Original Paper Open Access

Screening of phytic acid and inorganic phosphorus contents in corn inbred lines and F_1 hybrids in tropical environment

Pantipa Na Chiangmai[1]*, Phakatip Yodmingkhwan[1], Phrutiya Nilprapruck[1], Chokechai Aekatasanawan[2], Mana Kanjanamaneesathian[1]

[1]Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Cha-Am, Phetchaburi, 76120, Thailand
[2]National Corn and Sorghum Research Center, Pak-Chong, Inseechandrastitya Institute for Crops Research and Development, Kasetsart University, Nakhon Ratchasima, 30320, Thailand
*Corresponding author: E-mail: pantipa@su.ac.th

Abstract

Seeds of inbred lines and F_1 hybrids of corn (*Zea mays* L) grown from late rainy season of 2007 to early rainy season of 2009 at the National Corn and Sorghum Research Center, Thailand, were analyzed to determine the contents of phytic acid (PA) and inorganic phosphorous (InP). The PA contents in the tested inbred lines and F_1 hybrids ranged from 800 to 1,000 mg per 100 g seed. The two groups of Ki inbred lines (Nursery no. 122 in the 2007 late rainy season), based upon the analysis of PA, were categorized to low (L) (the value of <900 mg/100 g seed) and medium-high (M-H) (≥900 mg/100 g seed). The medium-high PA group gave the means of rating scores by visual selection of plant aspect and ear aspect better than those of the low PA group. Their correlations also revealed that the high PA inbreds possessed better both characteristics ($P < 0.05$) than those of the low PA inbreds. Most of negative and non-significant correlations were observed between PA and InP. Correlations between PA and grain yield of F_1 hybrids tested in the 2008 late rainy season and 2009 early rainy season were positive and non-significant. Correlations between InP and grain yield of the F_1 hybrids was negative and non-significant in the 2008 late rainy season, but highly significant ($P < 0.01$) in the 2009 early rainy season. From the results of genetic variation of the PA and InP contents of the Ki and new inbreds, medium PA inbred lines (M) (900≤x<950 mg/100g seed) were differentiated from high PA inbred lines (H) (≥950 mg/100g seed). The selected low PA hybrids with high grain yield can be achieved by using the M x L and M x M hybrid patterns which had lower PA contents than the means of the experiments in two Trials (Trial no. 916-918 and Trial no. 904-906) or two seasons.

Keywords: phytic acid, inorganic phosphorus, corn, inbred line, hybrid

Introduction

Corn has been utilized in producing feed for animal production in the countries around the world (Abbassian, 2006), including Thailand. Corn grain is a major component used in producing feedstuff to serve the poultry, pig and other livestock with the demand in Thailand at 3.1 million tons, worth around $US 406.1 million per annum (Abbassian, 2006). This demand for corn is expected to expand enormously in 2011 as Thailand has expected to increase the production of meat for export (Office of Agricultural Economics, 2011).

The important of animal production industry and the vast quantity of feedstuff used in Thailand means that management to alleviate the impact of waste from the animal industry is crucial (Cromwell and Coffey, 1991; Sharpley et al, 1994). In Thailand, there is a wide range of farm in which management to cope with waste problems is varied, dependent upon capital input, farm size and adopted technology. For instance, the large farms owned by a large agricultural conglomerate may deal with the waste by converting it into biogas which in turn can be utilized in the operation to run the farm. This practice minimizes environmental hazard and maximizes waste usage. In a relatively smaller farm, waste may be collected, processed and turned into organic fertilizers.

However, for the small household production in the backyard and the farms with no waste treatment facility, the waste may be disposed to the adjacent areas, causing environmental problems such as eutrophication (Sharpley et al, 1994). The phytic acid (PA), an organic compound in corn grains, is a substance which plays a role in causing this environmental hazard. The PA content in corn seeds was reported to account for about 75-80% of total phosphorus (P) (Raboy et al, 2000; Tongoona, 2005). If the content of this substance in corn grains is reduced, the environmental problem can be minimized.

The animal production industry has used the enzyme, synthetic phytase, to reduce the content of PA in this grain, the practice increasing the cost of feedstuff production (Cromwell et al, 1995; Liu et al, 1997; Liu et al, 1998). However, the negative pleiotropic effects caused by a low PA content in the kernel has been reported by several authors studying low phytic acid mutants; i.e. seed dry weight loss ranging from 4-23% (Raboy et al, 2000), decrease in germination

 RECEIVED 29/10/2011

capacity and rate (Pilu et al, 2005), decrease in kernel size (Lorenz et al, 2007; 2008) and low germination capacity (Doria et al, 2009). To solve this problem and alleviate the burden of the animal industry, the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University and the National Corn and Sorghum Research Center (NCSRC), Kasetsart University, Thailand has initiated a project with the aim to breed the corn hybrids with low PA trait and high yield.

This research aimed (1) to determine the contents of PA and inorganic phosphorous (InP) in available inbred lines and F_1 hybrids in the germplasm, (2) to categorize the corn inbred lines based upon the PA content, and (3) to investigate the correlations among the contents of these substances with some populations of both inbred lines and hybrids.

Materials and Methods

Genetic materials

Nine populations of inbred nurseries (Ki and new inbred lines) (Nursery no. 121, 122, 40, 41, 77, and 78-79), and F_1 hybrid trials (Trial no. 901-915, 916-918, and 904-906) (Figure 1) were planted from the late rainy season in 2007 until the early rainy season in 2009 at the NCSRC, Thailand.

Two seeds were planted in each hill with the distances of 0.20 m between hills within 5 m rows and spaced 0.75 m between rows. After planting 14 days, plants were thinned to one plant per hill. Fertilizer was broadcasted before planting at rates of 25 kg ha^{-1} of nitrogen (N) and 31.25 kg of P_2O_5 and 86.25 kg ha^{-1} of N was side dressed at the 6-8 leaf stage. Seeds from these planting were used in the chemical analysis.

Chemical analysis to determine PA and InP contents

The PA was analyzed in the protocol as described by Haug and Lantzsch (1983). The seed samples were grounded and incubated at 70°C for three days. The PA extraction was carried out by adding 10 ml 0.2 N HCl to 0.5 g and placed on the horizontal shaker at room temperature for 24 hours. Two ml of ferric ammonium sulfate solution was added to the 1 ml of an aliquot of the extract. The mixture of this solution was boiled at 100°C for 30 min and left it at room temperature. This solution was then centrifuged at 5,000 rpm at 4°C for 15 min. Two ml of this supernatant was mixed with 3 ml of 2,2'-bipyridine solution with a vortex. After the mixing, the mixture solution was incubated for 30 min and the light absorbance was measured with a spectrophotometer at 530 nm.

The InP was analyzed with the method as described by Chen et al (1956). Chemical stocks included (1) 50% trichloroacetic acid (TCA) stock solution, (2) 12.5% TCA: 25 mM $MgCl_2$, (3) 6 N Sulfuric acid, (4) Chen'reagent (6 N Sulfuric acid + 2.5% ammonium molybdate + 10% ascorbic acid + deionized water in ratio of 1:1:1:2), and (5) 0.1 mg P/ml standard solution stock (KH_2PO_4 was incubated in the oven at 105°C for two hours and 0.4394 g of this substance was taken and dissolved in 1,000 ml of deionized water).

The InP was determined by grounding the seeds sample and incubated in the oven at 70°C for three days. Ten ml of 12.5% TCA: 25 mM $MgCl_2$ (cooled) was added in 0.5 g of sample and place on a horizontal shaker for 24 hours at room temperature. The

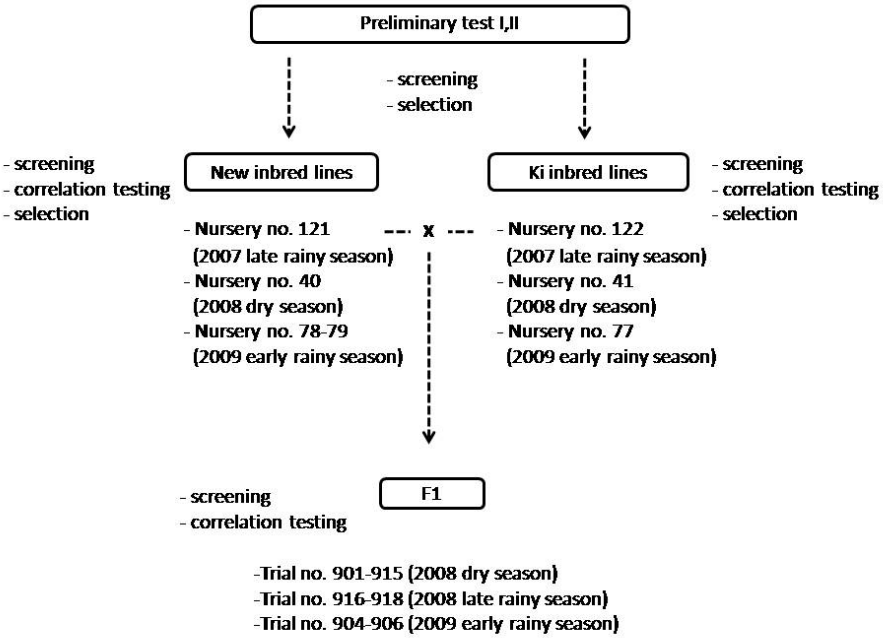

Figure 1 - The diagram outlining the protocol of this experiment.

solution was centrifuged at 5,000 rpm at 4°C for 15 min. The supernatant was adjusted to 25 ml with deionized water. This sample solution of 1.5 ml was added to Chen's reagent and then incubated at 37°C for one hour.

The concentrations of PA and InP at either 0, 5, 8, 10 or 15 µg P were used as standard solution. The light absorbance at 820 nm was measured.

Selection of inbred lines to form F_1 hybrids

Seeds of corn inbred lines from the germplasm bank at the NCSRC were used in this study. These inbred lines were subjected to chemical analysis to determine the values of PA and InP with the method as described by Haug and Lantzsch (1983) for the PA and by Chen et al (1956) for the InP. These preliminary chemical analysis were repeated twice (data not shown), in which the first round of the analysis carried out in 141 inbred lines followed by the second of 75 inbred lines.

After the first and second rounds of chemical analysis, seeds of the inbred lines which had high yield potential with resistance to corn downy mildew and other beneficial characteristics (such as drought and lodging resistance) were grouped based upon their genetic background [Ki (16 inbred lines) (Nursery no. 122, 41, and 77) and new inbred lines (26 lines) (Nursery no. 121, 40, and 78-79)] (Figure 1).

Seeds of both the Ki and new inbred lines were selected and planted in the 2007 late rainy season, and their kernels were subjected to the analysis to determine the PA content.

Some inbred lines from either Ki or new inbred lines with a combination of preferred characteristics (such as yield potential, resistance to downy mildew, drought and lodging) were selected for crossing to create F_1 population in the 2007 late rainy season. The seeds of these F_1 populations were planted in the following season (2008 dry season, 2008 late rainy season, and 2009 early rainy season) to evaluate the characteristics similar to their parents.

Correlation study between the contents of PA, InP, and grain yield

The results of the screening to determine PA and InP of the seeds from eight populations of the inbred nurseries and the hybrid trials (Nursery no. 122, 40, 41, 77, and 78-79, Trial no. 901-915, 916-918, and 904-906) were subjected to the correlation study between PA and InP. The correlation between either PA or InP with grain yield was studied in only two F_1 hybrids (Trial no. 916-918, 2008 late rainy season and

Table 1 - Means of PA, InP, plant aspect and ear aspect of 16 Ki inbred lines of corn growing in the Nursery no. 122 in the 2007 late rainy season at the NCSRC, Thailand.

Inbred lines	PA ± SE (mg/100g)	InP ± SE (mg/100g)	Plant aspect (1-5)[1]	Ear aspect (1-5)[1]
Low PA content				
Ki 6	879.80 ± 33.77	33.08 ± 0.87	4.0	2.5
Ki 20	885.78 ± 22.82	37.48 ± 1.84	3.0	2.0
Ki 52	886.23 ± 16.49	29.59 ± 0.69	3.0	1.5
Ki 15	891.78 ± 7.63	45.86 ± 0.71	4.0	4.0
Ki 23	894.57 ± 5.98	48.45 ± 2.14	3.0	1.5
Ki 10	898.21 ± 8.16	46.53 ± 0.69	3.0	2.0
Mean	889.40 ± 15.81	40.17 ± 1.16	3.3±0.5	2.3±0.9
Medium-High PA content				
Ki 12	908.92 ± 9.25	43.45 ± 1.68	3.5	2.0
Ki 48	912.13 ± 17.89	19.24 ± 0.49	1.0	1.0
Ki 11	915.04 ± 9.94	54.44 ± 1.76	3.5	2.0
Ki 16	915.51 ± 10.93	37.48 ± 2.10	4.0	2.5
Ki 3	919.39 ± 19.32	41.20 ± 0.33	2.0	1.0
Ki 17	922.64 ± 15.41	27.35 ± 0.26	4.0	1.5
Ki 47	925.74 ± 9.62	27.24 ± 0.90	1.0	1.5
Ki 25	928.51 ± 24.26	41.13 ± 0.82	3.0	2.0
Ki 46	933.17 ± 18.51	44.09 ± 1.21	1.0	1.0
Ki 51	953.41 ± 8.81	38.73 ± 1.67	1.0	1.0
Mean	923.45 ± 14.39	37.44 ± 1.12	2.4±1.3	1.6±0.6
Total mean	910.67 ± 4.39	38.47 ± 1.33	2.8±1.2	1.8±0.8
P value	0.1479	<0.0001		
F-test	ns	**		
t-test[2]	ns	ns	ns	ns

** Significant at the 0.01 probability level; ns, not significant
[1]Ratings 1-5; 1 = best, 5 = poorest
[2]Low PA vs Medium-High PA inbred lines

Trial no. 904-906, 2009 early rainy season).

The data from these populations for PA and InP contents was subjected to the correlation study. The diagram describing the whole experiment was shown in Figure 1.

Experimental design and data analysis

A Completely Randomized Design (CRD) was arranged in this experiment. After harvesting, three seed samples were collected from three selected plants. These seed samples were used for analyzing PA and InP in a laboratory.

Data of PA and InP was subjected to the analysis of variance (ANOVA), in which the F-test was used to test the statistical significance. Least Significant Difference (LSD) was employed to differentiate means of PA and InP ($P<0.05$), using R program version 2.13.0.

For F_1 hybrids Trial no. 916-918, and 904-906, the correlation between seed yield and PA was studied using the coefficient of correlation (r), in the formula as follows:

$$r = \frac{\frac{\sum(X-\overline{X})(Y-\overline{Y})}{n-1}}{\sqrt{\left[\frac{\sum(X-\overline{X})^2}{n-1}\right]\left[\frac{\sum(Y-\overline{Y})^2}{n-1}\right]}}$$

where X was the observed values in first variable, $\overline{X}$ was the average of mean of first variable, Y was the observed values in second variable, $\overline{Y}$ was the average of mean of second variable, *n* was the number for correlation analysis. The degree of freedom for the study in correlation was *n-2*.

Results

The screening test to determine the contents of PA and InP

The PA contents in seeds of both Ki and new inbred lines in these studies were 910.67 mg/100 g for PA in Nursery no. 122, 2007 late rainy season (Table 1) and they were 945.53 mg/100 g for PA in Nursery

Table 2 - Means of PA, InP, plant aspect and ear aspect of 26 new inbred lines of corn growing in the Nursery no. 40 in the 2008 dry season at the NCSRC, Thailand.

Inbred lines	PA ± SE (mg/100g)	InP ± SE (mg/100g)	Plant aspect (1-5)[1]	Ear aspect (1-5)[1]
Medium-High PA contents				
30A33-S10-140-1-5-6-3	904.23 ± 18.27	27.24 ± 0.90	1.5	1.0
30A10-S11-43-1-3	911.34 ± 19.47	20.20 ± 0.92	1.5	1.5
30A33-S9-13-1	914.34 ± 30.21	18.03 ± 1.38	1.0	1.0
C5218620-S7-154	926.16 ± 12.20	58.06 ± 0.24	1.0	2.0
SW1(S)C11(TC)C1-S9-21-3-2	926.31 ± 8.76	15.34 ± 0.10	2.0	1.0
30A33-S11-140-1-5-3-3	928.37 ± 16.70	32.31 ± 4.30	1.5	1.0
Suwan 3(S)C4(SF)-S11-150-1	934.10 ± 5.91	11.48 ± 0.16	1.0	2.0
KS6(S)C3(TC)C1-S9-19-2-2	938.12 ± 3.73	25.20 ± 1.12	3.5	2.5
Big949-S7-121-3	938.26 ± 18.61	30.00 ± 1.34	1.5	1.0
C5218620-S7-160	938.93 ± 3.43	23.38 ± 0.70	1.5	1.5
C5219041-S7-3-5	939.70 ± 4.03	18.15 ± 1.32	2.0	1.5
C5218620-S7-9-1	941.32 ± 13.83	19.76 ± 0.52	2.0	1.5
30A33-S9-87-1-2	945.87 ± 4.40	25.29 ± 0.68	1.0	1.5
C5219041-S7-62-4	947.12 ± 12.40	20.31 ± 0.82	1.5	1.5
Big949-S7-4	951.76 ± 8.60	23.49 ± 0.35	2.0	1.5
Big949-S7-30	952.83 ± 5.20	30.63 ± 0.63	1.5	1.0
C5218620-S7-20	952.84 ± 16.48	23.58 ±0.46	1.5	1.0
C5219041-S7-95	957.18 ± 8.96	26.50 ± 0.52	1.5	1.0
C5219041-S7-13-2	957.36 ± 10.79	27.78 ± 1.09	2.0	1.0
30A33-S12-84-2-6-2-3	957.51 ± 16.04	25.15 ± 0.40	1.5	1.5
30A33-S9-2-1	959.60 ± 6.12	18.94 ± 0.81	1.0	1.0
C5218620-S7-151	961.01 ± 5.57	31.54 ± 1.33	1.0	1.5
30A10-S11-44-1-2-1	964.02 ± 3.14	19.64 ± 0.46	1.0	1.0
C5219041-S7-51	972.27 ± 5.36	30.25 ± 1.48	1.0	1.0
C5219041-S7-45	977.11 ± 15.69	20.26 ± 0.38	1.0	1.0
Big949-S6-22	985.82 ± 7.43	31.79 ± 0.53	2.0	1.5
Total mean	945.53 ± 3.02	25.55 ± 0.99	1.5 ± 0.56	1.3 ± 0.40
P value	0.0029	<0.0001		
F test (0.05)	**	**		
LSD (0.05)	35.86	3.36		
LSD (0.01)	47.78	4.47		

** Significant at the 0.01 probability level
[1]Ratings 1-5; 1 = best, 5 = poorest
[2]Low PA vs Medium-High PA inbred lines

no. 40, 2008 dry season (Table 2).

The InP contents in seeds of both Ki (Nursery no. 122, 2007 late rainy season) and new inbred lines (Nursery no. 40, 2008 dry season) in these studies were 38.47 mg/100 g and 25.55 mg/100 g (Tables 1 and 2).

The two groups of these Ki inbred lines (Nursery no. 122 in the 2007 late rainy season), based upon the analysis of PA, were detected as corn inbreds with either low (the value of <900 mg/100 g seed) or medium-high (≥900 mg/100 g seed) (Table 1). The analysis of PA in new inbred lines resulted to one group of corn new inbred lines (Nursery no. 40 in the 2008 dry season) with only medium-high PA content (≥900 mg/100g seed) (Table 2). The medium-high PA group gave the means of rating scores by visual selection of plant aspect and ear aspect better than those of the low PA group (Table 1 and 2).

The mean values of the PA and InP of the Ki inbred lines, new inbred lines and F_1 hybrids was shown in Table 3. The mean values of PA of both Ki inbred lines (Nursery no. 41 in the 2008 dry season) (930.25 mg/100 g seed), new inbred lines (Nursery no. 40 in the 2008 dry season) (945.53 mg/100 g seed) and F_1 hybrid (Trial no. 901-915 in the 2008 dry season) (935.67 mg/100 g seed) were in the medium-high content category. The mean value of PA of this F_1 hybrid in Trial 901-915 (2008 dry season) (935.67 mg/100 g seed) was quite similar to those of F_1 hybrid in Trial 904-906 (2009 early rainy season) (940.40 mg/100 g seed). The value of PA of the F_1 hybrid in the Trial 904-906 was higher than that of Ki (Nursery no. 77 in the 2009 early rainy season) (919.44 mg/100 g seed), but was the same as that of the new inbred lines (Nursery no. 78-79 in the 2009 early rainy season) (940.40 mg/100 g seed). However, the value of PA of these two F_1 hybrids was higher than that of the F_1 hybrids (Trial no. 916-918) (the 2008 late rainy season) (892.71 mg/100 g seed) (Table 3).

The mean value of grain yield of hybrid in Trial 901-915 (2008 dry season) (6.99 t ha^{-1}) and that of the F_1 hybrid in Trial 904-906 (2009 early rainy season) (9.25 t ha^{-1}) was higher than that of the Trial 916-918 (2008 late rainy season) (3.60 t ha^{-1}) (Table 3).

Correlation study between the contents of PA, InP, and grain yield

The study showed that the value of correlation between PA and InP of the Ki inbred lines (from three populations) was negative with no statistically significant difference (Table 4). However, the correlation of new inbred lines was positive but not significant in one of the two populations (Table 4). In F_1 hybrid populations, two of the three populations had negative correlation. However, one of these populations had a positive correlation between a value of PA and InP with no statistically significant difference (Table 4).

The correlation study between the contents of PA and grain yield was positive in two F_1 hybrids populations. Nevertheless, there were no statistically significant differences in these correlations (Table 4). The correlation coefficients (*r*) between PA and grain yield of 98 F_1 hybrids evaluated in Trial no.916-918 in the 2008 late rainy seanson and of 46 F_1 hybrids grown in Trial no. 904-906 in the 2009 early rainy seanson were 0.107 and 0.172, respectively (Table 4, Figures 2 and 3). However, the study with InP and grain yield in F_1 hybrids showed that there was a negative correlation in two populations, with a statistical significant difference in one population (in Trial no. 904-906) and no statistically significant difference in the other (in Trial no. 916-918) (Table 4).

The correlations among PA, InP, plant aspect and ear aspect also revealed that the high PA inbreds possessed better both characteristics ($P < 0.05$) than those of the low PA ones (Table 5).

With a genetic variation of PA and InP contents of the Ki and new inbreds, a grouping of the tested corns into low PA inbred lines (L) (<900 mg/100 g seed), medium PA inbred lines (M) (900≤x<950 mg/100 g seed) and high PA inbred lines (H) (≥950 mg/100 g seed) was carried out. From the studies of the Trials no. 916-918 in 2008 late rainy season and

Table 3 - Means of the PA, InP and grain yield in mature seed of corn in nursery and trial numbers.

Nursery numbers/Trial numbers	PA ± SE (mg/100g)	InP ± SE (mg/100g)	Grain yield ± SE (t ha^{-1})
1. Ki inbred lines			
Nursery no. 122, 2007 late rainy season	910.67±20.14	38.47±9.23	na
Nursery no. 41, 2008 dry season	930.25±22.47	36.13±9.76	na
Nursery no. 77, 2009 early rainy season	919.44±25.99	28.43±9.08	na
2. New inbred lines			
Nursery no. 40, 2008 dry season	945.53±19.90	25.55±9.05	na
Nursery no. 78-79, 2009 early rainy season	940.40±23.43	28.73±9.69	na
3. F_1 hybrids			
Trial no. 901-915, 2008 dry season	935.67±24.27	40.78±20.56	6.99 ± 1.69
Trial no. 916-918, 2008 late rainy season	892.71±41.69	42.61 ± 1.24	3.60 ± 0.12
Trial no. 904-906, 2009 early rainy season	940.40±17.19	17.80±3.80	9.25 ± 0.14

na, not available

Table 4 - Correlation coefficients of PA and InP in inbred lines and F_1 hybrids in nursery and trial numbers.

Nursery numbers/Trial numbers	Traits	Correlation coefficient (r)
1. Ki inbred lines		
Nursery no. 122, 2007 late rainy season	PA vs InP	-0.019 ns
Nursery no. 41, 2008 dry season	PA vs InP	-0.098 ns
Nursery no. 77, 2009 early rainy season	PA vs InP	-0.080 ns
2. New inbred lines		
Nursery no. 40, 2008 dry season	PA vs InP	-0.069 ns
Nursery no. 78-79, 2009 early rainy season	PA vs InP	0.090 ns
3. F_1 hybrids		
Trial no. 901-915, 2008 dry season	PA vs InP	-0.023 ns
Trial no. 916- 918, 2008 late rainy season	PA vs InP	-0.151 ns
	PA vs Grain Yield	0.107 ns
	InP vs Grain Yield	-0.025 ns
Trial no. 904- 906, 2009 early rainy season	PA vs InP	0.081 ns
	PA vs Grain Yield	0.172 ns
	InP vs Grain Yield	-0.429 **

** Significant at the 0.01 probability level; ns, not significant.

Trials no. 904-906 in 2009 early rainy seasons, five hybrids with low PA content but with high grain yield can be selected (Table 6).

Discussion

The screening test to determine the contents of PA and InP

The PA content in this study is quite similar to that of the study conducted by Hidvegi and Lasztity (2002) which indicated that the PA contents in corn were 900 and 1,020 mg/100g in flint maize and yellow dent maize, respectively.

A narrow variation of the contents of PA and InP in corn seeds of the Ki and new inbred lines collected as a germplasm at NCSRC, Thailand was detected. Although environmental conditions may play a role in marginally changing the quantity of these substances between 2008 dry season and 2009 early rainy season both in the parents and F_1 hybrids, these conditions did affect the mean values of PA contents slightly.

The mean values of PA content in F_1 hybrids showed that the trial in 2008 dry season and that in 2009 early rainy season are not different. This means that the PA trait is genetically controlled. As the value of PA of this F_1 hybrids aligning toward better parents, this may indicate the role of genetic where dominant effect is exhibited. Nevertheless, environmental conditions may affect the PA phenotype as there is a difference between the PA value of the two populations of F_1 hybrids (Trial no. 901-915 and Trial no. 904-906) and the that of F_1 hybrids (Trial no. 916-918).

Although there is a narrow variation of the PA content in Ki and new inbred lines, it is believed that the PA trait is genetically controlled (as described above). The fact that there was a heterogeneity of the PA content in this study may warrant further genetical investigation and this better understanding would assist in inventing new corn lines with novel characteristics. As a result, we categorized these Ki inbred lines into two groups as either low- or medium-high PA contents, while the new inbred lines could be categorized into one group (as medium-high PA content).

The heterogeneity of the PA content in the matured corn seeds of Ki inbred lines may occur because the Ki inbred lines have a broad genetic background which has been developed from advanced popula-

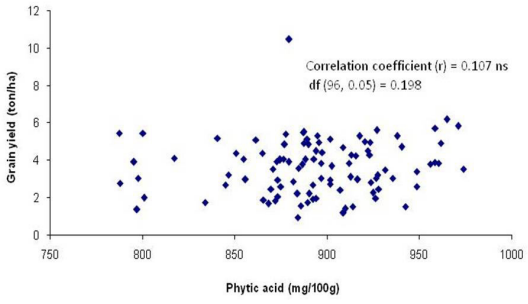

Figure 2 - Correlation coefficient (*r*) between traits of phytic acid vs grain yield of 98 F_1 hybrids evaluated in Trial no.916-918 in 2008 late rainy seanson at NCSRC, Thailand.

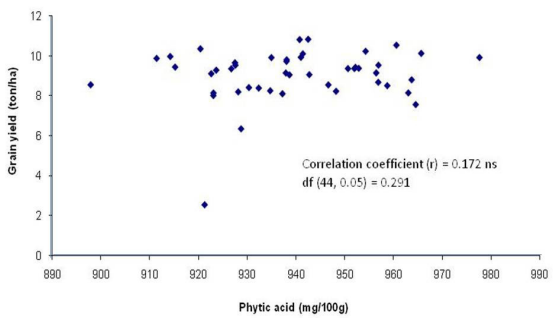

Figure 3 - Correlation coefficient (*r*) between traits of phytic acid vs grain yield of 46 F_1 hybrids evaluated in Trial no. 904-906 in 2009 early rainy seanson at NCSRC, Thailand.

Table 5 - Correlation coefficients among PA, InP, plant aspect and ear aspect of 16 Ki inbred lines and 26 new inbred lines growing in the Nursery no. 122 and 40 in the 2007 late rainy and 2008 dry seasons, respectively.

Traits	2007 late rainy season		2008 dry season	
	Plant aspect[1]	Ear aspect[1]	Plant aspect	Ear aspect
PA	-0.577*	-0.512*	-0.082	-0.148
InP	0.289	0.307	-0.089	0.150
Plant aspect		0.720**		0.421*

[1]Ratings 1-5; 1 = best, 5 = poorest

tions by S_1 recurrent selection for many cycles. This selection used various traits such as yield potential, plant performance, resistance to pests and drought tolerance as the characteristics for selecting potential lines to develop these inbred lines. In contrast, the homogeneity of the PA content in the new inbred lines is due to the fact that most of these lines were developed using the commercial corn hybrids having high yield potential (Goodman, 2005).

Correlation study between the contents of PA, InP, grain yield and some characteristics

The correlation between PA and InP contents showed the negative relationship in six populations (out of eight inbred lines and F_1 hybrids populations). This indicated that there may be a compensation of the P form in the corn seeds. The InP content may be also used as a chemical marker for the PA content in cereal grains including corn (Raboy et al, 2000; Ali et al, 2010).

The two groups of these Ki inbred lines (Nursery no. 122 in the 2007 late rainy season) were low PA (the value of <900 mg/100 g seed) and medium-high PA (≥900 mg/100 g seed) groups (Table 1). The medium-high PA group gave the means of rating scores by visual selection of plant aspect (i.e., root and stalk strength, leaf disease and insect resistance and husk cover) and ear aspects (i.e., ear and kernel size, rotten ears and insect damage) better than those of the low PA group. Their correlations also revealed that the high PA inbreds possessed better both characteristics ($P < 0.05$) than those of the low PA ones (Table 5). The results indicated that the Ki inbreds from the latter cycles of Suwan 1 improved by S_1 recurrent selection (Ki 25 from Suwan 1(S)C8 and Ki 46 from Suwan 1(S)C10 (HLT)C1) had higher PA contents and better performance than the earlier cycle inbreds (Ki 3, Ki 6, Ki 10, Ki 11, Ki 12, Ki 15, Ki 16 and Ki 17 from Suwan 1(S)C4 and Ki 23 from recycled Ki 11 with inbred from Suwan 1(S)C7). The correlations of the InP content and both traits (Table 5) showed that the low PA inbred group had higher mean of InP content but poorer both characteristics than the high PA inbred group (Tables 1 and 5).

The analysis of PA in new inbred lines resulted in one group of corn new inbred lines (Nursery no. 40 in the 2008 dry season) with only medium-high PA content (≥900 mg/100g seed) (Table 2). However, they had significantly different PA and InP which showed that there were genetic variation for both traits.

The correlation of PA and grain yield in two populations of F_1 hybrids (Trial no. 916-918 and Trial no. 904-906) showed positive relationship with no statistically significant difference. This means that the increased PA content in mature corn seeds may be the source of energy influencing seedling growth and development. These factors would contribute to the increment of corn yield. On the other hand, the correlation of InP and grain yield in two populations of F_1 hybrids (Trial no. 916-918 and Trial no. 904-906) showed the negative relationship. This indicated that the F_1 hybrids, with high PA and low InP, should have high yield potential. Thus, the InP should be used in conjunction with other traits for selecting corn with reduced PA as there are various pathways involving in reducing PA (Raboy et al, 2000). The negative pleiotropic effect caused by a low PA content in the kernel has been reported by several authors studying low phytic acid mutants (Raboy et al, 2000; Pilu et al, 2005; Lorenz et al, 2007; 2008; Doria et al, 2009).

In this study, the selected low PA hybrids with high grain yield can be achieved by using the cross between either M x L or M x M corn lines (Table 6).

Conclusions

We have detected significant PA and InP contents but narrow variation in corn seeds of the Ki and new inbred lines collected as a germplasm at NCSRC, Thailand. However, the environmental conditions may play a role in marginally changing the quantity of these substances between 2008 dry season and 2009 early rainy season both in the parents and F_1 hybrids. Nonetheless, the corn seeds of Ki can be arbitrarily categorized into two groups (as low and medium-high PA contents), while those of new inbred lines can be categorized into one group (as medium-high PA content). The narrow variation but discrete PA content among these corn germplasm may provide an opportunity to apply a conventional breeding program to create new corn hybrids with high yield and good agronomic traits as well as reduced PA content. The PA and InP contents were negative correlated, though not statistical significant difference in inbred lines and F_1 hybrids. The grouping may be utilized in the breeding program to produce corn varieties with preferred traits, such as an increased InP characteristic. The PA content in the F_1 hybrids may have a positive bearing on the yield. In contrast, the InP con-

Table 6 - Means of PA, InP and grain yield of five selected hybrids having PA contents less than the means of Trial no. 916-918 in 2008 late rainy season and Trial no. 904-906 in 2009 early rainy season.

No.	Pedigree	Type[1]	Trial 916-918 (2008 late rainy season) PA ± SE (mg/100g)	InP ± SE (mg/100g)	Yield ± SE (t ha^{-1})	Trial 904-906 (2009 early rainy season) PA ± SE (mg/100g)	InP ± SE (mg/100g)	Yield ± SE (t ha^{-1})
1	Ki 48 x Ki 10	M x L	797.47 ± 22.45	59.98 ± 1.12	3.05 ± 0.23	937.28 ± 4.25	14.18 ± 2.44	8.12 ± 1.02
2	Ki 48 x Ki 52	M x L	817.31 ± 17.32	52.99 ± 0.92	4.12 ± 0.26	926.80 ± 5.20	16.69 ± 3.39	9.37 ± 0.59
3	Ki 48 x KS6(S)C3(TC)C1-S7-19-2-2	M x M	840.68 ± 12.28	52.19 ± 0.08	5.20 ± 0.07	922.70 ± 22.03	14.03 ± 0.96	9.13 ± 0.29
4	Suwan 4452 = Ki 47 x Ki 48	M x M	874.26 ± 13.86	19.57 ± 3.49	4.03 ± 0.97	911.48 ± 8.12	15.39 ± 3.79	9.89 ± 0.26
5	Ki 47 x 30A10-S9-44-1-21	M x H	874.57 ± 25.16	24.26 ± 4.30	4.06 ± 0.29	927.65 ± 5.35	18.28 ± 1.03	9.55 ± 0.01
	Mean of Trials		892.71 ± 2.60	42.61 ± 1.24	3.60 ± 0.12	940.40 ± 2.12	17.80 ± 0.44	9.25 ± 0.14
	F-test		**	**	**	ns	**	**
	LSD (0.05)		31.80	4.16	2.75		7.04	1.65

[1]L = Low PA inbred lines (<900 mg/100g); M = Medium PA inbred lines (900≤x<950 mg/100g); H= High PA inbred lines (≥950 mg/100g)

tent in the F_1 hybrids was negatively correlated with grain yield, especially highly significant in the 2009 early rainy season. The positive correlation between a PA content and yield may be an obstacle to invent new high yield potential corn varieties with reduced PA content, a substance aimed to be reduced to address the environmental hazard.

However, this can be overcome by using M x L and M x M hybrid patterns to achieve the objective of having corn with high yield potential but with reduced PA content trait.

Acknowledgements

We would like to thank the National Corn and Sorghum Research Center (NCSRC), Inseechandrastitya Institute for Crops Research and Development, Kasetsart University, Thailand for assistance in a field study. This work was financially supported by the National Research Council of Thailand (NRCT) through the Silpakorn University Research and Development Institute (SURDI) (Project Number 35351), Thailand.

References

Abbassian A, 2006. Maize: International market profile. Trade and markets division. Economic and social department. Food and Agriculture Organization of the United Nations

Ali M, Shuja MN, Zahoor M, Qadri I, 2010. Phytic acid: How far have we come? Afr J Biotechnol 9: 1551-1554

Chen PS, Toribara TY, Warner H, 1956. Microdetermination of Phosphorus. Analyt Chemistry 28: 1756-1758

Cromwell GL, Coffey RD, 1991. Phosphorus-a key essential nutrient, yet a possible major pollutant-its central role in animal nutrition. "Biotechnology in the feed industry", pp 133-145. Lyons TP ed. Alltech Tech Publications, Nicholasville, KY

Cromwell GL, Coffey RD, Parker GR, Monegue HJ, Randolph JH, 1995. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorous in corn-soybean meal diets for pigs. J Amin Sci 73: 2000-2008

Doria E, Galleschi L, Calucci L, Pinzino C, Pilu R, Cassani E, Nielsen E. 2009. Phytic acid prevents oxidative stress in seeds: Evidence from a maize (*Zea mays* L.) low phytic acid mutant. J of Exp Bot 60: 967–978

Goodman MM, 2005. Broadening the US maize germplasm base. Maydica 50: 203-214

Haug W, Lantzsch H.-J, 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric 34: 1423-1426

Hidvegi M, Lasztity R, 2002. Phytic acid content of cereals and legumes and interaction with proteins. Periodica Polytechnica Ser Chem Eng 46: 59-64

Liu JD, Bollinger W, Ledoux DR, Ellersieck MR, Veum TL, 1997. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorous corn-soybean meal diet for growing pigs. J Anim Sci 75: 1292-1298

Lorenz AJ, Scott MP, Lamkey KR, 2007. Quantitative determination of phytate and inorganic phosphorus for maize breeding. Crop Sci 47: 598-604

Lorenz AJ, Scott MP, Lamkey KR, 2008. Genetic variation and breeding potential of phytate and inorganic phosphorous in a maize population. Crop Sci 48: 79-84

Liu JD, Bollinger W, Ledoux DR, Veum TL, 1998. Lowering the dietary calcium to total Phosphorus ration increases phosphorous utilization in low-Phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J Anim Sci 76: 808-813

Office of Agricultural Economics, 2011. Ministry of Agriculture and Cooperatives. [on-line]. Available: http://www.oae.go.th

Pilu R, Landoni M, Cassani E, Doria E, Nielsen E, 2005. The maize lpa241 mutation causes a remarkable variability of expression and some pleiotropic effects. Crop Sci 45: 2096-2105

Raboy V, Gerbasi PF, Young KA, Stoneberg SD, Pickett SG, Bauman AT, Murthy PPN, Sheridan WF, Ertl DS, 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1.

Plant Physiol 124: 355-368
Sharpley AN, Charpa SC, Wedepohl R, Sims JY, Danial TC, Reddy KR, 1994. Managing agricultural phosphorus for protection of surface waters: Issues and Options. J Environ Qual 23: 437-451
Tongoona P, 2005. Development of tropical maize varieties with low phytic acid to improve the nutritional status of poor communities in sub-Saharan Africa. [on-line]. Available: http://www.africancrops.net/ abstracts2/maize/tongoona.htm