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Research Article

Genetic diversity and decoding the genetics of phytic acid by investigating the inheritance of *lpa 2* allele in maize (*Zea mays* L.)

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Abstract

Developing biofortified maize varieties with increased bioavailability of nutrients is crucial for addressing global food challenges and meeting the diverse needs of growing population. Hence, to formulate need based breeding programme, a comprehensive multidimensional approach was employed to assess the variability among 49 inbreds. PCA analysis revealed that single plant yield along with hundred kernel weight, number of kernels per row, number of kernel rows, cob length, ear height, cob diameter and leaf length contributed maximum towards variability. However, phytic acid displayed a relatively lower contribution to the overall variability. Based on cluster analysis, inbreds in the cluster III were found to have maximum mean performance for yield and its associated traits. Cluster V showed maximum mean performance for Pi. Based on the results, the genotype *lpa 2* - UMI 395 from cluster V was selected as donor for hybridisation. From UMI 1201 x *lpa 2*-UMI 395 and UMI 1230 x *lpa 2*-UMI 395 crosses, a total of 11 and 10 F_{2:3} plants were identified respectively as homozygous for *lpa 2* using molecular markers. The selected plants with *lpa 2* allele were found to have Pi in the range of 1.1 and 1.4 mg/g. Inheritance pattern of phytic acid based on these observations confirmed the recessive nature of the trait. Hence, these selected plants with *lpa 2* allele can be selected for yield and its associated traits in further generations.

Keywords: Maize, PCA, Cluster, *lpa 2*, low phytic acid, Inheritance

INTRODUCTION

Maize is the highest yielding crop among cereals worldwide. It is a major human food crop in a number of nations, particularly in Sub-Saharan Africa, Latin America and a few Asian countries. Maize consumed as human food accounts for more than 20% of food calories in these regions (Shiferaw *et al.*, 2011). Cornmeal, grits, starch, flour, tortillas, snacks and morning cereals are among the many products made from it. Maize flour is used to manufacture chapatis or flat breads, which are mostly consumed in a few Northern Indian states (Mehta and

Dias, 1999). Maize is a good source of carbohydrates, fibre, vitamins and minerals. Exploring variability among maize genotypes can provide insights on breeding program to develop high yielding, nutritionally important and resilient cultivars. Breeding for biofortified maize is gaining momentum and the stumbling block is the presence of phytic acid in the kernel. Understanding the genetic behaviour of this trait will enhance breeding outcome. Hence, in the present study, a set of 49 inbreds were assessed for their variability for yield and

its associated traits along with phytic acid level. Besides inheritance of phytic acid controlled by *lpa 2* allele was studied in $F_{2,3}$ phytic acid allele introgressed population.

MATERIALS AND METHODS

Germplasm evaluation: The experimental material consisted of 49 maize inbreds were raised during *rabi*, 2020-21 at New Area Farm of the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. The experiment was framed using randomized complete block design with two replications. Each inbred was raised in two rows of 3 metre length at the spacing of 20 x 60 cm. A good crop stand was established with the recommended package of practices. Observations were recorded on five plants from each inbred for 16 quantitative traits viz., days to 50 per cent tasselling, days to 50 per cent silking, plant height (cm), ear height (cm), leaf length (cm), leaf width (cm), tassel length (cm), peduncle length (cm), cob length (cm), number of kernel rows, number of kernels per row, cob diameter (cm), rachis diameter (cm), single plant yield (g), hundred kernel weight (g) and inorganic phosphorous (mg/g). This data set was subjected to principal component analysis and cluster analysis using 'facto-extra' and 'cluster' library packages in R 4.1.2.

Development and evaluation of segregating population: Based on germplasm evaluation, the genotype UMI 395

with *lpa 2* allele was chosen as the low phytic acid parent and it was crossed with two elite inbreds viz., UMI 1201 and UMI 1230 during *rabi*, 2020-21. The F_1 s were raised and selfed during *kharif*, 2021. The F_2 populations were evaluated during *rabi*, 2021-22. The $F_{2,3}$ seeds were evaluated for inorganic phosphorus (P_i) content using high inorganic phosphorous (HIP) assay (Raboy *et al.*, 2000; Raboy, 2002) to find the genetic control and behaviour of *lpa 2* allele. Genotypic confirmation of the segregation was done using SSR marker umc 2230 (Suresh Kumar *et al.*, 2015) in UMI 1230 x UMI 395 and *lpa 2* specific *lpa 2-1* CAPS marker in UMI 1201 x UMI 395 (Abhijith *et al.*, 2020). Distribution pattern and segregation pattern among two populations were studied using 'moments' library package in R.4.1.2 and chi-square test using MS-Excel 2007, respectively.

RESULTS AND DISCUSSION

Reduction of phytic acid is a multifaceted approach with implications for human health, agriculture sustainability and global food security. Assessing variability in germplasm represents a critical step in the development of biofortified maize with enhanced nutritional value. Analysis of variance depicted significant variation among the genotypes for all the traits observed (Table 1). Variability acts as a tool in selection. To reduce the dimension of components in variability and to unravel

Table 1. Analysis of variance for 16 quantitative traits of 49 maize inbreds

Sources of variation	Treatment	Error
df	48.00*	48.00
DTT	3.51*	0.65
DTS	6.70*	0.63
PH	2124.76*	5.64
EH	589.75*	2.30
LL	214.82*	1.09
LW	4.00*	0.07
TL	129.62*	0.29
PL	13.94*	0.02
TB	9.41*	0.00
CL	20.55*	0.15
NKR	10.64*	0.06
NKPR	104.68*	0.36
CD	0.52*	0.00
RD	0.30*	0.00
SPY	3152.93*	5.98
HKW	109.76*	0.41
Pi	0.12*	0.00

Significant @ 5 % probability level -*

DTT – Days to 50 per cent tasseling (days), DTS – Days to 50 per cent silking (days), PH – Plant height (cm), EH- Ear height (cm), LL – Leaf length (cm), LW – Leaf width (cm), TL – Tassel length(cm), PL-Peduncle length(cm), CL- Cob length(cm), NKR – Number of kernel rows, NKPR – Number of kernels per row, CD -Cob diameter(cm), RD-Rachis diameter(cm), SPY- Single plant yield (g), HKW- Hundred kernel weight (g), Pi-Inorganic phosphorous (mg/g).

the intricate patterns of variability, principal component analysis was used. In the present study, total variation present among the 49 inbreds were partitioned into 16 principal components (PC). PCs with higher eigen value and variables with higher factor loadings better represent the variation attributed among genotypes studied (Kalagare *et al.*, 2022). Eigen values were depicted using scree plot (Fig. 1). Among 16 PCs, first five PCs possessed eigen value more than 1 and contributed cumulative variation of 75.98 per cent (Table 2 and Fig. 2). PC 1 represented maximum variability for yield (16.12 %) and its associated traits (Fig. 3). The variation among PC 1 was mostly contributed through single plant yield, ear height, leaf length, cob diameter, leaf width, hundred kernel weight, cob length, number of kernel rows and number of kernels per row. PC 2 accounted 15.44 per cent variation mostly through days to flowering, days to tasselling, inorganic phosphorous, peduncle length, plant height and leaf width. Among these traits, PC 2

prominently captures the genetic variability associated with phytic acid (14.51 %) making it a key component for this study (Fig. 4). Hence, two dimensional biplot was made with PC 1 and PC 2 (Fig. 5). Similarly, Ramya *et al.* (2017) and Jain and Diwan (2021) in pearl millet focussed on characters and genotypes that are in close proximity in biplot and exhibit higher PC 1 scores to capture the variability of a specific trait.

The vector direction in a biplot indicates the maximum change in a significant quantity and its length may be correlated with the rate of change. The acute coordinate angle (less than 90 degree) between the traits or principal component axis signifies a positive association, while an obtuse angle (greater than 90 degree) indicates a negative correlation. A right angle (equal to 90 degree) suggests no correlation between the traits (Govindaraj *et al.*, 2020). It is evident from the biplot that single plant yield exhibited maximum variability among the traits observed

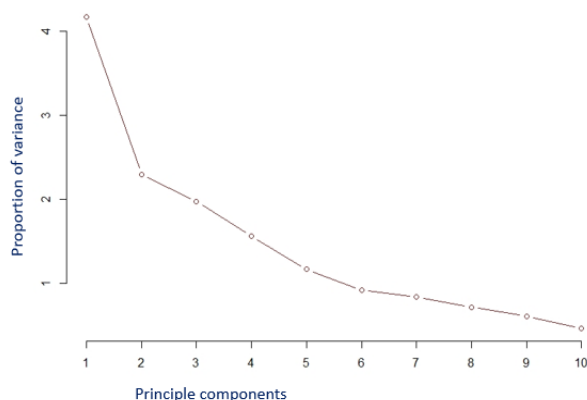


Fig. 1. Scree plot depicted using eigen value over principal components

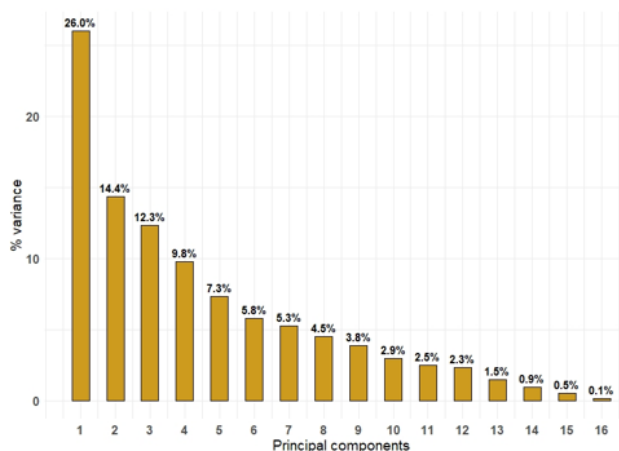


Fig. 2. Contribution of each of principal components to variability

Table 2. Eigen values and estimates of per cent variability accounted by the principal components

Components	Eigen value	Variance (%)	Cumulative variance (%)
PC1	5.2	32.65	32.65
PC2	2.4	15.44	48.10
PC3	1.7	11.23	59.33
PC4	1.5	9.78	69.12
PC5	1.0	6.85	75.98
PC6	0.9	5.67	81.65
PC7	0.6	4.35	86.01
PC8	0.5	3.37	89.38
PC9	0.4	2.63	92.02
PC10	0.3	2.46	94.48
PC11	0.3	2.06	96.55
PC12	0.2	1.56	98.12
PC13	0.1	0.65	98.78
PC14	0.0	0.56	99.34
PC15	0.0	0.51	99.85
PC16	0.0	0.14	100

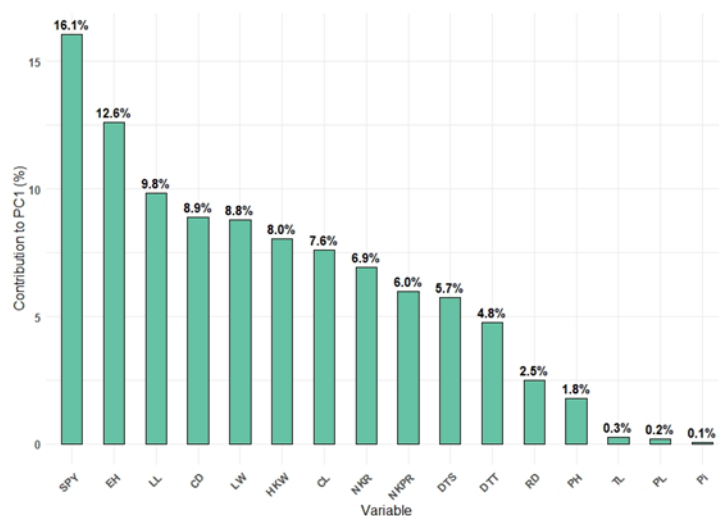


Fig. 3. Contribution of variables to principal component 1

and it is positively associated with hundred kernel weight, number of kernels per row, number of kernel rows, cob length, ear height, cob diameter and leaf length. Similar correlation pattern was observed in maize by Raghu *et al.* (2011) and Sinana *et al.* (2023). Since the relationship of these traits were similar in both PCs, it was placed in the IV quadrant of the biplot. Also, genotypes in the IV quadrant can be selected for improvement of yield and its associated traits. Pi (Inorganic phosphorus) exhibited less variability in comparison with yield and its associated traits. Also angle of vector of single plant yield towards Pi

was orthogonal. It represented that variation for Pi was very less to explain the relationship with single plant yield as explained by Govindaraj *et al.* (2020) in pearl millet. However, three inbreds *viz.*, *lpa 1-707*, *lpa 1-708* and *lpa 2-UMI 395* were aligned with the vector of Pi.

Cluster analysis grouped the genotypes into five clusters and is represented in dendrogram (Fig. 6). Cluster I contains eight genotypes, cluster II contains five genotypes, cluster III with 12 genotypes, cluster IV with 17 genotypes and cluster V with seven genotypes.

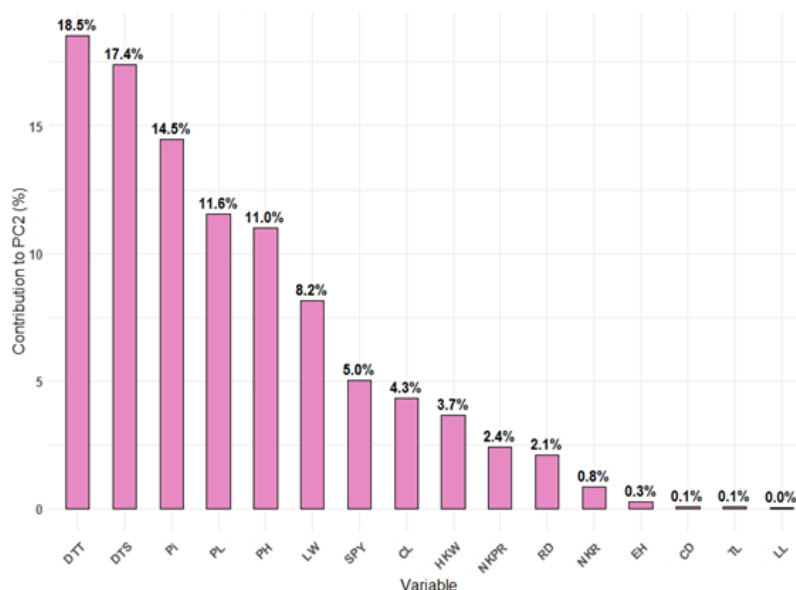


Fig. 4. Contribution of variables to principal component 2

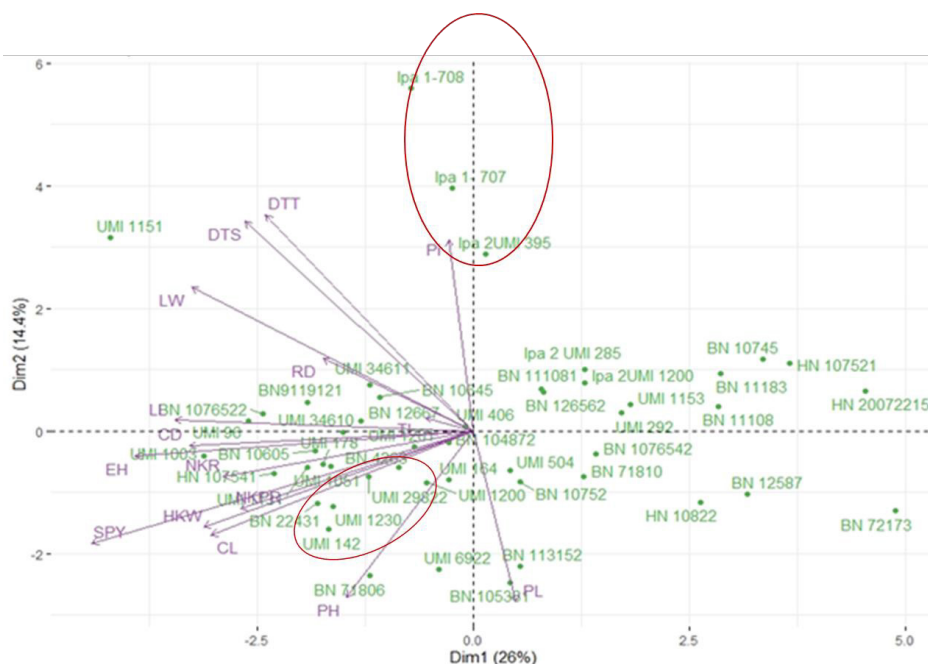


Fig. 5. 2D plot representing relationship pattern of genotypes with variables

DTT – Days to 50 per cent tasselling (days), DTS – Days to 50 per cent silking (days), PH – Plant height (cm), EH- Ear height (cm), LL – Leaf length (cm), LW – Leaf width (cm), TL – Tassel length(cm), PL-Peduncle length(cm), CL- Cob length(cm), NKR – Number of kernel rows, NKPR – Number of kernels per row, CD -Cob diameter(cm), RD-Rachis diameter(cm), SPY- Single plant yield (g), HKW- Hundred kernel weight (g), Pi-Inorganic phosphorous (mg/g). Genotypes in red ellipse represented grouping of genotypes along the angle of vector, where vector represented traits.

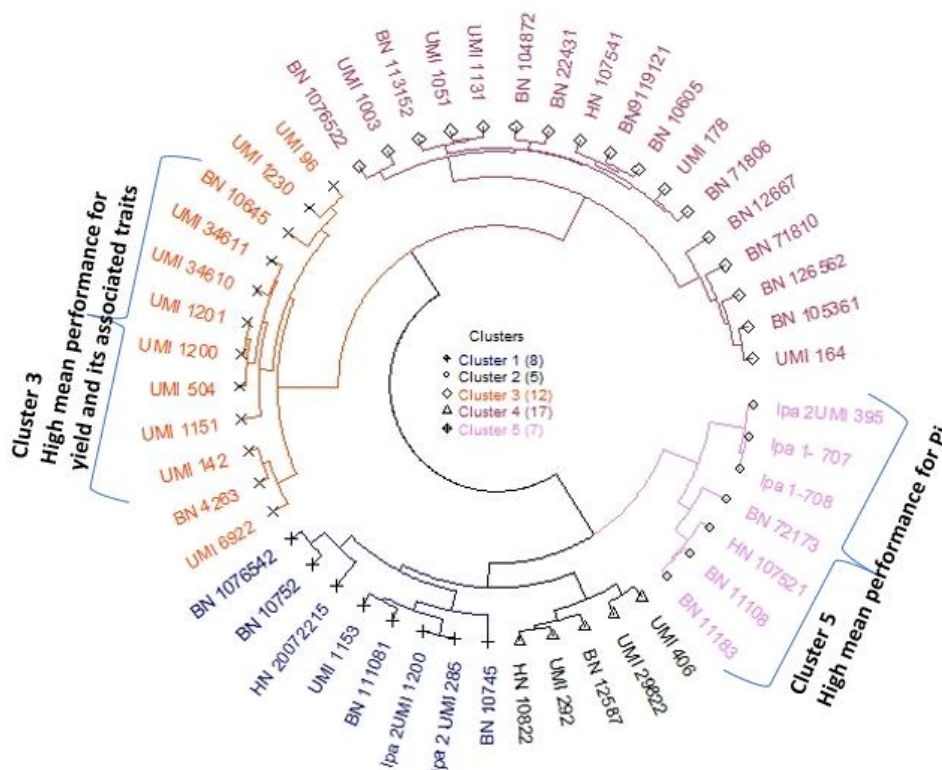


Fig. 6. Dendrogram representing grouping of inbreds based on similarity matrix of Euclidean distance

Table 3. Mean performance of genotypes for quantitative traits in each cluster

Clusters	DTT	DTS	PH	EH	LL	LW	TL	PL	CL	NKR	NKPR	CD	RD	SPY	HKW	Pi
Cluster I	57.38	58.13	154.50	71.25	71.69	7.50	28.38	3.38	14.50	11.00	14.25	3.24	1.45	30.26	19.46	0.58
Cluster II	57.00	58.80	152.40	71.20	63.60	6.80	36.40	6.80	14.06	12.60	23.80	3.00	1.34	61.30	20.83	0.71
Cluster III	57.83	58.75	139.92	77.17	78.25	8.33	31.88	4.54	16.76	14.17	24.83	3.49	1.70	86.48	26.15	0.63
Cluster IV	57.76	59.00	179.59	85.59	71.82	8.24	32.35	4.35	15.79	12.76	22.12	3.39	1.39	79.50	30.38	0.68
Cluster V	57.86	59.00	115.43	58.86	61.21	8.00	31.57	2.79	11.87	12.29	18.14	3.17	1.53	47.19	21.12	0.95

DTT – Days to 50 per cent tasseling (days), DTS – Days to 50 per cent silking (days), PH – Plant height (cm), EH- Ear height (cm), LL – Leaf length (cm), LW – Leaf width (cm), TL – Tassel length(cm), PL-Peduncle length(cm), CL- Cob length(cm), NKR – Number of kernel rows, NKPR – Number of kernels per row, CD -Cob diameter(cm), RD-Rachis diameter(cm), SPY- Single plant yield (g), HKW- Hundred kernel weight (g), Pi-Inorganic phosphorous (mg/g).

Genotypes in cluster II were found to have maximum mean performance for tassel length and peduncle length (Table 3). Cluster III recorded high mean performance for leaf length, leaf width, cob length, number of kernel rows, number of kernels per row, cob diameter, rachis diameter and single plant yield. Cluster IV was found to have maximum mean performance for plant height, ear height and hundred kernel weight. Cluster V recorded high mean performance for Pi, i.e., an indicator of low phytic acid. Among the 12 genotypes in cluster III, the two elite genotypes viz., UMI 1201 and UMI 1230 were

the parental inbreds of ruling hybrid CO H (M) 8. Among the genotypes in cluster V, *lpa 1-707*, *lpa 1-708* and *lpa 2- UMI 395* found to have highest Pi (>1.18 mg/g) and it was confirmed using *lpa* allele specific markers (Fig. 7). Among these selected genotypes, *lpa 2- UMI 395* was used in low phytic acid trait introgression programme.

In the process of low phytic acid introgression programme, *F₁s* were confirmed using molecular markers (Fig. 7a & b). *F₁s* of UMI 1201 x *lpa 2-UMI 395* were found to have amplification at 459 bp, 290 bp and 169 bp for the

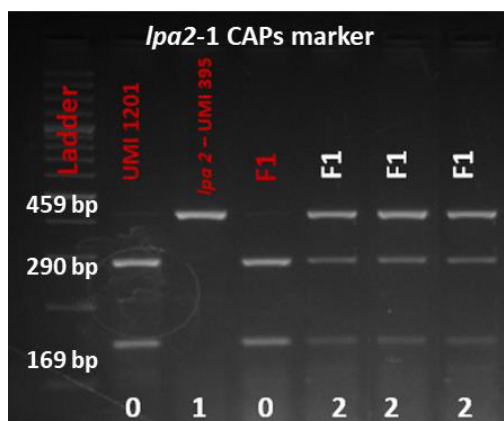


Fig. 7a. Confirmation of hybrids of UMI 1201 x *lpa 2*- UMI 395 for *lpa-2* allele using *lpa 2*-CAPs marker; 0 – UMI 1201, 1 – *lpa 2*- UMI 395, 2 – Heterozygote

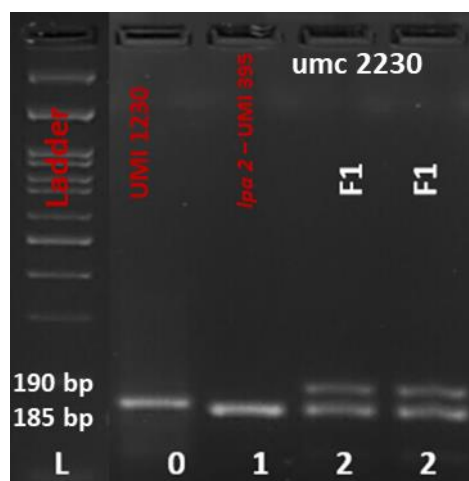


Fig. 7b. Confirmation of hybrids of UMI 1230 x *lpa 2*- UMI 395 for *lpa-2* allele using umc 2230 marker; 0 – UMI 1201, 1 – *lpa 2*- UMI 395, 2 – Heterozygote

marker umc 2230. Similarly, F_1 s of UMI 1230 x *lpa 2*- UMI 395 amplified at 175 and 180 bp for the *lpa 2* specific *lpa 2*-1 CAPs marker which confirmed the presence of allele from both the parents. These F_1 s derived F_2 populations were screened based on Pi (mg/g) and molecular markers (**Fig. 8b** and **Fig. 9**). Nature of distribution among the F_2 populations for low phytic acid based on Pi level is depicted in distribution insight plot (**Fig. 10**). Distribution insight plot of UMI 1201 x *lpa 2*- UMI 395 showcased that F_2 population was not normally distributed with skewness of -0.34 and platykurtic with kurtosis of 3.11 (**Fig. 10**). Empirical Cumulative Distribution Function curve (blue points) (ECDF) represented the distribution of Pi level and most of the F_2 segregants falls between 0.5 -1.0 mg/g. F_2 population of UMI 1230 x *lpa 2*- UMI 395 was not normally distributed. It was positively skewed (0.57) towards right since most of F_2 plants found to be present

below 1.0 mg/g (ECDF curve) and leptokurtic (2.81) (**Fig. 10**).

The low phytic acid allele was not expressed in a heterozygous condition. It implied the recessive nature of the gene. Both the F_2 populations were screened for *lpa 2* allele using phenotypic analysis and markers (**Fig. 8b & 9**). Among 71 plants screened for *lpa 2* in UMI 1201 x *lpa 2*- UMI 395 combination, 11 plants were found to have *lpa 2* allele in homozygous condition (**Fig. 9a**) with a mean Pi of 1.4 mg/g. Out of 60 plants screened in UMI 1230 x *lpa 2*- UMI 395 cross, 10 plants were found to have homozygous allele for *lpa 2* (**Fig. 9b**) with a mean Pi of 1.18 mg/g. However, Yathish *et al.* (2022) reported Pi of 1.22 mg/g in *lpa 2* introgressed BILs of maize. It depicted 3:1 (High: Low) segregation pattern for phytic acid level between 5-15 % probability level, where

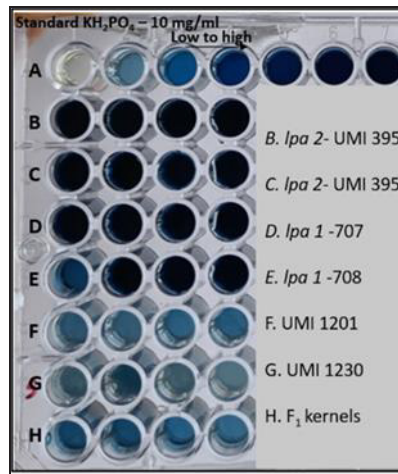


Fig. 8a. A1 – A7: Series of standards with stock concentration of 1mg/ ml;
B-G (1-4): Confirmation of parents using HIP assay

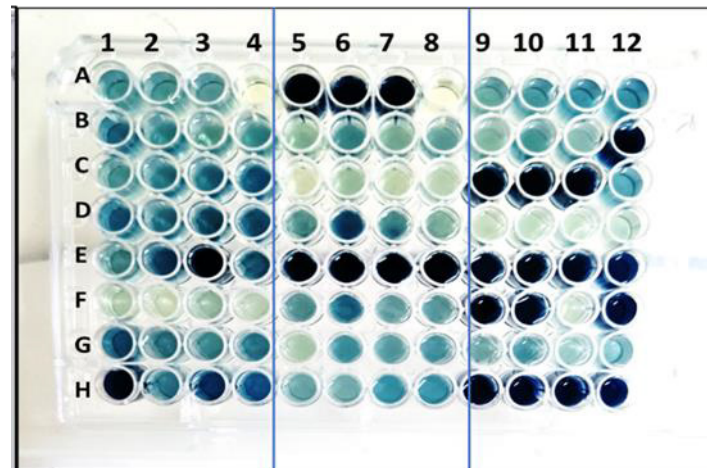


Fig. 8b. Screening of F_{2:3} plants using HIP assay
A1-A3 – UMI 1230, A5-A7 – lpa 2 UMI 395;
Low phytate plants: E5-E8 (F₂#059), E9-E12 (F₂#071), H9-H12 (F₂#074)

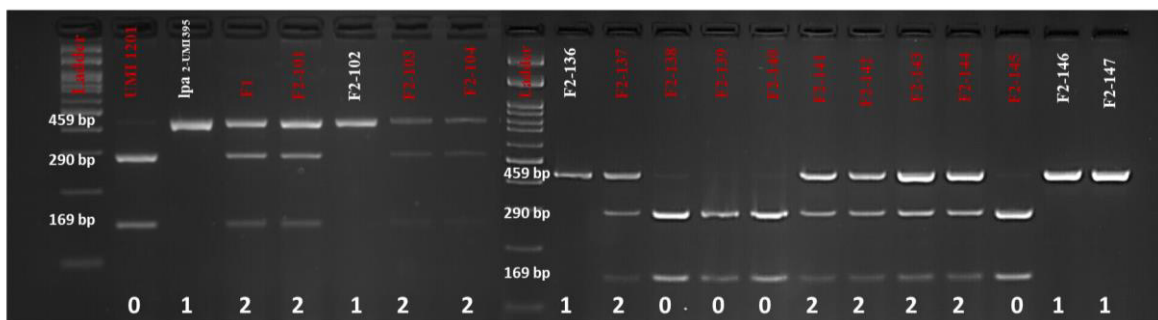


Fig. 9a. Profiling of F₂ plants of UMI 1201 x lpa 2- UMI 395 using lpa 2-1 CAPS marker for lpa 2;
0 – UMI 1201, 1 – lpa 2- UMI 395, 2 - Heterozygote

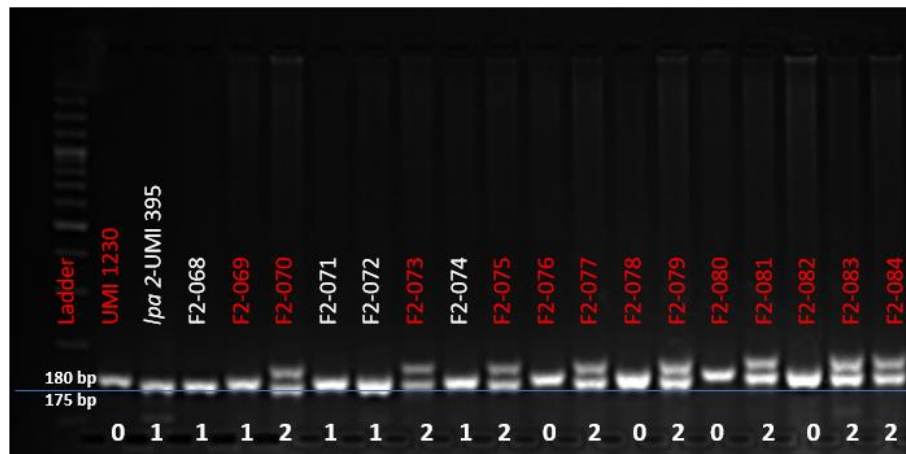


Fig. 9b. Profiling of F₂ plants of UMI 1230 x *lpa* 2- UMI 395 using SSR marker umc 2230; 0 – UMI 1230, 1 – *lpa* 2- UMI 395, 2 -Heterozygote

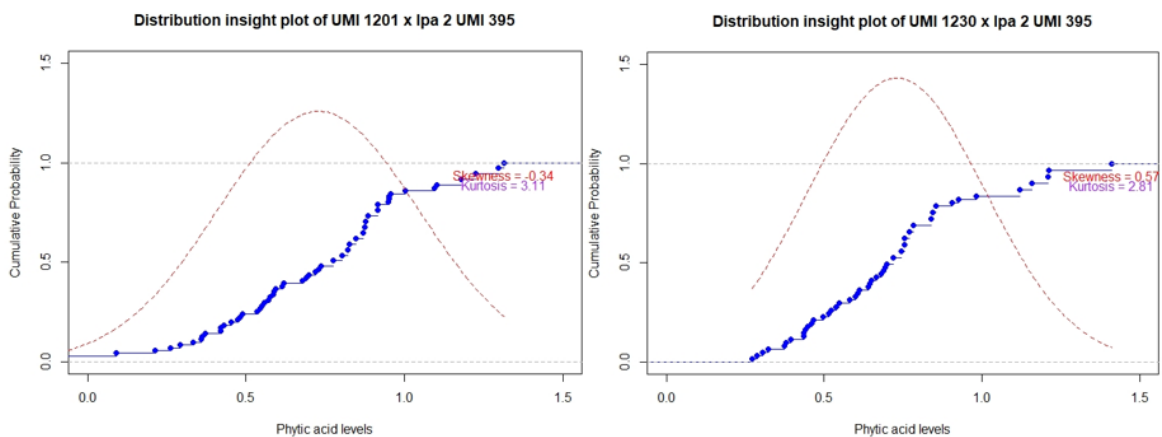


Fig. 10. Distribution insight plot of two F₂ populations

Table 4. Chi-square test for *lpa* trait in two F₂ populations

Cross combinations	Phenotype category	Mean P _i level (mg/g)	Observed individuals	Expected ratio	Expected individuals	Chi-square value	Probability level
UMI 1201 x <i>lpa</i> 2- UMI 395	High phytic acid	0.58	60	3	53	3.68 ns	5-10 %
	Low phytic acid	1.40	11	1	17		
UMI 1230 x <i>lpa</i> 2- UMI 395	High phytic acid	0.59	50	3	45	2.22 ns	10-15 %
	Low phytic acid	1.18	10	1	15		

Note: Phenotype categorized based on genotypic data; ns: non- significant

3/4th of the population was of high phytic acid (A-) and dominant in nature, whereas 1/4th of population was recessive for *lpa* 2 allele with low phytic acid (Table 4). The segregation analysis and chi square test showed the recessive nature of *lpa* 2 allele in maize (Tamil

Kumar *et al.*, 2014). A total of 11 and 10 homozygous plants for *lpa*-2 were identified from UMI 1201 x *lpa* 2-UMI 395 and UMI 1230 x *lpa* 2 -UMI 395, respectively. These homozygous lines could be exploited in future breeding programs.

To conclude, single plant yield exhibited maximum variability among the traits observed and it was positively associated with yield component traits viz., hundred kernel weight, number of kernels per row, number of kernel rows, cob length, ear height, cob diameter and leaf length. A total of 11 and 10 homozygous lines identified from the crosses UMI 1201 x lpa 2-UMI 395 and UMI 1230 x lpa 2-UMI 395 respectively could be exploited for development of low phytic acid inbreds in maize.

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