Note

Mapping of quantitative trait loci for phytic acid and phosphorus contents in seed and seedling of mungbean (Vigna radiata (L.) Wilczek)

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Phytic acid (PA) is the storage form of phosphorus (P) in seeds and plays an important role in the nutritional quality of food crops. There is little information on the genetics of seed and seedling PA in mungbean [Vigna radiata (L.) Wilczek]. Quantitative trait loci (QTL) were identified for phytic acid P (PAP), total P (TP), and inorganic P (IP) in mungbean seeds and seedlings, and for flowering, maturity and seed weight, in an F₂ population developed from a cross between low PAP cultivated mungbean (V1725BG) and high PAP wild mungbean (AusTRCF321925). Seven QTLs were detected for P compounds in seed; two for PAP, four for IP and one for TP. Six QTLs were identified for P compounds in seedling; three for PAP, two for TP and one for IP. Only one QTL co-localized between P compounds in seed and seedling suggesting that low PAP seed and low PAP seedling must be selected for at different QTLs. Seed PAP and TP were positively correlated with days to flowering and maturity, indicating the importance of plant phenology to seed P content.

Key Words: mungbean, Vigna radiata, phosphorus, phytic acid, QTL, SSR.

Introduction

Phytic acid (PA; myo-inositol-1, 2, 3, 4, 5, 6-hexaphosphate), is the storage form of P in seeds of cereals and legumes (Lott et al. 2000). Dietary PA inhibits the absorption of protein and certain mineral nutrients. It is not efficiently digested by human and nonruminant animals such as swine, poultry and fish. Seed-derived dietary PA can contribute to human mineral deficiencies. When PA is consumed as feeds, monogastric animals excrete a large fraction of phytate salts, which can contribute to water pollution (Sharpley et al. 1994).

Breeding for low PA (lpa) crops has recently been considered as a potential way to increase nutritional quality of crop products. Approaches include use of low phytic acid alleles (Cichy and Raboy 2008), genetic engineering (Shi et al. 2007) and QTL mapping for loci that impact seed P and PA (Blair et al. 2009, Cichy et al. 2009). Mungbean (Vigna radiata (L.) Wilczek) is an important food legume crop of Asia. Its seeds and flour are used in various foods. Another main use of mungbean is to be consumed as vegetable in the form of sprouts, either cooked or raw sprouts. To date in mungbean only naturally occurring germplasm that possesses low PA have been identified (Sompong et al. 2010). They reported that the high PA is controlled by dominant alleles at two independent loci showing duplicated recessive epistasis. In this paper, we report the first QTL mapping for PA and P contents in seed and seedling of mungbean.

Materials and Methods

Plant materials and DNA extraction

A mapping population of F₂ plants was developed from a cross between a high seed PA, V1725BG (as female) and a low seed PA, AusTRCF321925 (as male). V1725BG is a cultivated mungbean from Iran, while AusTRCF321925 is a wild mungbean from Australia. One hundred and seventy F₂₃ lines together with the parents were grown in an experimental field without replication at Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand during March–May, 2009. Each F₂₃ lines had at least 10 plants. F₄ seeds of each line were harvested in bulk and used for phenotypic measurement. DNA was extracted from young leaves of the F₂ plants and their parents using the method described by Lodhi et al. (1994) with a modification that absolute ethanol was used instead of 95% ethanol.

PAP, TP and IP assay in seed and seedling

F₄ seeds from 10-20 plants in each F₂₃ line were bulked and divided into two parts. The first part was prepared for
seed P determination and the second part was germinated on wet tissue papers at room temperature and watered daily for 3 days. The seeds and seedlings were dried at 60°C for 72 hr, milled to pass through a 40 mesh (0.5 mm) screen and stored in desiccators. The samples were separated into three parts, each for analysis of PAP, TP and IP with three determinations per part.

**Determination of Seed and Seedling P Constituents**

PAP content was assayed in seed via a modification of the methods as described (Haug and Lantzsch 1983). Fifty mg of ground seed or seedling were assayed in triplicate and sample PAP was calculated via comparison with PAP standard curves (0.0, 6.25, 12.5, 25, 50 and 75 µg PAP), prepared using reagent-grade PAP (Sigma Chem.). PAP can be converted to PA by multiplying by 3.548. To determine TP content, 50 mg of the ground seed or seedling was placed into a 15 mL tube, to which 1.0 mL of concentrated H2SO4 was added, and wet-digested to completion. P was then determined colorimetrically using a modification of the method of Chen et al. (1956). TP was calculated using the standard curve of KH2PO4 (0.0, 155, 465, 930 and 1395 ng P). IP content was assayed using a modification of the method as described (Raboy et al. 1984) and IP content calculated via comparison with the same standard curve as determination of TP content.

**Evaluation of agronomic traits**

Days to flowering, days to maturity and seed size were measured in the F2:3 lines. Days to flowering and days to maturity were recorded when 50% of the plants in the family reached the stage. Seed size was measured by weighing 100-seed sample.

**SSR marker analysis**

A total of 991 SSR primers were used to screen for polymorphism between the parents. Five hundred and forty-six primers were developed from mungbean (Gwag et al. 2006, Seelahak et al. 2009, Somta et al. 2008, 2009, Thangphatsornruang et al. 2009), 142 were from azuki bean (Wang et al. 2004), 189 were from cowpea (Kongjaimun et al., unpublished data, Li et al. 2001), and 114 were from common bean (Benchimol et al. 2007, Blair et al. 2009, Buso et al. 2006, Gaitán-Solís et al. 2002, Guerra-Sanz 2004). The polymorphic markers were used to analyze the F2 population. PCR was carried out following Somta et al. (2008) with the exception that annealing temperature varied from 47 to 65°C, depending on primers. The PCR products were separated on a 5% denaturing polyacrylamide gel and visualized by silver staining.

**Data analysis and QTL mapping**

Correlation among traits at 0.05 probability level was determined by Pearson correlation coefficient analysis using software R-2.10.0 (R Development Core Team 2008). The genetic linkage map was constructed using JoinMap 3.0 software (van Ooijen and Voorrips 2001). Chi-square tests for goodness of fit to the expected segregation ratio of 1:2:1 were determined for each SSR marker. The minimum LOD threshold of 8.0 and recombination frequency of 0.50 were used to assign markers into linkage groups. Recombination frequency was converted to genetic distance (cM) by Kosambi’s mapping function.

QTL analysis was performed with the Windows QTL Cartographer 2.5 software (Wang et al. 2007) using composite interval mapping (CIM) method, model 6 (forward and backward regression) with window size of 10 cM, background markers of 5 and 1-cM walkspeed. Significant LOD score threshold at 0.05 probability level was determined to identify the QTL by running a 1,000 permutation tests. Linkage groups were named following azuki bean linkage groups (Han et al. 2005). QTLs were named following Sompong et al. (2006).

**Results**

**Variation in P compound contents and agronomic traits**

Statistically significant variation between the parents and among the F2:3 families was observed for all traits (Supplemental Table 1). Observed continuous distribution in the F2 population indicates that these traits are inherited in a quantitative fashion (Fig. 1). TP and PAP in seed were normally distributed while the distributions of the other traits were skewed. Transgressive segregation was observed for PAP, IP and TP in both seed and seedling, revealing that both V1725BG and AusTRCF321925 contributed a combination of alleles that were either positive or negative for the traits.

Seed weight was not correlated with any of the three seed P fractions (Table 1). Therefore variation in these seed P fractions among the parents and F2:3 progenies was not an artifact of the large difference in seed size between the parents or variation for seed weight in the F2:3 progeny. With the exception of seed weight, both seed and seedling TP and PAP were positively correlated with nearly all other seed, seedling and agronomic traits. In contrast, seed IP was only correlated with seed and seedling TP. Seedling IP was positively correlated with seedling TP, seed TP and PA, days to flowering and maturity, but negatively correlated with seed weight. Seedling PAP was positively correlated with seed TP and PAP, seedling TP and seed weight.

**Linkage map construction**

A total of 101 SSR markers detected polymorphisms between the parents and were used in the construction of linkage map (Supplemental Table 2). The map consisted of 13 linkage groups (LGs) and spanned a total length of 855.8 cM (Fig. 2). The length of the LGs ranged from 19.8 (LG04B) to 104.6 cM (LG01). The number of markers per LG ranged from 2 (LG 11B) to 16 (LG 1). The average distance between the adjacent markers varied from 5.5 to 33.1 cM. LG09 and LG11B showed gaps wider than 20 cM.
QTL for phytic acid in mungbean

In total, 23 QTLs were found associated with the nine traits (Table 2 and Fig. 2). Two, four and one QTLs distributed on five linkage groups were found for seed PAP, IP and TP, respectively. The phenotypic variance explained (PVE) by these QTLs ranged from 3.43% to 11.24% of the trait variation. At QTLs SDIP1.1, SDIP8.1 and SDIP11.1, the alleles from AusTRCF321925 decreased trait values. QTLs SDPAP4.1 and SDTP4.1 overlapped.

For seedling P fraction QTLs, three were detected for PAP, while two and one were identified for TP and IP, respectively (Table 2 and Fig. 2). These QTLs were located on three linkage groups. Alleles of AusTRCF321925 at QTLs SLPAP7.1, SLPAP8.1 and SLTP4.1 reduced the value of the respective P compounds. QTLs SLIP4.1 and SLTP4.1 were co-localized to the marker interval CEDG088-CEDG091, whereas QTLs SLPAP7.2 and SLTP7.1 both mapped between IAC98 and Bmd26. Among the six QTLs identified for seed P fractions, only one (SLPAP4.1) co-localized with a QTL for a seed P fraction (SDIP8.1) (Fig. 2). Three QTLs for days to flowering and two for days to maturity were detected, while five QTLs were identified for seed weight (Table 2 and Fig. 2). PVE of the QTLs for days to flowering ranged from 5.01% to 33.38%, whereas those of days to harvesting varied from 7.24% to 25.67%. The PVE of the QTLs for seed weight were between 6.83% and 18.41%. Location of the QTLs DFL4.1 and DMT4.1 and DFL7.1 and DMT7.1 overlapped.

Table 1. Correlations among phytic acid P (PAP), inorganic P (IP) and total P (TP) in seed (SD) and seedling (SL); days to 50% flowering (DFL), days to 50% maturity (DMT), and 100-seed weight (SD100WT) in the population of 170 F_{2.3} families from the cross V1725BG×AusTRCF321925

|       | SLPAP | SDPAP | SDIP | SLP | SDTP | SLTP | DFL | DMT | SD100WT |
|-------|-------|-------|------|-----|------|------|-----|-----|---------|
| SLPAP | 0.521*** | 0.0802 | 0.0021 | 0.199** | 0.534*** | 0.303*** | 0.279** | 0.297** | −0.0533 |
| SDPAP | 0.0802 | 0.0021 | 0.0151 | 0.199** | 0.273*** | 0.188* | 0.0345 | 0.0395 | 0.2013 |
| SDIP | 0.199** | 0.0301 | 0.0515 | 0.534*** | 0.181* | 0.386*** | 0.0525 | 0.0751 | 0.0590 |
| SLP | 0.521*** | 0.0802 | 0.0021 | 0.199** | 0.273*** | 0.188* | 0.0345 | 0.0395 | 0.2013 |
| SDTP | 0.534*** | 0.273*** | 0.181* | 0.386*** | 0.188* | 0.647*** | 0.210** | 0.184* | 0.1420 |
| SLTP | 0.303*** | 0.298*** | 0.188* | 0.386*** | 0.188* | 0.518*** | 0.219** | 0.230** | 0.282** |
| DFL | 0.279** | 0.0345 | 0.0525 | 0.210** | 0.219** | 0.300** | 0.0590 | 0.0751 | 0.0590 |
| DMT | 0.297** | 0.0395 | 0.0751 | 0.184* | 0.230** | 0.282** | 0.0590 | 0.0751 | 0.0590 |
| SD100WT | −0.0533 | 0.2013 | 0.0590 | −0.223** | −0.1420 | −0.0858 | −0.0564 | −0.0186 |

*,** and *** are significant at 0.05, 0.01 and 0.001 probability level, respectively.

Fig. 1. Frequency distribution in F_{2.3} population from the cross V1725BG×AusTRCF321925, for (a) phytic acid P in seed, (b) phytic acid P in seedling, (c) inorganic P in seed, (d) inorganic P in seedling, (e) total P in seed, (f) total P in seedling, (g) days to 50% flowering, (h) days to 50% maturity and (i) 100-seed weight.
On LG 4, the maturity QTL overlapped with the seed PA QTL. This agrees with their statistically-significant correlation (Table 1). Of these major QTLs, the cultivated parent V1725BG provided the alleles for increased seed PA, as well as days to maturity. At these QTLs, alleles from V1725BG increased seedling PA, but decreased days to flowering and days to maturity.

**Discussion**

Continuous distribution for PAP, IP and TP in both seed and seedling of the F$_2$ population illustrates the quantitative component of these traits. Similarly, quantitative variation in seed PA and TP was observed in soybean (Raboy et al. 1984) and common bean (Blair et al. 2009, Cichy et al. 2009). The positive correlation between seed PA and seed IP concentrations observed here has been reported in studies of mungbean (Sompong et al. 2007), soybean (Raboy et al. 1984) and common bean (Blair et al. 2009). The typically highly positive correlation between seed TP and PAP can be attributed to the role of PAP as the storage form of P in seeds.

Variation in mineral concentrations in plant or seed tissues can be an outcome of agronomic traits such as yield, days to maturity and seed weight, and therefore these relationships must be evaluated in all QTL mapping populations (White and Broadley 2005). In a study of the variation in mineral composition in common bean cultivars, Moraghan and Grafton (2001) reported positive correlations between seed weight and seed TP. In a QTL mapping study of the model legume *Medicago truncatula*, Sankaran et al. (2009) similarly reported a positive correlation between seed weight and seed total P, but negative correlations between seed weight and seed Ca, Cu and Zn. The present study provides a good test of the relationship between seed weight and P content in that the mapping population parents differed greatly in seed weight and seed weight varied greatly in the F$_2$:3 population (Supplemental Table 1). In the present study, the lack of significant positive correlations between seed weight and seed constituents and the non-linkage of their QTLs indicate that selection for low seed P and PA and seedling PA can be expected to have no effect on seed size. A similar conclusion was reported in common bean (Blair et al. 2009).
al. 2009). The positive correlations between seed P fractions and both days to flowering and days to maturity observed in the present study confirm that agronomic traits such as plant phenology must also be considered when dissecting the genetics of seed mineral content.

In conclusion, the present study identified a few mungbean QTLs with low or moderate effect on PA and TP contents in seed and seedling. However, some QTLs for PA and TP contents of seed and seedling overlapped with QTLs for seed size, flowering and maturity, indicating that care must be taken in applying such results in a plant breeding program.

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| Trait QTL Linkage group Interval markers LOD %PVE from regression Additive effect |
|-----------------|-----------------|------------------|-----------------|-----------------|
| Seed PAP SDPAP4.1 4A CEDG139-MB-SSR179 3.78 11.24 14.39 0.0406 |
| SDPAP4.1 11A BM141-VR222 4.00 6.06 0.0670 |
| Seed IP SDIP1.1 1 Satt713-VR102 4.33 5.25 16.97 −0.0333 |
| SDIP8.1 8 CEDG257-IAC100 7.20 3.43 0.0737 |
| SDIP10.1 10 VR226-CEG907 3.45 5.99 −0.0006 |
| sSDIP11.1 11A VR216-CEG168 2.96 5.54 0.0285 |
| Seed TP SDTP4.1 4A Bmd25-MB-SSR179 2.64 10.58 0.1349 |
| Seedling PAP SLPPAP7.1 7 MB-SSR217-GBssr-MB7 5.35 7.56 8.11 −0.3350 |
| SLPPAP7.2 7 IAC98-Bmd26 4.13 7.57 0.1458 |
| SLPPAP8.1 8 MB58-IAC100 3.66 1.38 −0.5518 |
| Seedling IP SLPPAP4.1 4B CEGD088-CEG091 2.65 7.49 −0.2413 |
| Seedling TP SLTP4.1 4B CEGD088-CEG091 5.65 13.82 21.16 −0.5252 |
| SLTP7.1 7 IAC98-Bmd26 4.62 6.59 0.9745 |
| Days to 50% flowering DFL4.1 4A MB213-VR057 5.77 33.38 37.89 6.0000 |
| DFL4.2 4B CEDG154-CEG091 8.35 25.70 −1.3533 |
| DFL7.1 7 VR216-CEG143 4.43 5.01 −0.8575 |
| Days to 50% maturity DMT4.1 4A CEDG086-VR057 4.89 25.67 29.47 4.3978 |
| DMT7.1 7 GBssr-MB7-CEG143 2.64 7.24 −1.2200 |
| 100-Seed weight SD100WT1.1 1 CP1713-CP5137 6.52 18.41 48.03 −0.1465 |
| SD100WT2.1 2 VR078-CEG136 2.60 6.80 −0.0618 |
| SD100WT8.1 8 CP2470-CLM871 2.51 11.70 −0.1873 |
| SD100WT9.1 9 VRSSR010-MB36 2.90 17.88 −0.2141 |
| SD100WT10.1 10 CEDG097-CP2142 3.02 12.38 −0.2313 |

a PVE is phenotypic variance explain.

b Additive gene effect; where positive values indicate allelic contribution from the cultivated accession V1725BG and negative values from the wild accession AusTRCF321925
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