Songklanakarin J. Sci. Technol.
37 (5), 515-522, Sep. - Oct. 2015

http://www.sjst.psu.ac.th

Original Article

Correlations between the contents of phytic acid and inorganic phosphorous and downy mildew resistance of corn inbred lines

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Received: 24 November 2013; Accepted: 31 May 2015

Abstract

Seeds of corn inbred lines collected at the National Corn and Sorghum Research Center (NCSRC), Kasetsart University, were analyzed to determine the contents of phytic acid (PA) and inorganic phosphorous (InP). These 28 and 29 inbred lines were cultivated at the NCSRC (in the 2008 late rainy season and 2009 early rainy season) to evaluate their resistance to corn downy mildew caused by *Peronosclerospora sorghi*. Results showed that the values of the PA, InP contents and downy mildew infection were statistically different among these inbred lines in both seasons. However, there were no correlations between the contents of either PA or InP and downy mildew infection.

Keywords: corn, inbred line, phytic acid, inorganic phosphorus, corn downy mildew.

1. Introduction

Phytic acid (myo-inositol-1,2,3,4,5,6-hexa*kis*phosphate) (PA) is the main storage form of phosphorus (P) in seeds and grains, with around 1% or more of the dry weight and 50-80% of the total P (Ockenden *et al.*, 2004). This chemical agent is an essential precursor in several pathways in plant cells (Loewus and Murthy, 2000; Raboy, 2003).

In animal and human being, this substance may have a positive nutritional role as an anti-oxidant and anti-cancer agent (Lott *et al.*, 2000). However, PA is a strong chelating agent of the positively charged chemical substances (eg. zinc, iron, calcium), forming the insoluble compounds in the gastrointestinal tract (Abebe *et al.*, 2007). As a result, these substances may have negative effect as they can be the substance which may reduce the uptake of dietary minerals in both animals and human (Raboy, 2002; Shi *et al.*, 2003). They can also cause environmental hazard if the unabsorbed phytate passes through the animal gastrointestinal tract and is discharged to the environment causing eutrophication (Lott *et al.*, 2000; Shi *et al.*, 2003; Ockenden *et al.*, 2004). The normal practice in the animal production industry is to use phytase enzyme to reduce the PA content in the feedstuff, increasing the cost of animal production (Cromwell and Coffey, 1991). Alternatively, other grains such as barley which have a low PA content may be used as a substitution of corn to produce animal feedstuff (Veum *et al.*, 2002).

In Thailand, the primary industry involving poultry and swine production is dependent upon feeds in which grains of corn are considered as one of the major constituents. The value of imported corn for use in the feed industry for animal production is expected to cost 53,265 million Baht annually (Office of Agricultural Economics, 2010). To seek a better way to deal with this problem in the long run, a research to produce corn varieties, not only having low PA content trait

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but also possessing resistant characteristic to downy mildew disease of corn caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, has been initiated.

Corn downy mildew has always been a threat to corn production worldwide (Commonwealth Mycological Institute, 1975; Thurston, 1984; Cardwell, 1995; Jampathong *et al.*, 2013), particularly in the tropical Asian countries with a wide range of disease incidence (20-90%) (Sharma *et al.*, 1993; Sudha *et al.*, 2004). *P. sorghi* is the most important causal agent of downy mildew disease of sorghum and corn (Sharma *et al.*, 1993; Jampathong *et al.*, 2013). Resistant varieties have been developed and introduced to farmers to use, either alone or in combination with other control measures, for managing downy mildew disease of corn. The main advantage of using resistant varieties is that this measure entails low cost and can be easily adopted by the farmers (Renfro *et al.*, 1979; Cardwell *et al.*, 1997; Sudha *et al.*, 2004).

Mutated corn varieties were obtained (Shi *et al.,* 2003) and gene contributed to low PA content in seeds was identified (Raboy, 2002; Shi *et al.*, 2003). In mutated line of cereal grains, it was found that seeds with reduced PA had an increased InP level (Raboy *et al.*, 2000; Wilcox *et al.*, 2000; Hitz *et al.*, 2002). This InP in cereal grain could be utilized by the monogastric animals when additional phytase enzyme was supplemented to the animal feeds because these animals could not produce sufficient phytase in their digestive tract (Nelson *et al.*, 1968; Sharplay *et al.*, 2003; Shi *et al.*, 2007). On the other hand, ruminant animals harbored microorganisms in the rumen which possess the capacity to produce phytase enzyme, making PA available for use by their host (Frias *et al.*, 2003).

In terms of technology, it was reported that low PA corn varieties could be produced using genetic engineering technology, shortening the time and input used to obtain an acceptable varieties (Raboy and Gerbasi,1996; Raboy *et al.*, 2000; Spencer *et al.*, 2000; Raboy, 2002). However, there will be an issue of public acceptance if the PA-Genetically Modified Organism (GMO) corn will ever be produced. For this reason, we investigated the possibility of adopting a conventional breeding program to produce the low PA corn varieties with an acceptable yield potential and corn downy mildew resistance.

The objective of this research, which was a part of the project aimed to breed the corn for the low PA content, was to investigate the correlations between the contents of PA and inorganic phosphorus (InP) and the resistance to corn downy mildew.

2. Materials and Methods

2.1 Corn germplasm

This collaborative project between Faculty of Animal Science and Agricultural Technology, Silpakorn University (SU), and National Corn and Sorghum Research Center (NCSRC), Faculty of Agriculture, Kasetsart University (KU), was initiated in 2007. Corn inbred lines (listed in Table 1 and 2) from the corn gene bank at NCSRC were used throughout this experiment unless stated otherwise.

2.2 Evaluation of PA and InP in the corn seeds

Corn seeds of the inbred lines (listed in Table 1 and 2) were evaluated to determine the content of PA and InP. Sensitive and rapid method described by Haug and Lantzsch (1983) was used to determine the content of PA. Analysis of the InP content as reported by Chen *et al.* (1956) was used in this experiment. Each sample of the corn seeds was tested for PA and InP content using 3 replications. The PA and InP, in each sample were determined by spectrophotometer at the absorbance of 530 nm and 820 nm, respectively.

2.3 Downy mildew disease assessment

Assessment of downy mildew resistant was conducted at NCSRC, Thailand. CM109, a highly susceptible corn variety to downy mildew, was planted as a spreader row. In field trial, corn inbred lines (28 and 29 pure lines) were tested to determine the incidence of downy mildew disease in the late rainy season, 2008 and the early rainy season, 2009.

Inoculum of *Peronosclerospora sorghi* was prepared by washing the infected corn leaves to get rid of soil debris and old conidia. These leaves were then incubated in the sealed container at 20ºC for 8 hours. The new emerging conidia of the pathogen were rinsed with sterilized water and collected into the water container for inoculation. The number of conidia was enumerated with haemacytometer under compound microscope and the number of the conidia was adjusted to $1x10^4$ conidia/ml. This pathogen was used to inoculate corn seedlings planted in a spreader rows, twice consecutively 6 days and 7 days after seedling emergence (Cardwell *et al.*, 1997). The testing corns were planted in a 5-meter row with the spacing between rows at 75 cm and between plants at 25 cm. Three rows were planted for each corn variety. Downy mildew disease was assessed twice; first when the testing corns were 3 weeks old and second when they were 4 weeks old. Total number of the testing corns planted and the numbers of the infected corns were counted and infection percentage was calculated. Resistance to downy mildew disease was rated basing on the criteria as described by Craig *et al.* (1977), where 0% disease incidence was recorded as highly resistant, 1-10% as resistant, 11-25% as moderately resistant, 26-50% as moderately susceptible, 51-75% as susceptible, and 76-100% as highly susceptible.

2.4 Statistical analysis

Data on downy mildew infection percentage, and the content of PA and InP in Completely Randomized Design (CRD), each with 3 replicates, were analyzed using R program. Means were compared with either Duncan's New Multiple Range Test (DMRT) or Least Significant Difference (LSD).

Table 1. Values of phytic acid (PA), inorganic phosphorus (InP) and downy mildew infection (*Peronosclerospora sorghi*) of 28 corn inbred lines in the first season (2008, late rainy season)

Inbred lines	Mean ± SE PA (mg/100g)	Mean ± SE InP (mg/100g)	Mean ± SE Downy infection (%)
Big949-S_6-22	985.82 ± 7.43 a	31.79 ± 0.53 b	22.39 ± 3.44 defg
C5219041-S_7-45	977.11 ± 15.69 ab	20.26 ± 0.38 ghi	51.98 ± 11.59 abc
C5219041-S_7-51	972.27 ± 5.36 ab	30.25 ± 1.48 bc	22.22 ± 11.09 defg
30A10-S_{11}-44-1-2-1	964.02 ± 3.14 abc	19.64 ± 0.46 hi	7.94 ± 6.60 g
C5218620-S_7-151	961.01 ± 5.57 abc	31.54 ± 1.33 b	12.36 ± 3.32 fg
30A33-S_9-2-1	959.60 ± 6.12 abc	18.94 ± 0.81 ij	4.42 ± 2.61 g
30A33-S_{12}-84-2-6-2-3	957.51 ± 16.04 abcd	25.15 ± 0.40 ef	21.68 ± 5.29 defg
C5219041-S_7-13-2	957.36 ± 10.79 abcd	27.78 ± 1.09 cde	45.83 ± 19.69 abcde
C5219041-S_7-95	957.18 ± 8.96 abcd	26.50 ± 0.52 def	41.09 ± 15.18 abcdef
C5218620-S_7-20	952.84 ± 16.48 abcde	23.58 ±0.46 fg	59.23 ± 1.69 a
Big949-S_7-30	952.83 ± 5.20 abcde	30.63 ± 0.63 bc	27.31 ± 9.02 bcdefg
Big949-S_7-4	951.76 ± 8.60 abcde	23.49 ± 0.35 fg	21.38 ± 3.27 defg
C5219041-S_7-62-4	947.12 ± 12.40 abcdef	20.31 ± 0.82 ghi	18.30 ± 10.83 efg
30A33-S_9-87-1-2	945.87 ± 4.40 abcdef	25.29 ± 0.68 ef	16.98 ± 8.49 efg
C5218620-S_7-9-1	941.32 ± 13.83 bcdef	19.76 ± 0.52 ghi	55.05 ± 2.60 ab
C5219041-S_7-3-5	939.70 ± 4.03 bcdef	18.15 ± 1.32 ij	24.17 ± 12.78 cdefg
C5218620-S_7-160	938.93 ± 3.43 bcdef	23.38 ± 0.70 fgh	15.01 ± 6.03 fg
Big949-S_7-121-3	938.26 ± 18.61 bcdef	30.00 ± 1.34 bcd	19.28 ± 2.56 efg
KS6(S)C3(TC)C1-S_9-19-2-2	938.12 ± 3.73 bcdef	25.20 ± 1.12 ef	11.42 ± 6.23 fg
Suwan3(S)C4(SF)-S_{11}-150-1	934.10 ± 5.91 bcdef	11.48 ± 0.16 k	4.84 ± 1.48 g
30A33-S_{11}-140-1-5-3-3	928.37 ± 16.70 cdef	32.31 ± 4.30 b	19.86 ± 6.62 efg
SW1(S)C11(TC)C1-S_9-21-3-2	926.31 ± 8.76 cdef	15.34 ± 0.10 j	12.64 ± 4.02 fg
C5218620-S_7-154	926.16 ± 12.20 cdef	58.06 ± 0.24 a	25.57 ± 8.91 cdefg
30A33-S_9-13-1	914.34 ± 30.21 def	18.03 ± 1.38 ij	19.33 ± 5.28 efg
30A10-S_{11}-43-1-3	911.34 ± 19.47 ef	20.20 ± 0.92 ghi	4.85 ± 1.53 g
30A33-S_{10}-140-1-5-6-3	904.23 ± 18.27 f	27.24 ± 0.90 cdef	29.77 ± 13.85 bcdefg
30A33-S_{11}-84-2-6-2-4	nd	nd	19.34 ± 5.82 efg
KS6(S)C3(TC)C1-S_6-246-11	nd	nd	50.56 ± 17.36 abcd
Mean	945.52 ± 3.02	25.17 ± 0.99	24.46 ± 2.17
F test (0.05)	**	**	**
LSD (0.05)	35.86	3.36	25.18
LSD (0.01)	47.78	4.47	33.52

Means in each column followed with the same letter are not significantly different according to Duncan's New Multiple Range Test (DMRT).
** = highly significant different at 99% confidence. nd = not detectable.
Note: The PA value of 28 inbred lines of corn in this table was listed based upon the concentration of these substances starting from the highest concentration to the lowest concentration

The relationship between % downy mildew infection and the content of PA and InP were analyzed using correlation coefficient (r) with Microsoft Office Excel version 2003.

3. Results

3.1 Values of PA and InP contents and the infection of corn downy mildew

The values of the PA and InP contents of the seeds of the 28 inbred lines (in the first season testing) and the 29 inbred lines (in the second season testing) are presented in Tables 1 and 2. The percentage of corn downy mildew incidence of the 28 and the 29 inbred lines are also shown in Tables 1 and 2, respectively.

In the 2008 late rainy season, the inbred line Big949-S_6-22 had the highest PA content while 30A33-S_{10}-140-1-5-6-3 had the lowest PA content (Table 1), with the difference of the PA contents at 81.59 mg/100 g. The inbred lines from similar genetic backgrounds had a wide range of the PA values such as Big949 XXX, C5219041 XXX, 30A10 XXX, and 30A33 XXX. Big949-S_6-22 had the highest content of PA

Table 2. Values of phytic acid (PA), inorganic phosphorus (InP) and downy mildew infection (*Peronosclerospora sorghi*) of 29 corn inbred lines in the second season (2009, early rainy season)

Inbred lines	Mean ± SE PA (mg/100g)	Mean ± SE InP (mg/100g)	Mean ± SE Downy infection (%)
30A33-S_7-87-1	997.65 ± 8.01 a	27.84 ± 1.22 hij	16.07 ± 15.41 cde
Suwan3(S)C4(SF)-S_{10}-150-1	972.10 ± 4.95 ab	16.14 ± 0.96 q	0.00 ± 0.00 e
C5218620-S_7-9-1-2	969.23 ± 10.53 ab	23.96 ± 0.83 klmn	45.60 ± 20.06 bc
Big949-S_7-121-3	952.28 ± 7.61 bc	29.51 ± 0.27 ghi	14.17 ± 6.28 de
Big949-S_6-22	952.14 ± 13.82 bc	37.35 ± 1.11 c	26.97 ± 7.33 bcde
C5219041-S_7-95	951.84 ± 9.79 bc	27.07 ± 0.34 ijk	41.04 ± 10.26 bcd
Big949-S_7-4	948.58 ± 12.89 bc	21.44 ± 0.73 nop	25.95 ± 10.98 bcde
C5218620-S_7-151	947.97 ± 10.98 bc	62.22 ± 1.87 a	8.63 ± 3.64 e
30A33-S_9-13-1	947.95 ± 4.70 bc	25.14 ± 0.48 jklm	4.25 ± 0.91 e
C5218620-S_6-20-1	947.77 ± 12.64 bc	32.38 ± 0.91 efg	29.99 ± 10.01 bcde
30A10-S_{11}-44-1-2-1	946.97 ± 9.18 bc	23.75 ± 0.28 lmn	2.78 ± 2.78 e
C5219041-S_7-62-4	946.85 ± 4.01 bc	18.72 ± 0.47 pq	0.76 ± 0.76 e
C5218620-S_8-160-1	945.86 ± 13.79 bc	33.19 ± 0.19 def	31.08 ± 13.16 bcde
30A33-S_{11}-140-1-5-3-3-1	945.08 ± 7.87 bc	47.72 ± 1.91 b	0.00 ± 0.00 e
C5219041-S_7-51	944.43 ± 11.34 bc	19.20 ± 2.44 op	26.50 ± 5.61 bcde
Big949-S_7-30	944.13 ± 1.37 bc	35.98 ± 0.47 cd	21.53 ± 4.97 bcde
SW1(S)C11(TC)C1-S_{10}-21-3-2-2	941.12 ± 5.82 bc	30.80 ± 2.29 fgh	8.05 ± 8.05 e
C5218620-S_7-154	937.53 ± 4.59 bc	30.23 ± 0.47 fgh	8.57 ± 5.10 e
KS6(S)C3(TC)C1-S_6-246-11	937.51 ± 9.63 bc	31.77 ± 0.33 efg	20.07 ± 9.04 bcde
30A33-S_{13}-84-2-6-2-3-3	936.55 ± 7.79 bc	30.47 ± 0.65 fgh	15.36 ± 10.51 cde
30A33-S_9-2-1	932.42 ± 10.04 bcd	23.07 ± 0.91 lmn	0.81 ± 0.81 e
C5219041-S_7-13-2	925.99 ± 9.79 cde	24.43 ± 0.21 klmn	50.60 ± 10.53 ab
KS6(S)C3(TC)C1-S_9-19-2-2	924.37 ± 7.33 cde	22.08 ± 0.45 mno	2.10 ± 1.20 e
C5219041-S_7-45	922.21 ± 1.76 cde	25.56 ± 0.26 jkl	76.12 ± 2.51 a
30A10-S_{11}-43-1-3	896.50 ± 12.15 def	25.70 ± 0.70 jkl	2.16 ± 1.14 e
30A33-S_{10}-53-2-5	890.52 ± 16.03 ef	34.21 ± 0.79 de	8.51 ± 7.05 e
C5218620-S_6-70-4-1	885.28 ± 35.97 f	15.72 ± 0.29 q	49.93 ± 8.84 ab
30A33-S_{11}-84-2-6-2-4	nd	nd	29.17 ± 23.20 bcde
Tuxpeño (Check)	nd	nd	76.20 ± 2.51 a
Mean	940.40 ± 3.16	28.73 ± 1.08	22.17 ± 2.66
F test (0.05)	**	**	**
LSD (0.05)	32.99	2.892	25.68
LSD (0.01)	49.34	3.853	34.65

Same corrections means with the same letter are not significantly different by Duncan's New Multiple Range Test (DMRT).
** = highly significant different at 99% confidence. nd = not detectable.
Note: The PA value of 29 inbred lines of corn in this table was listed based upon the concentration of these substances starting from the highest concentration to the lowest concentration

(985.82 mg/100g) while Big949-S_7-30 had a PA content of 952.83 mg/100g and Big949-S_7-121-3 had a PA content of 938.26 mg/100g (Table 1).

The value of InP was statistically different among the corn inbred lines in this study, with the average value of the InP in their seeds 37.57 times less than that of the PA (Table 1). C5218620-S_7-154 had the highest value of InP (58.06 mg/100g), while Suwan3(S)C4(SF)-S_{11}-150-1 had the lowest value (11.48 mg/100g), with a difference between the two varieties of 46.58 mg/100g.

The lower values of downy mildew infection in the 2008 late rainy season were obtained from the inbred lines 30A33-S_9-2-1 (4.42%), Suwan3(S)C4(SF)-S_{11}-150-1 (4.84%) and 30A10-S_{11}-43-1-3 (4.85%). Three inbred lines which had the higher percentage of infection included C5219041-S_7-45 (51.98%), C5218620-S_7-9-1 (55.05%) and KS6(S)C3(TC)C1-S_6-

246-11 (50.56%) (Table 1). The difference of downy mildew incidences among inbred lines that had the highest and lowest values in this season was 50.81%.

In the 2009 early rainy season; 29 inbred lines were planted but seeds from only 27 inbred lines were collected and subjected to the analysis to determine the values of the PA and InP. Almost all of the corn inbred lines planted in this season were similar to those planted in the 2008 late rainy season. The corn seeds planted in this season, however, had a lower PA content than those in the 2008 late rainy season. For instance, 30A33-S_7-87-1 had the highest PA content (997.65 mg/100g), while C5218620-S_6-70-4-1 had the lowest value (885.28 mg/100g) (Table 2), with the difference at 112.37 mg/100g. This difference was greater than that of the previous season (81.59 mg/100g).

C5218620-S_7-151 had the highest content of InP at 62.22 mg/100g, while C5218620-S_6-70-4-1 had the lowest content (15.72 mg/100g) (Table 2). The difference between the two inbred lines was 46.50 mg/100g. This value was quite similar to that of the previous crop at 46.58 mg/100g. Seeds from the corn planted in the 2009 early rainy season had difference value of the InP, with statistical difference among the inbred lines. The average value of the InP in corn seeds in this season was 32.73 times less than that of the PA (Table 2). The distinction between the InP and PA values of the 2009 trial was quite similar to that of the 2008 trial, in which the InP content was 37.57 times less than that of the PA.

Suwan3(S)C4(SF)-S_{11}-150-1 (0%) and 30A33-S_{11}-140-1-5-3-3-1 (0%) had the lowest values of downy mildew infection, followed by C5219041-S_7-62-4 (0.76%) and 30A33-S_9-2-1 (0.81%). Tuxpeño, a susceptible variety used as a check, had the value of downy mildew infection at 76.20%. The other susceptible inbred lines included C5219041-S_7-13-2 (50.60%), C5218620-S_6-70-4-1 (49.93%) and C5218620-S_7-9-1-2 (45.60%) in the 2009 trial (Table 2). The difference value between the highest and lowest values of the downy mildew infection in this season was 50.60%.

3.2 Relationship of the levels of the PA and InP contents and the downy mildew infection

The correlations between the levels of the PA and InP contents and the downy mildew infection of the 28 inbred lines (in the first season) and the 29 inbred lines (in the second season) are presented in Table 3. The values of the PA, InP and the percentage of downy mildew incidence were statistically different among inbred lines (Table 1 and Table 2). However, there was no correlation between the level of PA (r = 0.236) or the InP content (r = 0.144) and downy mildew incidence for the 2008 late rainy season (Table 3). Big949-S_6-22, which had the high PA content (985.82 mg/100g), had moderate level of downy mildew incidence (22.39%). This level of disease incidence was quite similar to the average of downy mildew incidence in the other inbred lines (at 24.46%) (Table 1). Nevertheless, C5218620-S_7-20 had the highest downy mildew infection (59.23%) in the 2008 late rainy season, with a moderate content of PA (952.84 mg/100g) (ranked 10 among all inbred lines tested) (Table 1).

In the 2008 late rainy season; C5218620-S_7-154 which had the highest content of InP (58.06 mg/100g), had a moderate level of downy mildew incidence (25.57%). This value was quite similar to those of 28 varieties in this test (at 24.46%) (Table 1). C5218620-S_7-20, which had the highest downy mildew incidence (59.23%), also had moderate level of InP content (23.58 %) (ranking 14 among all inbred lines tested) (Table 1). However, Suwan3(S)C4(SF)-S_{11}-150-1, having the lowest downy mildew incidence (4.84%), also had the lowest content of InP (11.48 mg/100g) (Table 1).

In the 2009 early rainy season, there was no correlation between the level of PA and InP content and downy mildew incidence (r = -0.124 and -0.166, respectively) (Table 3). In this season, the level of PA decreased compared with that of the 2008 late rainy season (Tables 1 and 2). However; in both tests the-inbred lines with the highest PA, had moderate level of downy mildew incidence. For instance; 30A33-S_7-87-1, a corn variety with highest content of PA (997.65 mg/100g) in the 2009 early rainy season, had downy mildew infection (16.07%). This value was quite similar to that of the average of downy mildew incidence of the other corn varieties inbred lines (22.17%) (Table 2). However; C5218620-S_6-70-4-1, which had the lowest content of PA (at 885.28 mg/100g), had quite high downy mildew incidence (49.93 %) (Table 2). C5218620-S_7-151, which had the highest InP content (62.22 mg/100g), had the lowest downy mildew incidence (8.63 %) (Table 2), while C5218620-S_6-70-4-1, which had the lowest InP (15.72 mg/100g), had quite high incidence of downy mildew (49.93 %) (Table 2).

The correlation coefficients (r) between the value of PA and downy mildew infection were both positive (r = 0.236 in the 2008 late rainy season) and negative (r = -0.124 in the 2009 early rainy season), but the correlations were not statistically significant (Table 3).

The correlation coefficients (r) between the value of InP and % downy mildew incidence were also both positive (r = 0.144 in the 2008 late rainy season) and negative (r = -0.166 in the 2009 early rainy season), but they were not statistically significant (Table 3).

4. Discussion

4.1 Values of PA and InP contents and the infection of corn downy mildew

Selected inbred lines were tested in two seasons with respect to the values of PA, InP contents, plant and ear aspects (Na Chiangmai *et al.*, 2011). These inbred lines were collected and used for improving open-pollinated varieties, inbred lines and hybrids at NCSRC, KU (Aekatasanawan *et al.*, 1997; Aekatasanawan *et al.*, 2001; Jenweerawat *et al.*, 2009). The value of PA was highly varied among the seeds of these inbred lines because these inbred lines had different genetic background. This characteristic may be beneficial as

Table 3. The correlations among three characteristics; i.e. the percentage of downy mildew infection, the phytic acid (PA) content and the inorganic phosphorus (InP) content

Correlations between two characteristics	Correlation coefficients (r)
Downy mildew infection vs the concentration of PA (mg/100g) of 26 inbred lines (first season test, 2008 late rainy season)	0.236 ns
Downy mildew infection vs the concentration of InP (mg/100g) of 26 inbred lines (first season test, 2008 late rainy season)	0.144 ns
Downy mildew infection vs the concentration of PA (mg/100g) of 27 inbred lines (second season test, 2009 early rainy season)	-0.124 ns
Downy mildew infection vs the concentration of InP (mg/100g) of 27 inbred lines (second season test, 2009 early rainy season)	-0.166 ns

ns = non-significantly different at 95% confidence.

the variation among the lines may be a source of diverse genetic material for genetic improvement to obtain high yielding corn with low PA content (Aekatasanawan *et al.*, 2001; Aekatasanawan *et al.*, 2003).

The difference between the highest and the lowest of the PA and InP values in the two field trials (2008 late rainy season and 2009 early rainy season) in Thailand was smaller than that reported by Queiroz *et al.* (2011). This may be because corn was not indigenous in Thailand and the varieties with high yield characteristic had been selectively introduced to the country (Hufford *et al.*, 2012). As a result, genetic variation was very narrow, demanding more research in inventing new hybrid with preferred characteristic (Na Chiangmai *et al.*, 2011). Nevertheless, the inbred lines - extracted from a similar genetic background at NCSRC, KU - showed a wide range of the PA contents, providing an opportunity to use as genetic materials in the conventional breeding program for downy mildew resistance (Table 1).

The average downy mildew infection in the 2008 late rainy season (24.46%) was quite similar to that in the 2009 early rainy season (22.17%) (Tables 1 and 2), indicating that genetic control rather than environmental conditions, strongly influenced the expression of downy mildew resistance of these inbred lines. George *et al.* (2003) also reported that downy mildew resistance of corn in subtropical and tropical regions in Asia was at the middle to high level of heritability. Nevertheless, many genes and interaction between genetic and environment were also identified as the factors contributing to the phenotypic variance in disease resistance (George *et al.,* 2003). The genetical approach using conventional breeding should still be continually adopted to improve corn varieties for downy mildew resistance.

4.2 Relationship of the levels of PA and InP contents and downy mildew infection

No correlation between the PA, InP and downy mildew infection (either in the 2008 late rainy season or the 2009 early rainy season) (Table 3) means that these phenotypes are likely to be controlled by different genes with no linkage among these three traits. This indicated that the values of the PA and InP contents were not suitable to be used as a predictor of downy mildew resistance in these inbred corns. This may also indicate that the contents of either PA or InP content may be improved (either for a reduced or an increased values) independently, with no effect on the downy mildew resistant characteristic. It is possible that there is no linkage or no epistasis between genes that control either PA or InP content with downy mildew resistance. This event may give an opportunity for the corn breeder to develop the new corn varieties with desirable traits such as low PA or high InP content in the seeds for high resistance to downy mildew disease.

Although there was no correlation between the PA and InP contents in this study, a negative correlation between the PA and InP contents was reported in both mutant maize (Raboy *et al.*, 2000) and in some of the similar inbred lines (Na Chiangmai *et al.*, 2011). It is possible that the lack of correlation was due to the numbers of inbred lines used for correlation test in this study being relatively smaller than those used by Na Chiangmai *et al.* (2011), resulting in the difference in the results of these two studies. In other plant species, it was reported that seeds of plants with low PA content had relatively higher content of translocable InP (Strother, 1980). Lorenz *et al.* (2008) also showed that the PA content was positively non-significant with the InP in the Western normal lines germplasm.

The difference between the correlation coefficient values of 2008 late rainy season (positive correlation coefficient) and 2009 early rainy season (negative correlation coefficient) (Table 3) indicated that the PA and InP were the quantitative characteristics which were likely to fluctuate based upon the environmental conditions. The PA content was reported to have less broad-sense heritability value than the InP content (Lorenz *et al.*, 2008), indicating that environment factors may influence the PA trait more than they influence the InP trait.

Acknowledgements

We would like to thank the National Corn and Sorghum Research Center (NCSRC), Faculty of Agriculture, Kasetsart University (KU), 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 35380), Thailand.

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