0.027	0.8450±0.039
IPs870	0.4264±0.034	1.1460±0.023
IPs872	0.8677±0.033	0.9917±0.046
IPs883	0.4428±0.051	1.1949±0.026
IPs819	0.9219±0.041	0.6965±0.045
IPs908	0.4324±0.034	0.4900±0.084
IPs919	0.5458±0.017	1.1061±0.008
IPs928	0.5774±0.061	0.7126±0.031

Supplementary Table 03: Estimation of phytic acid (g/100 gm) in dehusked kodo millet grains cultivated in the two seasons

Accession Identifier (Core germplasm samples)	Rabi season - dehusked kodo millet grains- Phytic acid (g/100 g)	Kharif season - dehusked kodo millet grains - Phytic acid (g/100 g)
IPs04	0.1742±0.021	0.7578±0.042
IPs05	0.5491±0.011	0.5948±0.005
IPs13	0.2637±0.043	0.4878±0.018
IPs 68	0.3166±0.007	0.7207±0.023
IPs69	0.8157±0.017	0.5007±0.044
IPs91	0.7124±0.029	0.5950±0.058
IPs105	0.4131±0.022	0.5471±0.06
IPs155	0.8896±0.004	0.5711±0.059
IPs159	0.6652±0.027	0.5663±0.002
IPs172	0.4726±0.027	0.4981±0.023
IPs176	0.8865±0.061	0.4051±0.050
IPs181	0.4108±0.024	0.6449±0.084
IPs207	0.7667±0.051	0.4175±0.018
IPs208	0.8573±0.081	0.6051±0.002
IPs236	0.3409±0.061	0.6421±0.020
IPs240	0.3455±0.017	0.7019±0.009
IPs245	0.7271±0.084	0.5817±0.015
IPs254	0.5708±0.034	0.8611±0.016
IPs274	0.4767±0.024	0.6545±0.008
IPs275	0.7640±0.011	0.6063±0.022
IPs279	0.4957±0.061	0.2784±0.013
IPs280	0.3340±0.071	0.5988±0.026
IPs287	0.7361±0.005	0.4071±0.007
IPs292	0.7772±0.007	0.4046±0.009
IPs293	0.2778±0.009	0.4949±0.020
IPs318	0.5981±0.611	0.5710±0.027
IPs319	0.8710±0.034	0.6911±0.019
IPs329	0.8449±0.041	0.8911±0.021
IPs344	0.6066±0.052	0.6701±0.081
IPs358	0.3778±0.034	0.4014±0.081
IPs368	0.5821±0.072	0.5881±0.029
IPs388	0.7344±0.011	0.7715±0.031
IPs415	0.8639±0.034	0.8154±0.066
IPs429	0.8276±0.027	0.6301±0.041
IPs585	0.8191±0.067	0.5841±0.024
IPs593	0.8575±0.094	0.6141±0.517
IPs597	0.9913±0.037	0.8814±0.047
IPs599	0.7746±0.051	0.7715±0.051
IPs606	0.8766±0.017	0.3711±0.081
IPs614	0.5650±0.005	0.6271±0.099

Accession Identifier (Core germplasm samples)	Rabi season - dehusked kodo millet grains- Phytic acid (g/100 g)	Kharif season - dehusked kodo millet grains - Phytic acid (g/100 g)
IPs622	0.6639±0.064	0.6017±0.044
IPs627	0.7134±0.081	0.5418±0.841
IPs628	0.3113±0.014	0.4710±0.241
IPs630	0.8513±0.002	0.6914±0.841
IPs645	0.7271±0.081	1.1057±0.571
IPs648	0.4526±0.009	0.6881±0.441
IPs653	0.2082±0.051	0.8021±0.755
IPs654	0.5019±0.080	0.6551±0.141
IPs669	0.4387±0.043	0.3342±.0841
IPs670	0.4839±0.067	0.5084±0.084
IPs694	0.8534±0.019	0.9127±0.035
IPs695	0.7523±0.046	0.7751±0.051
IPs699	0.5847±0.039	0.8821±0.049
IPs706	0.6839±0.051	1.1174±0.057
IPs709	0.8318±0.067	0.6227±0.117
IPs730	0.7590±0.003	0.7751±0.077
IPs741	0.7049±0.191	0.9907±0.025
IPs744	0.6586±0.040	0.8041±0.051
IPs764	0.5807±0.028	0.5160±0.078
IPs777	0.7439±0.084	0.4814±0.060
IPs782	0.4644±0.064	0.5001±0.054
IPs785	0.3590±0.028	0.3511±0.081
IPs793	0.4752±0.024	0.5771±0.035
IPs795	0.3263±0.030	0.6804±0.041
IPs803	0.3264±0.051	0.5803±0.011
IPs814	0.7677±0.071	0.5914±0.011
IPs828	0.4428±0.039	0.5803±0.026
IPs862	0.8219±0.049	0.5175±0.028
IPs870	0.3134±0.061	0.5296±0.018
IPs872	0.7948±0.017	0.6335±0.007
IPs883	0.3714±0.077	0.6731±0.033
IPs819	0.4544±0.054	0.5285±0.011
IPs908	0.3461±0.011	0.4801±0.015
IPs919	0.5714±0.040	0.5568±0.013
IPs928	0.3548±0.066	0.2450±0.038