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Biochemical estimation of phytic acid and inorganic phosphate in diverse maize germplasm to identify potential donor for low phytic acid (LPA) trait in tropical genetic background

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Abstract

Phytic acid (PA), an anti-nutritional factor in maize (*Zea mays* L.) grains form various salts by chelating essential vital mineral elements namely, Fe^{3+}, Zn^{2+}, Mg^{2+}, etc. affecting their bioavailability. Low phytic acid (LPA) maize can play a vital role as an important food and feed crop to diversify the existing food basket to address micronutrient malnutrition. Globally efforts to identify LPA maize genotypes in the existing germplasm are limited. Keeping this in view, the present experiment was designed using the diverse set of maize germplasm including landraces, composites, inbred lines and hybrids of field corn and also some genotypes of quality protein maize and sweet corn to explore the extent of variability for PA in the existing germplasm and to identify the LPA maize genotypes. The mean PA content across 104 genotypes excluding LPA mutants varied from 1.7 mg/g (CML150) to 4.5 mg/g (VMH45) whereas inorganic phosphate (P_i) varied from 0.07 mg/g (LM16) to 0.95 mg/g (PMH9). The correlation coefficient between PA and Pi across genotypes was negative but moderate (-0.35) (P = 0.00024). The present study has identified two inbred lines namely, CML150 (1.7 mg/g) and CML176 (1.8 mg/g) which do not differ significantly with either of the two LPA mutants namely, LPA1 (1.3 mg/g) and LPA2 (1.7 mg/g). The study further indicated that PA content is generally low in white kernel colour germplasm as compared to other kernel colours. The identified genotypes could be potential donor for developing LPA maize genotypes and also their utilization in the breeding for development of LPA maize cultivars.

Keywords: Phytic acid, inorganic phosphate, micronutrients, LPA, maize

Introduction

Globally, maize (*Zea mays* L.) is an important cereal food crop with highest production (1147.6 million tons) among cereals (FAOSTAT 2018). It is one of the major staple food crops in most of the developing countries of Latin America, Africa and some Asian countries including India, particularly in the tribal areas of states like Himachal Pradesh, Jammu & Kashmir, Rajasthan, Gujarat, Madhya Pradesh, Chhattisgarh, North-east hill region etc. Maize contributes approximately 9-10% of the total food basket of the country after rice and wheat. The demand for maize in India is increasing continuously and it is expected to continue in near future as well. As a result, a significant proportion of children below the age of 5 are suffering from one or the other type of malnutrition like stunting (34.7%), underweight (33.4%), wasting (17.3%) and severe wasting (4.9%) (UNICEF/WHO/World Bank 2020). 54% of the children under the age of five are suffering from one or the other type of malnutrition in India (UNICEF 2020). Among the malnourished children, a certain percentage of children suffer from multiple malnutrition namely stunted and underweight (18.1%), wasted and underweight (7.9%), stunted, wasted and underweight (6.4%). More than 50% of the women between 15-49 years of age are deficient in iron; iron deficiency-related anaemia is one of the major health concerns in India (FNSA 2019). The major reason for micronutrient malnutrition was due to lack of essential

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micronutrients in sufficient quantity in staple food crops and the narrow food basket. There are several approaches to overcome the micronutrient malnutrition namely enrichment of staple food crops with essential micronutrients through biofortification, diversification of food basket with different staple food crops, reduction of anti-nutritional factors which affect the availability of essential micronutrients in staple food crops etc. (Gyani et al. 2021; Siwela et al. 2020; Bollinedi et al. 2020).

Maize can play an important role in diversifying the food basket in India. Corn-flakes and other value-added maize-based snacks are slowly making inroads in regular dietary habit. However, it may be noted that the population which largely depends on the maize-based diets suffer from acute iron and zinc deficiency even though maize contains adequate amounts of iron and zinc which are sufficient to meet the dietary requirements of human beings. The main reason is due to the presence of phytic acid (IP6 or PA), an anti-nutritional factor in maize grains, reducing the overall nutritional value of maize. PA forms insoluble salts by chelating essential vital mineral elements especially polyvalent cations namely, Fe^{3+} (Monoferricphytate, $C_6H_{18}FeO_{24}P_6^{+3}$), Zn^{2+} (Zinc phytate, $C_6H_6O_{24}P_6Zn_6$), Mg^{2+} (Magnesium 2,3,4,5,6-pentakis (phosphonooxy) cyclohexyl phosphate, $C_6H_{16}MgO_{24}P_6$), Ca^{2+} (Calcium phytate, $C_6H_6Ca_6O_{24}P_6$), Na^{2+} (Phytate sodium, $C_6H_9Na_9O_{24}P_6$), etc. Chemically PA ($C_6H_{18}O_{24}P_6$) is composed of carbon, hydrogen, oxygen and phosphorous; Hence, PA is a major source of phosphorous but the phytate-phosphorous is not hydrolysable, thus not in an available form for mono-gastric animals like poultry, swine, etc. (Golam Masum Akond et al. 2011). A large proportion (0.3 to 2.4%) of phosphorous is excreted in poultry litter without its utilization and poultry farmers are forced to add inorganic P in the poultry feed to meet the P needs of the birds (de Souza et al. 2012). The dispose of poultry waste into the environment leads to eutrophication and the addition of P in the poultry feed increases the cost of production and also makes P a costly input (Bolan et al. 2010).

Caoulibaly et al. (2011) reported that PA reduction increases the bioavailability of iron (Fe), zinc (Zn), and magnesium (Mg) by 12, 20 and 60 % respectively. It is also reported that the reduced PA does not affect the total phosphorous reserve in the grains (Raboy et al. 2000). The above reports encourage the development of low phytic acid (LPA) maize to enhance the bioavailability of major essential mineral elements like Fe, Zn, Mg and Ca, and improving the biological value of animal feeds. LPA maize can play a vital role as an important food and feed crop to diversify the existing food basket to address micronutrient malnutrition (Raboy 2001) and can also reduce the environmental pollution due to eutrophication (Holt et al. 1970). Globally several efforts have been made to develop LPA maize using different approaches like mutagenesis (Raboy et al. 2000), conventional breeding (Lorenz et al. 2008), marker-assisted introgression of LPA mutants into elite cultivars (Tamilkumar et al. 2014; Sureshkumar et al. 2008) and transgenics (Chen et al. 2008). However, efforts to understand the genetic variability for PA and possible identification of LPA in maize genotypes in the existing germplasm, is a prerequisite for breeding for LPA maize. Such studies are limited, especially in tropical germplasm (Pramitha et al. 2019; Chiangmai et al. 2011; Lorenz et al. 2008). The present study was therefore, formulated to understand the genetic variability for PA in the existing germplasm, to explore the extent of variability for PA, and to identify the LPA maize genotypes as a potential donor from the available maize germplasm.

Materials and methods

Plant materials and experimental design

A set of 106 diverse maize germplasm comprising eight landraces (LR), five composites or open-pollinated varieties (OPV), 47 elite inbred lines (IL), two LPA mutant genetic stocks (Mu), and 44 single cross hybrids (SCH) were used for PA screening (Supplementary (Suppl.) Table S1). The diverse maize germplasm composed of different kinds of maize namely normal maize (N, 87), quality protein maize (Q, 15), sweet corn (SC, 2) and LPA mutant stocks (Mu, 2) procured from ICAR-VPKAS, Almora. The selected germplasm were of different kernel colours namely orange (50), red (2), yellow (47) and white (7). The inbred lines comprised of parental lines of hybrids used in the study. The hybrids and OPVs used in the study were released and notified during the past 15-20 years for commercial cultivation in different agro-ecological zones of India. The germplasm are genetically diverse with distinguished geographical adaptation (Suppl. Table S1).

The experimental materials were grown for two seasons one each during *kharif* 2018 at ICAR-Indian Agricultural Research Institute, New Delhi (28° 64'02.48"N, 77p 15'25.06"E) and the other during *rabi*

Table 1. The summary of the analysis of variance (ANOVA) for phytic acid (PA) and inorganic phosphate (P_i) content

Particular	Df	SS PA	MS	F value	Pr(>F)	SS P_i	MS	F value	Pr(>F)
Genotypes	105	251.04	2.3909	35.44	$<2e^{-16***}$	57.23	0.545	18.93	$<2e^{-16***}$
Germplasm Type	3	23.27	7.757	23.39	$1.53e^{-14***}$	5.23	1.7442	18.41	$1.57^{e-11***}$
Maize Type	3	67.02	22.34	79.97	$<2e^{-16***}$	40.02	13.338	278	$<2^{e-16***}$
Kernel Colour	3	7.64	2.5475	7.271	$8.11e^{-05***}$	0.48	0.1613	1.596	0.189

DF = Degree of freedom; SS = Sum of square; MS = Mean square

2018-19 at Regional Maize Research and Seed Production Centre, Kushmahut, Begusarai (25° 46'53.45"N, 86° 23'03.16"E); the genetic integrity of the material is maintained through selfing and sib-mating. Each genotype was harvested and bulked separately, the random sample form the bulk was taken in three replicates for biochemical analysis. The material was planted in four rows of 4 m length and the spacing between rows × plants was kept at 75 cm × 20 cm.

Biochemical estimation of phytic acid (PA) and inorganic phosphate (P_i)

The standards curves drawn using OD values obtained against the respective known concentrations of PA and P_i standards were used to estimate the PA and P_i content in the samples, respectively. The standard curves were linear in the respective assays with R^2 values of 0.959-0.996 for PA and 0.982-0.999 for P_i. The PA or P_i of samples (x) was estimated by substituting the values in the respective linear equation of PA and P_i standards curve, $y = ax + b$ where y is the OD value of the sample, a is the slope, b = constant or intercept. The frequency distribution of residues of estimated PA across genotypes, germplasm types, maize types and kernel colour showed approximately near-normal distribution (Suppl. Fig. 1a-d). However, for P_i, the frequency distribution curve of residuals showed near approximately normal distribution for genotypes (Suppl. Fig. 2a) but slightly right-skewed for germplasm types, maize types and kernel colour (Suppl. Fig. 2b-d).

The PA and P_i were estimated using a colorimetry by following the protocol of Lorenz et al. (2007). The reagents required for PA and P_i estimation were different and prepared separately (Suppl. Information 1). The seed sample of 10 g was drawn randomly from the bulk seeds of each genotype. Three samples of each genotype were taken from the same source of bulk seed to represent three analytical replicates per genotype. The whole kernels were ground into a fine powder using Mixer Mill (MM 400; Retsch GmbH, Germany) in Teflon cylinder using zirconium balls and stored in airtight tubes at 4 p C for further biochemical analysis of PA content. Ten milligrams (mg) maize flour from each of the above ~10 g stock was taken as sub-sample and placed in a 2 mL centrifuge tubes, 200 µL of 0.65 M HCl was added to the tubes. The mixture was kept ~12-hour incubation at room temperature on shaker/rocker. After the incubation, the tubes were centrifuged at 3000 rpm for 20 minutes. For PA estimation 30 µL extract was taken-out after centrifugation into 96-well microplate, then 200 µL diluted (1:4) wades reagent was added to each well. For P_i estimation, another 30 µL extract was taken-out in a separate 96-well microplate to which 130 µL deionized water and 100 µL Chen's reagent was added to each well. The sodium phytate (HiMedia, GRM6226) and potassium dihydrogen phosphate (Merch 104873 Supelco) were used for the preparation of PA and P_i control standard respectively. The three analytical replicates of control standards of PA and P_i were also prepared as described above and placed in the assigned wells of respective 96-well microplates. For measurement of PA and P_i, the 96-well plates containing control standards and samples of different genotypes were kept for 15-20 minutes and then the OD_{490nm} and OD_{820nm} were recorded for PA and P_i estimation respectively using BioTek Epoch 2 Microplate Spectrophotometer (BioSPX B.V., LA Abcoude, The Netherlands).

Statistical analysis

The OD values were used to estimate the PA and P_i based on the linear equation obtained using the respective standard. The PA and P_i values thus obtained were analysed by fitting the following model.

$$Y_{ij} = \mu + \tau_i + \varepsilon_{oj} = \bar{y}_{-} + (\bar{y}_{i.} - \bar{y}_{-}) + Y_{ij} - \bar{y}_{i.})$$

where Y_{ij} is the j^{th} observation of the i^{th} treatment; μ is the overall mean; τ_i is the treatment effect of the i^{th} treatment and ε_{ij} is the random error.

The one-way analysis of variance (ANOVA) was performed for both the traits (PA and P_i) separately for genotypes (106 genotypes), germplasm types (LR, OPV, IL, SCH), maize types (N, Q, SC, Mu), and kernel colour (Orange, Red, White, Yellow) by partitioning the corrected total sum of squares. The means were compared for genotypes, germplasm types, maize types and kernel colour separately using the post hoc test namely Fisher's LSD and Tukey's HSD. However, Tukey's HSD used to compare different groups for significant differences. The normality of residues and homogeneity of variance were also tested using Shapiro-Wilk's W test and Levene's test, respectively. The Pearson's correlation between PA and P_i was also performed over genotypes, within sub-groups of germplasm types, maize types and kernel colour. The statistical analysis was performed in R environment using different packages and codes (R Core Team, 2020).

Results

Phytic acid

The ANOVA for PA showed a significant difference among genotypes (Table 1). The differences were also significant between germplasm types, different maize types and kernel colour. The mean PA content in LPA mutants was the lowest (1.3 mg/g in LPA1 and 1.7 mg/g in LPA2), while across all the genotypes excluding LPA mutants, it varied from 1.7 mg/g (CML150) to 4.5 mg/g (VMH45). Among ILs excluding LPA mutants, the mean PA content varied from 1.7 mg/g (CML150) to 4.1 mg/g (HKI1128). Similarly, the PA level within different types of germplasm namely, LR, OPVs and SCHs varied from 2.5 mg/g in Desi Summan to 3.7 mg/g in MakkaDhebri, 2.8 mg/g {Gaurav (D931)} to 4.1 mg/g (Win Orange Sweet Corn) and 2.1 mg/g (HM8) to 4.5 mg/g (VMH45), respectively (Table 2). The mean PA of ILs was 2.8 mg/g, the lowest mean among different germplasm types namely LR (3.0 mg/g), OPVs (3.5 mg/g) and SCH (3.1 mg/g) (Table 3). In case of maize types excluding mutants (Mu), the PA content in sweet corn was 3.4 mg/g, whereas for normal (N) and QPM (Q) it was 3.0 mg/g (Table 3). The mean PA content between different classes of colour maize varied from 2.6 mg/g (white) to 3.0 mg/g (orange, red and yellow) (Table 3).

The pair-wise multiple comparisons between genotypes based on Tukey's HSD for PA content showed that two inbred lines namely CML176 (1.8 mg/g) and CML150 (1.7 mg/g) do not differ significantly with either of the two LPA mutants namely LPA1 (1.3 mg/g) and LPA2 (1.7 mg/g) (Supplementary Table S2). Similarly, V346 (2.0 mg/g) did not differ significantly with one of the two LPA mutant, LPA2. However, it showed significant difference with other LPA mutant, LPA1. The pair-wise multiple comparisons between different germplasm types, maize types and kernel maize types showed significant differences among groups. The PA content of inbred lines differed significantly with OPVs and SCHs, whereas in LR it was comparable with ILs and SCHs. The sweet corn PA content differed significantly with other types of maize. The PA content of white maize differed significantly with orange colour maize but not with red and yellow colour maize (Table 3).

Inorganic phosphate

The inorganic phosphate (P_i) content showed a significant difference among genotypes, germplasm types, maize types but not among kernel colours (Table 1). The mean P_i content across different maize germplasm varied from 0.07 mg/g (LM16) to 0.95 mg/g (PMH9). P_i content in inbred lines excluding LPA mutants varied from 0.07 mg/g (LM16) to 0.74 mg/g (CML176). The mean P_i content of landraces varied from 0.14 mg/g (Chutri Makka) to 0.33 mg/g (Desi Ridhhi). Similarly for OPVs and SCH, it varied from 0.18 mg/g (African Tall) to 0.34 mg/g (Narmada Moti) and 0.10 mg/g (VMH27) to 0.95 mg/g (PMH9) respectively (Table 2). The mean P_i of different germplasm types also differed which was 0.43 mg/g for IL and 0.20, 0.24 and 0.29 mg/g for LR, OPV, and SCH respectively (Table 3). Whereas for different types of maize namely mutants, normal maize, QPM, and sweet corn the mean P_i was 1.62, 0.32, 0.32 and 0.18 mg/g respectively (Table 3).

The pair-wise multiple comparisons of genotypes based on Tukey's HSD showed that two mutants namely LPA1 and LPA2 differed significantly for P_i content with the rest of the genotypes (Suppl. Table S3). However, similar comparisons within different germplasm types (Table 3) and maize types (Table 3) did not show significant differences within sub-groups except IL with rest of the groups (Table 3) and mutants with rest of the maize types (Table 3). Further, comparison of mean among different classes of kernel colour could not reveal any significant differences (Table 3).

Table 2. The mean PA and Pi content in diverse tropical maize germplasm

Genotypes	GT	MT	KC	PA(mg/g)	R	LCL-UCL	Pi(mg/g)	R	LCL-UCL
LPA1	IL	Mu	Y	1.3±0.4	15	1.2-1.5	1.61±0.21	12	1.52-1.71
LPA2	IL	Mu	Y	1.7±0.1	15	1.6-1.8	1.63±0.31	12	1.54-1.73
BML2	IL	NM	O	3.5±0.2	9	3.4-3.7	0.27±0.22	8	0.16-0.39
BML45	IL	NM	O	3.4±0.2	6	3.2-3.6	0.24±0.04	6	0.10-0.37
BML7	IL	NM	O	3.0±0.1	9	2.9-3.2	0.72±0.19	8	0.60-0.84
CM139	IL	NM	O	3.4±0.1	9	3.2-3.5	0.21±0.13	8	0.09-0.32
CM140	IL	NM	O	2.7±0.1	9	2.6-2.9	0.34±0.11	8	0.22-0.46
CML163	IL	NM	O	2.9±0.3	9	2.7-3.1	0.14±0.01	6	0.01-0.28
CML165	IL	NM	O	2.2±0.2	9	2.1-2.4	0.14±0.07	8	0.02-0.26
HKI1128	IL	NM	O	4.1±0.3	9	3.9-4.2	0.38±0.25	8	0.26-0.49
HKI323	IL	NM	O	2.7±0.2	9	2.5-2.9	0.67±0.23	8	0.55-0.79
LM13	IL	NM	O	2.9±0.3	15	2.7-3.0	0.44±0.21	14	0.35-0.53
LM14	IL	NM	O	3.0±0.4	15	2.8-3.1	0.34±0.15	14	0.25-0.43
LM15	IL	NM	O	3.1±0.6	9	2.9-3.3	0.25±0.26	8	0.13-0.37
LM17	IL	NM	O	2.5±0.1	6	2.3-2.7	0.11±0.02	6	0.02-0.25
LM19	IL	NM	O	2.7±0.3	15	2.6-2.9	0.30±0.18	14	0.21-0.38
UMI1200	IL	NM	O	3.5±0.7	9	3.3-3.7	0.38±0.02	6	0.24-0.52
UMI1201	IL	NM	O	2.8±0.3	9	2.6-2.9	0.44±0.24	8	0.33-0.56
UMI1205	IL	NM	O	2.8±0.1	9	2.6-2.9	0.49±0.24	8	0.37-0.60
UMI1210	IL	NM	O	3.0±0.5	9	2.8-3.2	0.65±0.32	8	0.54-0.77
UMI1220	IL	NM	O	3.2±0.1	9	3.0-3.4	0.45±0.26	8	0.33-0.56
UMI1230	IL	NM	O	3.0±0.8	9	2.8-3.2	0.72±0.47	8	0.60-0.84
LM18	IL	NM	R	2.8±0.2	9	2.7-3.0	0.26±0.21	8	0.15-0.38
CML142	IL	NM	W	2.9±0.3	9	2.7-3.1	0.19±0.14	8	0.08-0.31
CML150	IL	NM	W	1.7±0.0	9	1.5-1.9	0.16±0.10	8	0.04-0.28
CML176	IL	NM	W	1.8±0.2	9	1.6-1.9	0.74±0.04	6	0.61-0.88
BML15	IL	NM	Y	2.8±0.1	9	2.6-3.0	0.56±0.38	8	0.44-0.68
BML6	IL	NM	Y	3.5±0.3	15	3.4-3.7	0.31±0.14	14	0.22-0.40
CM145	IL	NM	Y	3.2±0.3	9	3.0-3.3	0.36±0.22	8	0.24-0.48
CM152	IL	NM	Y	3.4±0.3	9	3.2-3.6	0.37±0.27	8	0.25-0.48
CM212	IL	NM	Y	2.8±0.1	9	2.6-3.0	0.28±0.02	6	0.14-0.42
CML161	IL	NM	Y	2.8±0.2	9	2.6-2.9	0.25±0.13	8	0.13-0.37
CML169	IL	NM	Y	2.9±0.4	9	2.8-3.1	0.25±0.29	8	0.13-0.37
CML186	IL	NM	Y	2.5±0.1	9	2.4-2.7	0.60±0.47	8	0.49-0.72
HKI1105	IL	NM	Y	2.2±0.2	9	2.0-2.4	0.49±0.13	8	0.37-0.61
LM16	IL	NM	Y	3.3±0.1	9	3.2-3.5	0.07±0.05	8	0.05-0.19
V335	IL	NM	Y	3.1±0.4	9	2.9-3.2	0.23±0.24	8	0.11-0.35
V341	IL	NM	Y	2.6±0.2	9	2.4-2.7	0.34±0.22	8	0.22-0.46
V345	IL	NM	Y	2.5±0.1	9	2.4-2.7	0.28±0.11	8	0.16-0.40
V346	IL	NM	Y	2.0±0.1	9	1.9-2.2	0.37±0.29	8	0.25-0.49

Contd ....

Genotypes	GT	MT	KC	PA(mg/g)	R	LCL-UCL	Pi(mg/g)	R	LCL-UCL
V372	IL	NM	Y	3.1±0.5	9	2.9-3.3	0.18±0.13	8	0.06-0.30
V373	IL	NM	Y	3.0±0.2	9	2.8-3.2	0.17±0.11	8	0.05-0.29
HKI163	IL	QPM	O	3.1±0.5	9	2.9-3.2	0.24±0.19	8	0.12-0.36
VQL2	IL	QPM	O	3.2±0.2	9	3-3.3.0	0.36±0.27	8	0.24-0.48
DMR QPM106	IL	QPM	Y	2.9±0.1	9	2.7-3.1	0.29±0.14	8	0.17-0.41
HKI161	IL	QPM	Y	3.1±0.2	9	2.9-3.3	0.57±0.39	8	0.45-0.69
HKI193-1	IL	QPM	Y	3.2±0.1	9	3.0-3.4	0.45±0.26	8	0.33-0.56
HKI193-2	IL	QPM	Y	3.3±0.4	9	3.2-3.5	0.28±0.20	8	0.16-0.40
VQL1	IL	QPM	Y	2.8±0.4	9	2.6-3.0	0.53±0.03	2	0.30-0.77
Bad Makai	LR	NM	Y	2.6±0.3	9	2.4-2.7	0.19±0.01	6	0.06-0.33
Chota Kanchan	LR	NM	Y	3.2±0.1	9	3.0-3.4	0.16±0.01	6	0.03-0.30
Chutri Makka	LR	NM	Y	3.0±0.2	9	2.9-3.2	0.14±0.01	6	0.00-0.27
Desi Ridhhi	LR	NM	Y	3.1±0.2	9	2.9-3.3	0.33±0.02	6	0.20-0.47
GD Bad	LR	NM	Y	2.7±0.1	9	2.6-2.9	0.19±0.01	6	0.05-0.32
Makka Dhebri	LR	NM	Y	3.7±0.3	9	3.6-3.9	0.28±0.02	6	0.15-0.42
Desi Summan	LR	NM	O	2.5±0.1	9	2.4-2.7	0.16±0.01	6	0.02-0.29
Madhur Makka	LR	SC	Y	3.0±0.2	9	2.8-3.1	0.15±0.01	6	0.01-0.28
African Tall	OPV	NM	W	2.9±0.2	6	2.7-3.1	0.18±0.03	6	0.05-0.32
Narmada Moti	OPV	NM	W	3.7±0.2	6	3.5-3.9	0.34±0.05	6	0.20-0.47
Amar [D941]	OPV	NM	Y	4.1±0.3	6	3.9-4.3	0.19±0.03	6	0.06-0.33
Gaurav [D931]	OPV	NM	Y	2.8±0.2	6	2.6-3.0	0.26±0.04	6	0.13-0.40
Win orange sweet corn	OPV	SC	Y	4.1±0.3	6	3.8-4.3	0.20±0.03	6	0.07-0.34
CO6	SCH	NM	O	2.5±0.1	6	2.2-2.7	0.21±0.04	6	0.08-0.35
COH[M]10	SCH	NM	O	2.6±0.1	6	2.4-2.8	0.33±0.05	6	0.19-0.46
COH[M]7	SCH	NM	O	3.0±0.2	6	2.8-3.2	0.25±0.04	6	0.12-0.39
COH[M]8	SCH	NM	O	3.0±0.2	6	2.8-3.2	0.21±0.03	6	0.07-0.34
COH[M]9	SCH	NM	O	2.8±0.2	6	2.6-3.0	0.17±0.03	6	0.03-0.30
DHM113	SCH	NM	O	3.0±0.2	6	2.8-3.3	0.15±0.03	6	0.02-0.29
DHM117	SCH	NM	O	3.6±0.2	6	3.4-3.9	0.40±0.06	6	0.26-0.54
DHM119	SCH	NM	O	2.9±0.2	6	2.7-3.1	0.23±0.04	6	0.09-0.37
DHM121	SCH	NM	O	3.9±0.2	6	3.7-4.1	0.27±0.04	6	0.14-0.41
HM10	SCH	NM	O	3.7±0.2	6	3.5-3.9	0.33±0.05	6	0.19-0.46
HM11	SCH	NM	O	2.9±0.2	6	2.7-3.1	0.40±0.06	6	0.26-0.54
HM4	SCH	NM	O	2.9±0.2	6	2.6-3.1	0.19±0.03	6	0.06-0.33
HM8	SCH	NM	O	2.1±0.1	6	1.9-2.3	0.12±0.02	6	0.02-0.25
HM9	SCH	NM	O	3.3±0.2	6	3.0-3.5	0.34±0.05	6	0.20-0.47
PMH1	SCH	NM	O	2.5±0.1	6	2.3-2.7	0.47±0.08	6	0.33-0.60
PMH2	SCH	NM	O	3.1±0.2	6	2.9-3.3	0.14±0.02	6	0.00-0.27
PMH3	SCH	NM	O	2.6±0.1	6	2.4-2.8	0.35±0.06	6	0.21-0.48
PMH6	SCH	NM	O	2.7±0.2	6	2.5-2.9	0.47±0.08	6	0.33-0.60
PMH7	SCH	NM	O	2.6±0.1	6	2.4-2.8	0.47±0.08	6	0.34-0.61

Contd ....

Genotypes	GT	MT	KC	PA(mg/g)	R	LCL-UCL	Pi(mg/g)	R	LCL-UCL
PMH8	SCH	NM	O	3.0±0.2	6	2.8-3.2	0.29±0.05	6	0.15-0.43
PMH9	SCH	NM	O	3.4±0.2	6	3.2-3.7	0.95±0.15	6	0.81-1.09
Prakash	SCH	NM	O	3.6±0.2	6	3.4-3.9	0.22±0.04	6	0.08-0.36
PMH5	SCH	NM	R	3.2±0.2	6	3.0-3.4	0.37±0.06	6	0.24-0.51
HM12	SCH	NM	W	3.6±0.2	6	3.3-3.8	0.43±0.07	6	0.29-0.57
Bajaura Makka1	SCH	NM	Y	4.0±0.2	6	3.7-4.2	0.23±0.04	6	0.09-0.36
Pantsankar Makka1	SCH	NM	Y	4.0±0.2	6	3.8-4.2	0.35±0.06	6	0.22-0.49
Pratap Kanchan2	SCH	NM	Y	3.2±0.1	9	3.1-3.4	0.23±0.01	6	0.09-0.36
VMH15	SCH	NM	Y	2.9±0.2	6	2.7-3.1	0.23±0.04	6	0.09-0.37
VMH25	SCH	NM	Y	2.9±0.2	6	2.6-3.1	0.39±0.06	6	0.25-0.53
VMH27	SCH	NM	Y	4.0±0.2	6	3.8-4.2	0.10±0.02	6	0.04-0.23
VMH33	SCH	NM	Y	2.8±0.2	6	2.6-3.1	0.14±0.02	6	0.01-0.28
VMH35	SCH	NM	Y	3.2±0.2	6	2.9-3.4	0.30±0.05	6	0.16-0.44
VMH39	SCH	NM	Y	3.9±0.2	6	3.7-4.2	0.24±0.04	6	0.10-0.37
VMH43	SCH	NM	Y	2.8±0.2	6	2.6-3.0	0.26±0.04	6	0.12-0.39
VMH45	SCH	NM	Y	4.5±0.3	6	4.3-4.7	0.27±0.04	6	0.13-0.40
VMH9	SCH	NM	Y	2.7±0.2	6	2.5-2.9	0.31±0.05	6	0.18-0.45
HQPM4	SCH	QPM	O	3.4±0.2	6	3.2-3.6	0.15±0.03	6	0.02-0.29
HQPM5	SCH	QPM	O	2.8±0.2	6	2.6-3.0	0.35±0.06	6	0.21-0.48
Shaktiman3	SCH	QPM	O	2.9±0.2	6	2.7-3.1	0.20±0.03	6	0.07-0.34
Shaktiman4	SCH	QPM	O	2.6±0.1	6	2.4-2.8	0.15±0.03	6	0.01-0.28
Shaktiman5	SCH	QPM	O	3.0±0.2	6	2.8-3.2	0.11±0.02	6	0.02-0.25
Shaktiman1	SCH	QPM	W	2.2±0.1	6	2.0-2.5	0.33±0.05	6	0.19-0.46
Pratap QPM Hybrid1	SCH	QPM	Y	3.2±0.2	6	3.0-3.4	0.26±0.04	6	0.13-0.40
Vivek QPM9	SCH	QPM	Y	3.3±0.2	6	3.1-3.5	0.22±0.04	6	0.08-0.35

GT = Germplasm type; MT = Maize type; KC = Kernel colour; PA = Phytic acid; R = Number of replicates; LCL-UCL = Lower control limit-upper control limit and Pi = Inorganic phosphate

Correlation between PA and P_i

The correlation coefficient between PA and Pi across genotypes was moderate negative (-0.35) but highly significant (P = 0.00024). However there was no significant correlation between PA and P_i in sub-classes namely inbred lines (R=-0.12, P=0.42), landraces (R=0.48, P=0.23), OPVs (R=-0.069, P=0.91) and SCHs (R=0.025, P=0.87). The correlation coefficient between PA and P_i when calculated within different sub-groups of germplasm types, maize types and kernel colour, it was not consistent and varied across subgroups.

Discussion

The normal distribution of residuals for PA estimates of genotypes, germplasm types, maize types and kernel colour indicates the fulfilling the assumption of one-way ANOVA and reliability of the PA estimates and its comparison between genotypes, germplasm types, maize types and kernel colour types. Similarly, the estimates and comparison for P_i are also valid for genotypes. However, the non-normal distribution of germplasm types, maize types and kernel colour for P_i content could be attributed to the inherent genetic heritable property of the chosen germplasm which needs further validation in a larger number of samples. The results of the present study also indicate that the relationship between PA and P_i content may not be constant but may vary depending on the genetic background.

The maize germplasm in the current study possessed significant variability for PA content.

Table 3. The mean PA and P_i in different germplasm types of maize

Class	PA (mg/g)			Pi (mg/g)		
	Mean	SD	R	Mean	SD	R
IL	2.8[c]	0.6	471	0.43[a]	0.40	406
LR	3.0[bc]	0.4	72	0.20[b]	0.07	48
OPV	3.5[a]	0.6	30	0.24[b]	0.07	30
SCH	3.1[b]	0.5	267	0.29[b]	0.15	264
Mu	1.5[c]	0.3	30	1.62[a]	0.26	24
N	3.0[b]	0.6	684	0.32[b]	0.22	614
Q	3.0[b]	0.4	111	0.30[b]	0.21	98
SC	3.4[a]	0.6	15	0.18[b]	0.04	12
Orange	3.0[a]	0.5	381	0.34[a]	0.23	354
Red	3.0[ab]	0.3	15	0.31[a]	0.17	14
White	2.6[b]	0.8	51	0.33[a]	0.20	46
Yellow	3.0[ab]	0.7	393	0.39[a]	0.40	334

IL=inbred lines; LR=landraces; OPV=open-pollinated varieties; SCH=single cross hybrids, Mu=low phytic acid (LPA) mutants; N=normal maize; Q=quality protein maize (QPM); SC=sweet corn

Previous studies on screening of maize germplasm for PA content have classified the germplasm into high, medium and low PA lines based on the phytic acid content. However, the PA levels which were considered to classify the germplasm into high, medium, and low differed across different studies (Pramitha et al. 2019; Chiangmai et al. 2011; Lorenz et al. 2007, 2008). The classification of germplasm into high, medium and low PA varies depending on the germplasm analysed. The present study classified 106 genotypes into high (>3.5 mg/g), medium (2.5-3.5 mg/g) and low (<2.5 mg/g) based on the PA level, which largely in conformity with the classification made by Lorenz et al. (2007) (Suppl. Table S2). The sub-classification within different germplasm types, maize types and kernel colour types is given in Table 2. Interestingly PA content of one inbred line CML150 has the same numerical value of PA content as that of one of the LPA mutant i.e. LPA2, whereas other inbred lines, CML176 did not show a significant difference between either of the two LPA mutants. Similarly V346, another inbred line did not differ statistically with one of the LPA mutant lines i.e. LPA2. To ascertain the genetic relatedness of these lines, the genetic background was looked into and found that all three lines are from a different genetic background (Supplementary Table S1). Interestingly both the inbred lines namely CML176 and CML150 are of white kernel colour. However, there is a need to ascertain the heterotic behaviour of the lines as CML150 and CML176 do not share the common pedigree. CML176 was derived through recycling involving two different pedigrees namely population 63 (P63) and population 67 (P67) but the phytate level is numerically higher than the CML150. On the contrary, CML186 do share the pedigree of CML176 but the phytate level is higher than the CML176. Probably due to the mixing of the genetic background of population 63 (P63) during recycling to develop CML176, the phytate got reduced. Thus it can safely be attributed that P63 might have contributed to the reduction of phytic acid in the CML176. However, further studies are required to understand the molecular basis for the low level of PA in these inbred lines.

In was interesting to note that sweet corn genotype recorded higher PA content than other types of maize. In maize, unlike in other cereals like rice and wheat, the PA is largely concentrated in the germ (O'Dell et al. 1972). Since sweet corn contains a negligible proportion of endosperm, which could be the reason for increased PA content in sweet corn. However, there is a need to study the PA accumulation in different stages of kernel development in sweet corn. Some studies have been carried out in different crops to correlate PA content with other biochemical components of the kernel (Raboy et al. 1991). In the present study the mean range in PA content did not differ significantly between QPM and normal maize. On the contrary, the white kernel maize mostly preferred for food differed significantly from other kernel colour maize with lower PA content. Probably PA might also contribute to the organoleptic rejection of coloured maize and acceptance of white maize as human food.

In contrast to PA, the P_i content did not show a significant difference between different kernel colour maize. The highest Pi among the inbred lines was in CML176, it is also the line with the lowest PA content. Even though it did not differ significantly with LPA mutants for PA content but differed significantly for P_i content. However, in contrast to LPA mutant, the P_i content in CML176 was significantly lower. The underlying mechanism needs to be explored which requires further investigation. Among OPVs, Narmada Moti, a white kernel colour composite had relatively higher Pi as compared to others.

Although there is a moderate correlation between PA and Pi, the study confirms that phosphate levels do not get affected due to reduced phytate. Besides,

the results also indicated that each category of germplasm or maize types and kernel colour can have equal probability to show variability for the P_i content. It depends on the genetic background as well thus relative skewness in the residues for P_i content observed in the present study was due to the low frequency of LPA maize germplasm. The results of the present study opens-up several follow-up studies which require further investigation on the correlation between seed phytate and phosphorous uptake by the genotype, seed phytate and its relation with available phosphorous in the soil, relationship between seed phytate and phosphorous use efficiency of genotype, genotype × environment interaction for total seed phytate content, relationship between total seed phytate and other biochemical components of seeds like protein and other secondary metabolites. The decrease in phytic acid in mature seeds is accompanied by a corresponding increase in inorganic phosphate (Pi) (Raboy et al. 2000). It is concluded that the development of LPA maize assumes much-more significance in the context of increasing uses of maize as diverse value-added food products. Maize is very rich in genetic diversity, the present study showed the possibility to get genotypes with reduced PA, if large-scale screening for reduced phosphate is undertaken.

Authors' Contribution

Conceptualization of research (CGK); Designing of the experiments (GGK); Contribution of experimental materials (CGK); Execution of field/ lab experiments and data collection (SSG, YKR, TG); Analysis of data and interpretation (CGK, AS, RKP, AKD, FH); Preparation of the manuscript (CGK, SR, SN, SBS, AK, RNG, FH).

Declaration

The authors declare no conflict of interest

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Supplimentary Table S1.

Genotypes	PA (mg/g)	Groups	Classification
Vivek Maize Hybrid-45	4.5	a	High
Amar [D-941]	4.1	ab	High
HKI-1128	4.1	ab	High
Win Orange Sweet Corn	4.1	ab	High
Bajaura Makka-1	4.0	abcd	High
Pantsankar Makka-1	4.0	abc	High
Vivek Maize Hybrid-27	4.0	abc	High
DHM-121	3.9	abcde	High
Vivek Maize Hybrid-39	3.9	abcd	High
HM-10	3.7	bcdefg	High
Makka Dhebri	3.7	bcdef	High
Narmada Moti	3.7	bcdefgh	High
DHM-117	3.6	bcdefghi	High
HM-12	3.6	bcdefghij	High
Prakash	3.6	bcdefghi	High
BML-2	3.5	bcdefghij	Intermediate
BML-6	3.5	bcdefghij	Intermediate
UMI-1200	3.5	bcdefghij	Intermediate
BML-45	3.4	cdefghijkl	Intermediate
CM-139	3.4	defghijklm	Intermediate
CM-152	3.4	cdefghijk	Intermediate
HQPM-4	3.4	cdefghijklm	Intermediate
PMH-9	3.4	bcdefghijk	Intermediate
HKI-193-2	3.3	efghijklm	Intermediate
HM-9	3.3	efghijklmno	Intermediate
LM-16	3.3	efghijklmn	Intermediate
Vivek QPM-9	3.3	efghijklmn	Intermediate
Chota Kanchan	3.2	ghijklmno	Intermediate
CM-145	3.2	ghijklmnop	Intermediate
HKI-193-1	3.2	fghijklmno	Intermediate
PMH-5	3.2	fghijklmno	Intermediate
Pratap Kanchan-2	3.2	fghijklmno	Intermediate
Pratap QPM Hybrid-1	3.2	fghijklmno	Intermediate
UMI-1220	3.2	ghijklmno	Intermediate
Vivek Maize Hybrid-35	3.2	ghijklmnop	Intermediate
VQL-2	3.2	ghijklmnop	Intermediate
Desi Ridhhi	3.1	hijklmnopq	Intermediate
HKI-161	3.1	hijklmnopq	Intermediate
HKI-163	3.1	ijklmnopqr	Intermediate
LM-15	3.1	ijklmnopq	Intermediate
PMH-2	3.1	ghijklmnopq	Intermediate

V-335	3.1	ijklmnopqr	Intermediate
V-372	3.1	ijklmnopq	Intermediate
BML-7	3.0	jklmnopqrs	Intermediate
Chutri Makka	3.0	jklmnopqrs	Intermediate
COH[M]-7	3.0	klmnopqrs	Intermediate
COH[M]-8	3.0	jklmnopqrs	Intermediate
DHM-113	3.0	ijklmnopqrs	Intermediate
LM-14	3.0	klmnopqrs	Intermediate
Madhur Makka	3.0	klmnopqrs	Intermediate
PMH-8	3.0	jklmnopqrs	Intermediate
Shaktiman-5	3.0	jklmnopqrs	Intermediate
UMI-1210	3.0	jklmnopqrs	Intermediate
UMI-1230	3.0	jklmnopqrs	Intermediate
V-373	3.0	jklmnopqrs	Intermediate
African Tall	2.9	klmnopqrs	Intermediate
CML-142	2.9	klmnopqrst	Intermediate
CML-163	2.9	klmnopqrs	Intermediate
CML-169	2.9	klmnopqrs	Intermediate
DHM-119	2.9	klmnopqrs	Intermediate
DMR QPM-106	2.9	klmnopqrs	Intermediate
HM-11	2.9	klmnopqrs	Intermediate
HM-4	2.9	lmnopqrst	Intermediate
LM-13	2.9	lmnopqrst	Intermediate
Shaktiman-3	2.9	klmnopqrst	Intermediate
Vivek Maize Hybrid-15	2.9	klmnopqrst	Intermediate
Vivek Maize Hybrid-25	2.9	lmnopqrst	Intermediate
BML-15	2.8	lmnopqrstu	Intermediate
CM-212	2.8	mnopqrstu	Intermediate
CML-161	2.8	nopqrstu	Intermediate
COH[M]-9	2.8	lmnopqrstu	Intermediate
Gaurav [D-931]	2.8	mnopqrstu	Intermediate
HQPM-5	2.8	lmnopqrstu	Intermediate
LM-18	2.8	lmnopqrst	Intermediate
UMI-1201	2.8	nopqrstu	Intermediate
UMI-1205	2.8	nopqrstu	Intermediate
Vivek Maize Hybrid-33	2.8	lmnopqrst	Intermediate
Vivek Maize Hybrid-43	2.8	lmnopqrstu	Intermediate
VQL-1	2.8	lmnopqrstu	Intermediate
CM-140	2.7	opqrstuv	Intermediate
GD Bad	2.7	opqrstu	Intermediate
HKI-323	2.7	opqrstuvw	Intermediate
LM-19	2.7	opqrstu	Intermediate
PMH-6	2.7	opqrstuv	Intermediate

Vivek Maize Hybrid-9	2.7	opqrstuvw	Intermediate
Bad Makai	2.6	qrstuvwx	Intermediate
COH[M]-10	2.6	opqrstuvwx	Intermediate
PMH-3	2.6	opqrstuvwx	Intermediate
PMH-7	2.6	pqrstuvwx	Intermediate
Shaktiman-4	2.6	opqrstuvw	Intermediate
V-341	2.6	pqrstuvwx	Intermediate
CML-186	2.5	rstuvwx	Low
CO-6	2.5	stuvwx	Low
Desi Summan	2.5	rstuvwx	Low
LM-17	2.5	rstuvwx	Low
PMH-1	2.5	rstuvwx	Low
V-345	2.5	qrstuvwx	Low
CML-165	2.2	uvwxy	Low
HKI-1105	2.2	vwxy	Low
Shaktiman-1	2.2	tuvwxy	Low
HM-8	2.1	wxyz	Low
V-346	2.0	xyz	Low
CML-176	1.8	yzA	Low
CML-150	1.7	yzA	Low
LPA-2	1.7	zA	Low
LPA-1	1.3	A	Low

Supplimentary Table S2.

Genotypes	Pi (mg/g)	Groups
LPA-2	1.63	a
LPA-1	1.61	a
PMH-9	0.95	b
CML-176	0.74	bc
BML-7	0.72	bc
UMI-1230	0.72	bc
HKI-323	0.67	bcd
UMI-1210	0.65	bcde
CML-186	0.60	bcdef
HKI-161	0.57	bcdefg
BML-15	0.56	bcdefgh
VQL-1	0.53	bcdefghi
HKI-1105	0.49	cdefghi
UMI-1205	0.49	cdefghi
PMH-7	0.47	cdefghi
PMH-1	0.47	cdefghi
PMH-6	0.47	cdefghi
UMI-1220	0.45	cdefghi
HKI-193-1	0.45	cdefghi
UMI-1201	0.44	cdefghi
LM-13	0.44	cdefghi
HM-12	0.43	cdefghi
DHM-117	0.40	cdefghi
HM-11	0.40	cdefghi
Vivek Maize Hybrid-25	0.39	cdefghi
UMI-1200	0.38	cdefghi
HKI-1128	0.38	cdefghi
PMH-5	0.37	cdefghi
V-346	0.37	cdefghi
CM-152	0.37	cdefghi
VQL-2	0.36	cdefghi
CM-145	0.36	cdefghi
Pantsankar Makka-1	0.35	cdefghi
PMH-3	0.35	cdefghi
HQPM-5	0.35	cdefghi
CM-140	0.34	defghi
V-341	0.34	defghi
LM-14	0.34	efghi
Narmada Moti	0.34	efghi
HM-9	0.34	efghi
Desi Ridhhi	0.33	efghi
HM-10	0.33	efghi
COH[M]-10	0.33	efghi
Shaktiman-1	0.33	efghi
Vivek Maize Hybrid-9	0.31	efghi
BML-6	0.31	fghi
Vivek Maize Hybrid-35	0.30	fghi
LM-19	0.30	fghi
PMH-8	0.29	fghi
DMR QPM-106	0.29	fghi
Makka Dhebri	0.28	fghi
V-345	0.28	fghi
CM-212	0.28	fghi
HKI-193-2	0.28	fghi
BML-2	0.27	fghi
DHM-121	0.27	fghi
Vivek Maize Hybrid-45	0.27	fghi
LM-18	0.26	fghi
Gaurav [D-931]	0.26	fghi
Pratap QPM Hybrid-1	0.26	fghi
Vivek Maize Hybrid-43	0.26	fghi
CML-161	0.25	fghi
LM-15	0.25	fghi
CML-169	0.25	fghi
COH[M]-7	0.25	fghi
BML-45	0.24	fghi
HKI-163	0.24	fghi
Vivek Maize Hybrid-39	0.24	fghi
V-335	0.23	ghi
Vivek Maize Hybrid-15	0.23	ghi
DHM-119	0.23	ghi
Bajaura Makka-1	0.23	ghi
Pratap Kanchan-2	0.23	ghi
Prakash	0.22	ghi
Vivek QPM-9	0.22	ghi
CO-6	0.21	ghi
COH[M]-8	0.21	ghi
CM-139	0.21	ghi
Win Orange Sweet Corn	0.20	ghi
Shaktiman-3	0.20	ghi
Amar [D-941]	0.19	ghi
Bad Makai	0.19	ghi

Genotypes	Pi (mg/g)	Groups
HM-4	0.19	ghi
CML-142	0.19	hi
GD Bad	0.19	hi
African Tall	0.18	hi
V-372	0.18	i
V-373	0.17	i
COH[M]-9	0.17	i
CML-150	0.16	i
Chota Kanchan	0.16	i
Desi Summan	0.16	i
HQPM-4	0.15	i
DHM-113	0.15	i
Madhur Makka	0.15	i
Shaktiman-4	0.15	i
Vivek Maize Hybrid-33	0.14	i
CML-163	0.14	i
CML-165	0.14	i
PMH-2	0.14	i
Chutri Makka	0.14	i
HM-8	0.12	i
Shaktiman-5	0.11	i
LM-17	0.11	i
Vivek Maize Hybrid-27	0.10	i
LM-16	0.07	i