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Kernel density as a population-screening tool for low phytic acid content in maize (*Zea mays* L.)

S.M. Indhu[1], R. Ravikesavan[1], N. Senthil[2], C. Thiyagarajan[3], F. Hossain[4] and A.J. Joel[2]*

[1]*Department of Genetics and Plant Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641 003, India*
[2]*Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, India*
[3]*Department of Sericulture, Forest college and Research Institute, Tamil Nadu Agricultural University, Mettupalayam – 641 301, India*
[4]*Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India*

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***Corresponding Author Email :** jnjoel@gmail.com ***ORCiD:** https://orcid.org/0000-0002-7994-7365

Abstract

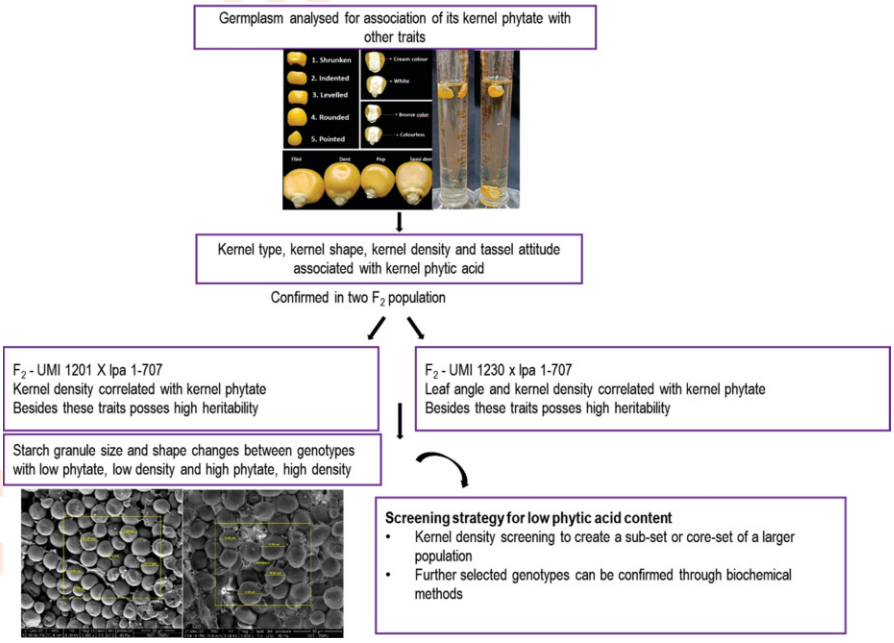
Aim: The present study was performed to find association of morphological traits with kernel phytic acid.

Methodology: A set of 20 qualitative phenotypic observations were studied in 29 diverse germplasms and two F_2 populations. Further correlated traits were quantitatively verified for their correlation and heritability.

Results: Maize kernels characterized by low phytate content correlated with reduced seed density traits. Further insights from scanning electron microscopic studies revealed alterations in the distribution pattern of starch granules, among low and high phytate genotypes. Heritability studies indicated a strong heritable nature of the traits *viz.*, kernel density, inorganic phosphorous and phytic acid, and were found to be inter correlated.

Interpretation: Intercorrelation and heritable nature of the trait is viewed as a 'risk index' that directly influences the success of genetic selection for the phytic acid trait which is highly complex and involves more laboratory experimentation. The selected genotypes may be further screened using biochemical analysis for confirmation.

Key words: Correlation, F_2 population, Germplasm, Kernel density, Maize, Phytic acid



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Introduction

Maize serves as a significant source of nutrition and contributes valuable phytochemical compounds *viz.,* carotenoids, phenolic compounds, zein, lectin and resistant starch and phytosterols (Rouf Shah *et al.,* 2016). Maize is a major energy source in animal nutrition. Industrial application includes production of corn-starch, cereals, adhesives, sweeteners and alcohol (Ranum *et al.*, 2014). Corn, one among the prominent cereal crops, plays a vital role in producing a diverse range of products for human consumption, animal feed and industrial applications. In spite of its beneficial properties, populations that rely on maize-based diet, experience acute iron and zinc deficiencies (Yathish *et al.,* 2021). Because it possesses an anti-nutritional compound called phytic acid. Phytate is a composite of various cationic salts derived from phytic acid, specifically myo-inositol hexakisphosphate (dihydrogen phosphate). It serves as the principal reservoir of phosphorous in seeds and grains, constituting as much as 85% of the overall seed phosphorus content and representing a substantial proportion of seed dry weight (Lott, 1994). Grains play a crucial role in animal nutrition worldwide. However, the phosphorus bound phytate, a significant component of grains, remains inaccessible to monogastric animals like pigs, poultry and fish. The negative charge of phytic acid strongly binds to essential metallic cations such as Ca, Fe, Mg and Zn, rendering them nutritionally unavailable (Cromwell and Coffey, 1991). Additionally, undigested phytate phosphorus is excreted, which leads to eutrophication.

To mitigate these issues, animal diets can be enriched with phytase (Cromwell, 2005). However, it adds to cost of production. Alternatively, low phytate maize can be incorporated to address these challenges. Various approaches have been employed by various institutes at global level to develop low phytic acid maize. It includes mutagenesis (Raboy and Gerbasi, 1996; Raboy *et al.,* 2000 and Pilu *et al.,* 2003), marker assisted selection approaches (Sureshkumar *et al.,* 2015; TamilKumar *et al.,* 2014) and germplasm screening (Lorenz *et al.,* 2008; Chiangmai *et al.,* 2011; Pramitha *et al.,* 2020 and Yathish *et al.,* 2021). However, screening these larger populations for low phytic acid using colorimetry-based techniques like High Inorganic Phosphorous assay, Wades assay and Davies and Reid method are laborious and time-consuming processes. Besides, interrelationships among kernel density, inorganic phosphorus (IP), and phytic acid content in seeds are evident across several crops, including sorghum, wheat, mungbean and soybean. Studies on sorghum identified genotypes with low phytic acid and high yield, impact mineral bioavailability (Badigannavar *et al.*, 2015). Wheat breeding lines exhibited wide variation in phytic acid content, with a negative correlation observed between phytic acid and hundred kernel weight (HKW) (Shitre *et al.*, 2015; Dhole *et al.*, 2015). Similarly, mungbean genotypes with low phytic acid demonstrated potential for enhanced nutrient assimilation, showing a negative correlation between phytic acid and seed size (Badigannavar *et al.*, 2015). Soybean germplasm studies revealed negative correlations between seed physical characteristics and phytic acid content, suggesting the potential for selecting low phytate lines (Abirami *et al.*, 2014). These findings emphasize the genetic control and potential for breeding programs to develop seeds with desirable nutritional profiles by targeting these interrelated traits. In these contexts, the present study was conducted to understand the morphological changes associated with kernel phytate. It could provide insights into developing an effective screening strategy.

Materials and Methods

A total of 29 maize genotypes were evaluated during *Rabi* 2020-21 (November – February) at No. 2G New area farm, Department of Millets, TNAU, Coimbatore for twenty plant morphological traits based on Maize descriptors (IBPGR, 1991). The list of genotypes and source from which it was obtained are

Table 1: List of genotypes and source from which it was obtained

Genotype	Source	Genotypes	Source
BN 1048	DOM, TNAU, Coimbatore.	*lpa* 1- 708	IARI, New Delhi
BN 1053	DOM, TNAU, Coimbatore.	UMI 1003	DOM, TNAU, Coimbatore.
BN 1076	DOM, TNAU, Coimbatore.	UMI 1131	DOM, TNAU, Coimbatore.
BN 1118	DOM, TNAU, Coimbatore.	UMI 1151	DOM, TNAU, Coimbatore.
BN 1131	DOM, TNAU, Coimbatore.	UMI 1153	DOM, TNAU, Coimbatore.
BN 1253	DOM, TNAU, Coimbatore.	UMI 1201	DOM, TNAU, Coimbatore.
BN 1258	DOM, TNAU, Coimbatore.	UMI 1230	DOM, TNAU, Coimbatore.
BN 1265	DOM, TNAU, Coimbatore.	UMI 142	DOM, TNAU, Coimbatore.
BN 2243	DOM, TNAU, Coimbatore.	UMI 29	DOM, TNAU, Coimbatore.
BN 426	DOM, TNAU, Coimbatore.	UMI 298	DOM, TNAU, Coimbatore.
BN 71806	DOM, TNAU, Coimbatore.	UMI 346	DOM, TNAU, Coimbatore.
BN 9119	DOM, TNAU, Coimbatore.	UMI 504	DOM, TNAU, Coimbatore.
HN 1075	DOM, TNAU, Coimbatore.	UMI 692	DOM, TNAU, Coimbatore.
HN 1082	DOM, TNAU, Coimbatore.	UMI 96	DOM, TNAU, Coimbatore.
lpa 1 -707	IARI, New Delhi		

DOM: Department of Millets; TNAU: Tamil Nadu Agricultural University; IARI: Indian Agricultural Research Institute

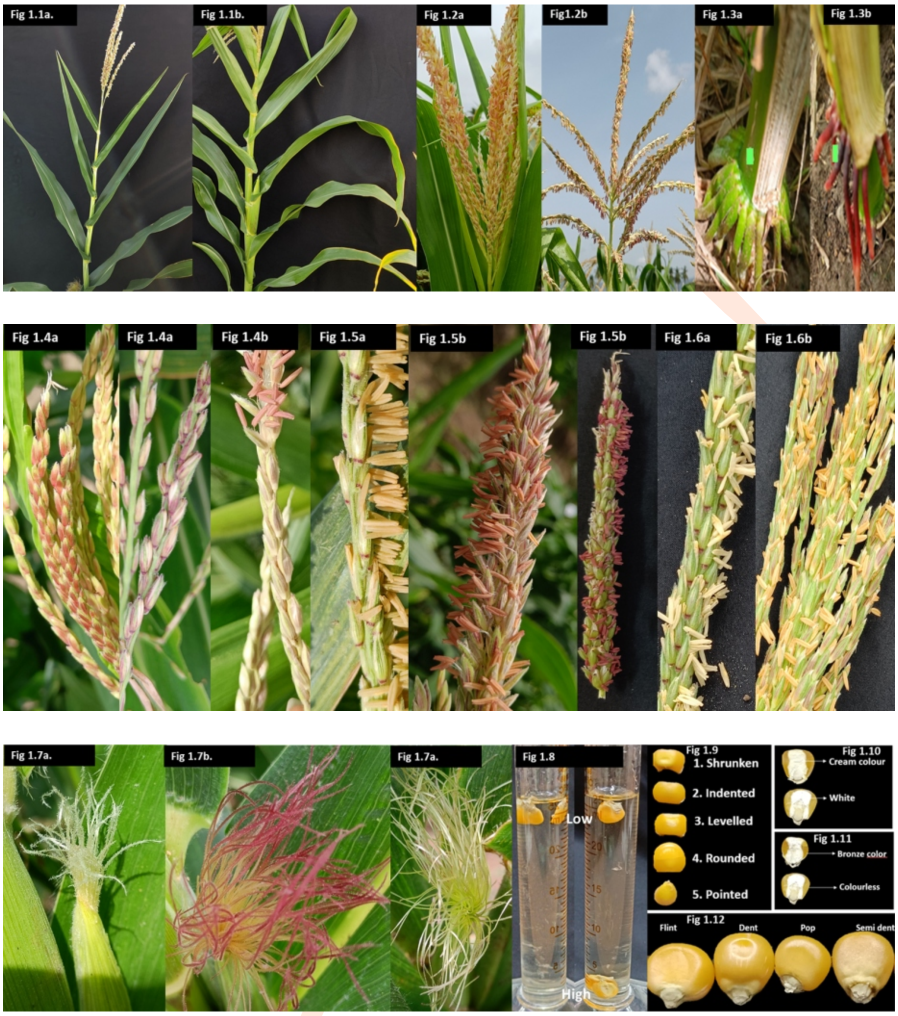

Fig. 1: Variability among qualitative traits 1.1-Leaf angle and attitude a.Narrow+erectb.wide+droopy, 1.2 -Tassel angle & attitude a. narrow and straight b. wide and curved, 1.3- Stem brace root anthocyanin pigmentation a. Absent b. Present 1.4 – Tassel anthocyanin pigmentationa. present b. absent, 1.5- Anther coloura. Not pigmented b. pigmented, 1.6 -Glume base coloura. Pigmented b. not pigmented, 1.7 Silk coloura. Not pigmented b. pigmented, 1.8 Kernel density, 1.9 Kernel type, 1.10 Endosperm colour, 1.11 Aleurone colour, 1.12 Kernel shape.

presented in Table 1. Out of 20 traits, four vegetative traits *viz.,* leaf angle, leaf attitude, stem brace root colour and leaf sheath colour were measured. The remaining sixteen reproductive traits were further classified as inflorescence traits and kernel traits. The inflorescence traits studied were: tassel anthocyanin pigmentation, glume colour, glume base colour, silk colour, anther colour, spikelet density, tassel angle and tassel attitude. The kernel traits studied were: kernel type, kernel colour, kernel shape, pericarp colour, aleurone colour, endosperm colour, kernel density and kernel phytate content. The data obtained was subjected to correlation analysis to determine the relationship of the traits with kernel phytate. Further, the correlated traits were used for grouping genotypes using cluster analysis. Genetically distant genotypes viz., *lpa 1*-707, UMI 1201 and UMI 1230 were selected from diverse cluster. They were subjected to hybridization programme which constituted two crosses *viz.,* UMI 1201 × *lpa 1*-707 and UMI 1230 × *lpa 1*-707 during *Kharif* 2021 (April – July). The F_1 population was raised and selfed during *Kharif* (August – November) 2021. The F_2 population was raised and evaluated to validate the relationship of variables tested in the germplasm with kernel phytate level during *Rabi* 2021-2022. The kernel density parameter was estimated using sucrose solution as suggested by Landoni *et al.* (2013). It was further quantified by calculating floaters percentage as suggested by Crawford and Gould (1957). Kernel phytate was estimated using HIP assay (S1) as suggested by Raboy and Gerbasi (1996).

Statistical analysis was performed using corrplot (correlation), factoextra (cluster) and ggplot (chi-square test) in R.4.2.0. Variability estimates *viz.,* variances (Lush, 1940), heritability (%) (Lush, 1940) and genetic advance as percent of mean (%) (Johnson,1955) were calculated using MS-excel.

Results and Discussion

Morphological characters, including leaf characters, tassel characteristics and kernel characteristics play a pivotal role in identifying and classifying maize genotypes. In breeding, these traits are crucial for selecting plants with desirable features. Morphological studies also aid in assessing genetic diversity within maize populations, offering insights into the genetic makeup and relationships between different genotypes. The morphological variability exhibited among genotypes are depicted in Fig. 1. It implies the sufficient variation present among the genotypes for further study. Stewart *et al.* (2003) reported that the leaf angle and leaf attitude were the key traits involved in deciding maize plant architecture. Upright leaf arrangement and narrow angle of orientation minimizes shading effect on its neighbouring plants. It increases the photosynthetic efficiency of plants and reflects in final yield. Conversely, the plants with droopy leaves and a wider angle of orientation are not well-suited for high-density planting. This observation was correlated with other traits such as tassel angle, tassel attitude and spikelet density. Most of the plants with upright and narrow leaf angle were found to have upright tassel branches and narrow angle between tassel branches with dense spikelet. The interplay of these characteristics highlights the importance of leaf architecture in optimizing planting density, consequently influencing the overall performance and yield of maize plants. At the same time, these traits were not correlated with kernel phytate level. Pigmentation in the brace roots, glume, anther and silk depends on anthocyanin accumulation in the cells and it was found to be tissue specific.

Anthocyanin accumulation in various tissues plays a role in numerous physiological functions, including the modulation of hormone responses, providing protection against damage from ultraviolet radiation, and eliciting defence responses to both biotic and abiotic stresses (Ithal and Reddy, 2004). Glume colour was correlated with tassel anthocyanin colour, leaf sheath colour, and endosperm colour. This relationship pattern was purely dependent on genotype and environmental factors, particularly light intensity (Ithal and Reddy, 2004). The relationship of these traits to phytate content were found minimal and in negative direction. Only four traits *viz.,* kernel density, kernel shape, kernel type, and tassel attitude showed significant positive correlation with kernel phytate levels. Landoni *et al.* (2013) studied lpa 1 mutation using lpa 1 mutants and they reported that lpa 1 mutations produced pleiotropic effects on seed density, ion content and antioxidant compounds in maize kernels. These effects were not directly related to phytic acid synthesis pathway.

Table 2: List of genotypes and kernel characteristics of genotypes in each of the clusters

Cluster	No. of genotypes	Name of genotypes	Kernel characteristic features
Cluster 1	4	UMI 298, UMI 1131, BN 71806, UMI 692	High phytate, high density, semi – flint, semi – dent
Cluster 2	10	UMI 1153, BN 1048, BN 9119, BN 1265, BN 1131, UMI 29 HN 1075, BN 426, UMI 1003, UMI 1230	High phytate, high density, levelled and rounded, flint and pop type
Cluster 3	13	UMI 1151, HN 1082, BN 1118, BN 2243, UMI 142, UMI 96 BN 1076, BN 1258, UMI 504, BN 1053, BN 1253, UMI 346 UMI 1201	High phytate, high density, flint and semi-flint, indented
Cluster 4	2	lpa 1 -707, lpa 1- 708	Low phytate, low density, pointed and pop type

Table 3: Distinguishing features observed among parents involved in the study

Morphological characteristics	UMI 1201	*lpa 1* -707	UMI 1230
Leaf angle	Narrow	Wide	Wide
Leaf attitude	Erect	Droopy	Droopy
Tassel anthocyanin	Present	Absent	Absent
Glume base	Absent	Present	Absent
Spikelet density	Dense	Sparse	Dense
Tassel angle	Narrow	Wide	Wide
Tassel attitude	Straight	Curved	Curved
Silk colour	Pigmented	Not pigmented	Not pigmented
Kernel type	Flint	Pop	Flint
Kernel shape	Indented	Pointed	Rounded
Kernel density	High	Low	High
Kernel phytate	High	Low	High

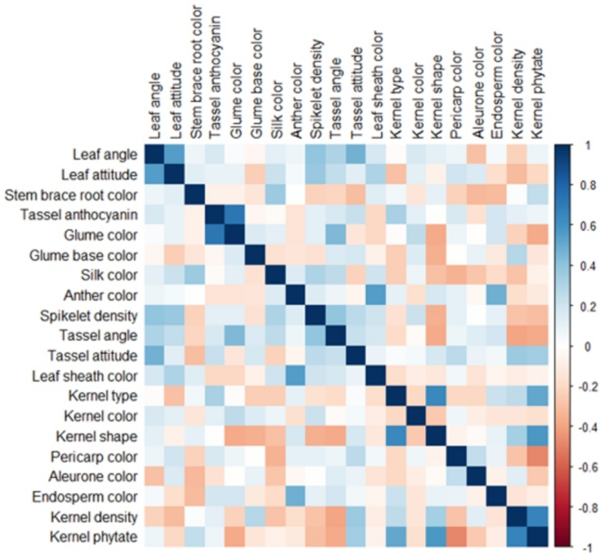

Fig. 2: Correlation using qualitative traits among the germplasm set.

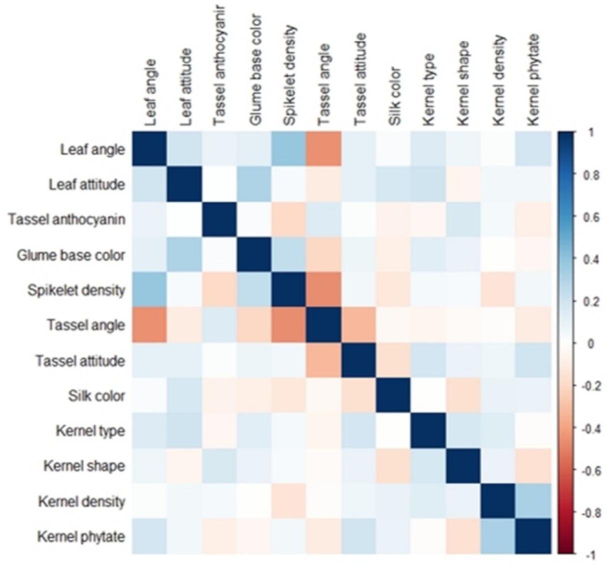

Fig. 3: Correlation among F_2 individuals of UMI 1201 ×*lpa* 1-707.

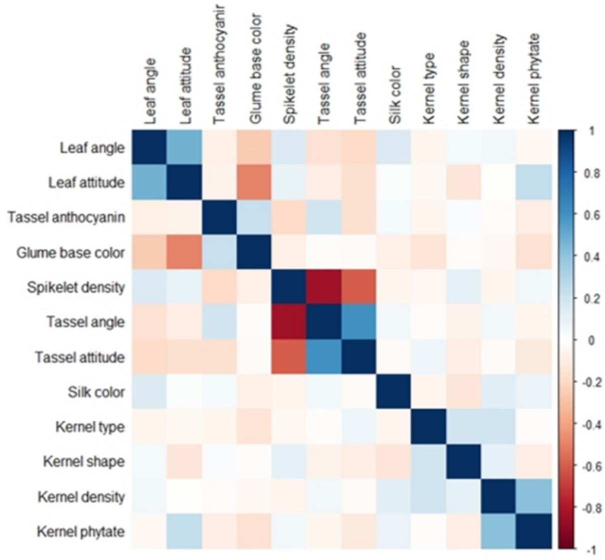

Fig. 4: Correlation among F_2 individuals of UMI 1230 ×*lpa* 1-707.

Lydia *et al.* (2020) observed positive correlation between phytic acid content and traits such as plant stand, germination percentage, kernel yield, ear length, ear diameter, and number of kernels per row. It emphasized the negative pleiotropic effects of low phytic acid lines on germination and seed set (Lydia *et al.*, 2020). Notably, kernels with low phytate were associated with lower kernel density, a pointed shape, and a popcorn type of kernel with shrivelled embryo (Fig. 2). Further clustering of the germplasm set based on kernel phytate and the four associated traits revealed four distinct clusters (Table 2).

Within the four grouped clusters, cluster 4 showed a distinct pattern characterised by low phytate, low seed density, pointed and pop type kernel and consisting of two genotypes *viz., lpa* 1 -707 and *lpa* 1-708. In contrast, the other clusters displayed high phytate and high density, with further subdivisions based on kernel type and shape. Cluster 1 featured genotypes with semi dent and shrunken type kernel. While cluster 2 represented genotypes having flint and pop type with levelled and rounded type kernel. Cluster 3 represented genotypes having kernels with a flint and indented type. To explore the association between kernel traits and kernel phytate, a hybridization experiment was undertaken. This involved selecting a low phytate genotype from cluster 4, specifically lpa 1-707, and two high phytate lines from clusters 2 and 3, namely UMI 1201 and 1230, respectively.

The contrasting morphological features of parents involved in crossing ispresented in Table 3. The two high phytate lines selected for hybridization are notably prominent parents of the elite Hybrid CoH (M) 8 released from TNAU during the year 2013. Low phytate genotypes *viz., lpa* 1 -707 and *lpa* 1 -708 were observed to exhibit wider leaf angle, droopy leaf attitude, pigmented glume base and sparse spikelet density. To explore this association with low phytate levels, two F_2 populations were analysed, with the contrasting features among parents presented in Table 3.

In F_2 population of UMI 1201 × *lpa* 1 -707, leaf angle showed a significant correlation with spikelet density, while in the F_2 of UMI 1230 x *lpa* 1-707, wider leaf angle exhibited a significant correlation with droopy leaf attitude and low kernel phytate. However, none of the vegetative and inflorescence traits were found to be correlated with kernel phytate level. Thus, among the 12 contrasting features studied, seed density alone was

Table 4: Starch granule size of high and low phytate genotypes measured using scanning electron microscope.

Genotypes	Starch granule size (µm)
UMI 1201	10.13
UMI 1230	10.22
lpa 1-707	13.47

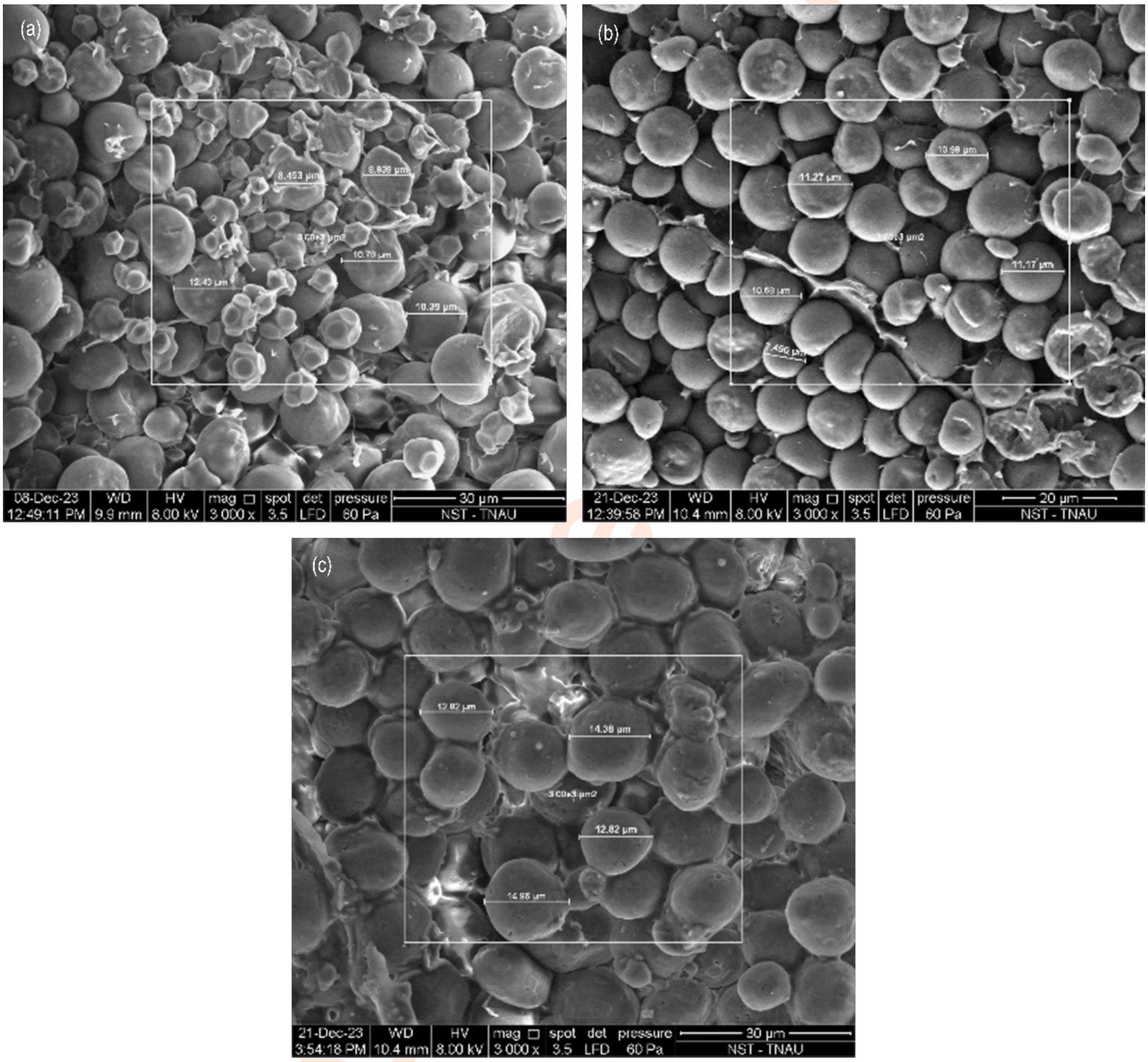

Fig. 5: Differences among starch granules among parents; (a) Starch granules of UMI 1201 which are uniform sized with protein bodies; (b) Starch granules of UMI 1230 which are uniform sized and (c) Starch granules of lpa 1-707 which are irregular in shape and less in number in comparison with high phytate lines viz., UMI 1201 and UMI 1230.

associated with phytate level, which was evident from the correlation analysis of two F_2 populations *viz.,* UMI 1201 ×*lpa* 1-707 and UMI 1230 ×*lpa* 1-707 (Fig. 3, 4). Similar findings were reported by Borlini *et al.* (2019) in a mutant population.

Density is defined as mass per volume and it was assessed using a sucrose solution with more than 1 of specific gravity was more than 1. When seeds possess high density, they easily sink in the sucrose solution due to its high specific gravity.

Table 5: Testing the significance of Mendelian inheritance using Chi-square test

Population	**Trait**	**Class**	**No. of plants**	**Chi-square**
UMI 1201 × *lpa 1* 707	Kernel density	High	80	1.80^{ns}
		Low	35	
UMI 1201 × *lpa 1* 707	Kernel phytate	High	93	2.11^{ns}
		Low	22	
UMI 1230 × *lpa 1* 707	Kernel density	High	64	1.89^{ns}
		Low	29	
UMI 1230 × *lpa 1* 707	Kernel phytate	High	73	0.60^{ns}
		Low	20	

Table 6: Variability estimates for the kernel traits between two F_2 populations

Kernel traits	**F_2-UMI 1201 x *lpa* 1-707**		**F_2–UMI 1230 x *lpa* 1-707**	
Variability estimates	h^2 (%)	GAM (%)	h^2 (%)	GAM (%)
100 - kernel weight	89	29	86	19
Inorganic phosphorous	96	67	92	73
Phytic acid	82	22	63	31
Kernel density	64	77	88	10

h^2 – heritability (%); GAM – Genetic Advance as percentage of Mean (%)

Table 7: Simple Pearson correlation among F2 of UMI 1201 x lpa 1-707

Traits	**Hundred kernel weight**	**Inorganic phosphorous**	**Phytic acid phosphorous**	**Kernel density**
Hundred kernel weight	1			
Inorganic phosphorous	0.23	1		
Phytic acid phosphorous	0.32	-0.32*	1	
Kernel density	0.12	0.74*	-0.32*	1

* - Significant @ probability level of 0.05

Table 8: Simple Pearson correlation among F_2 of UMI 1230 x *lpa 1*-707

Traits	**Hundred kernel weight**	**Inorganic phosphorous**	**Phytic acid phosphorous**	**Kernel density**
Hundred kernel weight	1			
Inorganic phosphorous	0.10	1		
Phytic acid phosphorous	0.02	-0.54*	1	
Kernel density	0.06	0.57*	-0.57*	1

* - Significant @ probability level of 0.05

It was mostly associated with the quality of seed, which affects germination (Baskin, 1990). In the present study it was influenced by phytate level. This association was further studied using scanning electron microscope by dissecting kernels of high and low phytate genotypes (Fig. 5), which provided insights on endosperm morphological changes associated with phytate level (Table 4). Starch granules were uniform in shape and size in the high phytate lines *viz.,* UMI 1201 and UMI 1230. However, the shape of the starch granules in *lpa* 1-707 was not uniform and were oblong. It is in accordance with the study of Landoni *et al.* (2013), where shape and size of starch granules were altered in *lpa* 1 mutants. Starch granules of high phytate lines were uniform and spherical in shape. The consistency in varying size of the starch granules vary between species. The resulting F_2

population correlations between kernel phytate and kernel density, suggested a link between seed density and changes in starch granule. Further, genetic segregation for these two traits were analysed in the two F_2 population which revealed that both kernel density and kernel phytate were segregating in monogenic fashion (Table 5). Kumar *et al.* (2010) reported the monogenic inheritance of kernel phytate, which corroborates with the findings of this study. This establishes it as a heritable qualitative trait that transfers from one generation to the next. Further, quantitative analysis of seed density was conducted to explore its relationship with 100-kernel weight (g), inorganic phosphorus, and phytic acid. Density is defined as the ratio of mass to volume. Therefore, the 100-kernel weight was considered to establish a relationship with kernel density, phytic acid and inorganic phosphorus. Variability estimates *viz.,* heritability % and genetic advance as percentage of mean were calculated for the four traits. All four traits viz., showed high 100-kernel weight, inorganic phosphorous, phytic acid phosphorous and kernel density possessed to have high heritability coupled with high genetic advance (Table 6). Similar results were reported by Chandana *et al.* 2018. Also, Kahriman *et al*. (2021) reported high heritability for phytic acid. This indicates the predominance of additive gene action for controlling these traits (Johannsen *et al.,* 1995). This indicates the predominance of additive gene action for controlling these traits. Consequently, selection is expected to be effective for improving these traits. Further, Simple Pearson correlation coefficients among these traits in two F_2 populations are presented in Table 7, 8. Kernel density alone exhibited a significant negative correlation with phytic acid phosphorous. This suggests an inverse relationship between kernel density and inorganic phosphorus with phytic acid. Hence, decreasing phytic acid was found to have a positive effect on kernel phosphorous availability and less seed density. It is consistent with the findings reported by Gerbasi and Raboy (1996) and Raboy *et al.* (2000). On the other hand, kernel density showed significant positive correlation with inorganic phosphorous. It can be used as a preliminary selection tool to narrow down the larger population. Further confirmation of the shortlisted genotypes can be made through biochemical analysis like HIP, Wades, and Davis and Reid methods. Therefore, utilizing kernel density for the rapid identification of low phytate genotypes can serve as a selection index for efficiently screening large populations. This approach may prove valuable in the development of core set or working collections in maize breeding programmes.

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Authors' contribution: A.J. Joel: Supervisor, Conceptualization of research, designing of experiments and manuscript preparation; **S.M. Indhu:** Designing of experiments, execution of field/lab experiments, Data collection, Data analysis and interpretation, Preparation of manuscript; **R. Ravikesavan** and **N. Senthil:** Co-supervisors, contribution of research materials, guided in conducting experiments and field monitoring; **T. Chitdeshwari:** Guided in conducting lab experiments; **F. Hossain:** Contribution of research material.

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