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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 F_2 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.

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Materials and Methods

Plant materials and DNA extraction

A mapping population of F_2 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 $F_{2:3}$ 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 $F_{2:3}$ lines had at least 10 plants. F_4 seeds of each line were harvested in bulk and used for phenotypic measurement. DNA was extracted from young leaves of the F_2 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

F_4 seeds from 10-20 plants in each $F_{2:3}$ 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 H_2SO_4 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 KH_2PO_4 (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 $F_{2:3}$ 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, Seehalak *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 F_2 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 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 Voorips 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 Somta *et al.* (2006).

Results

Variation in P compound contents and agronomic traits

Statistically significant variation between the parents and among the $F_{2:3}$ families were observed for all traits (Supplemental Table 1). Observed continuous distribution in the F_2 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 $F_{2:3}$ progenies was not an artifact of the large difference in seed size between the parents or variation for seed weight in the $F_{2:3}$ 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.

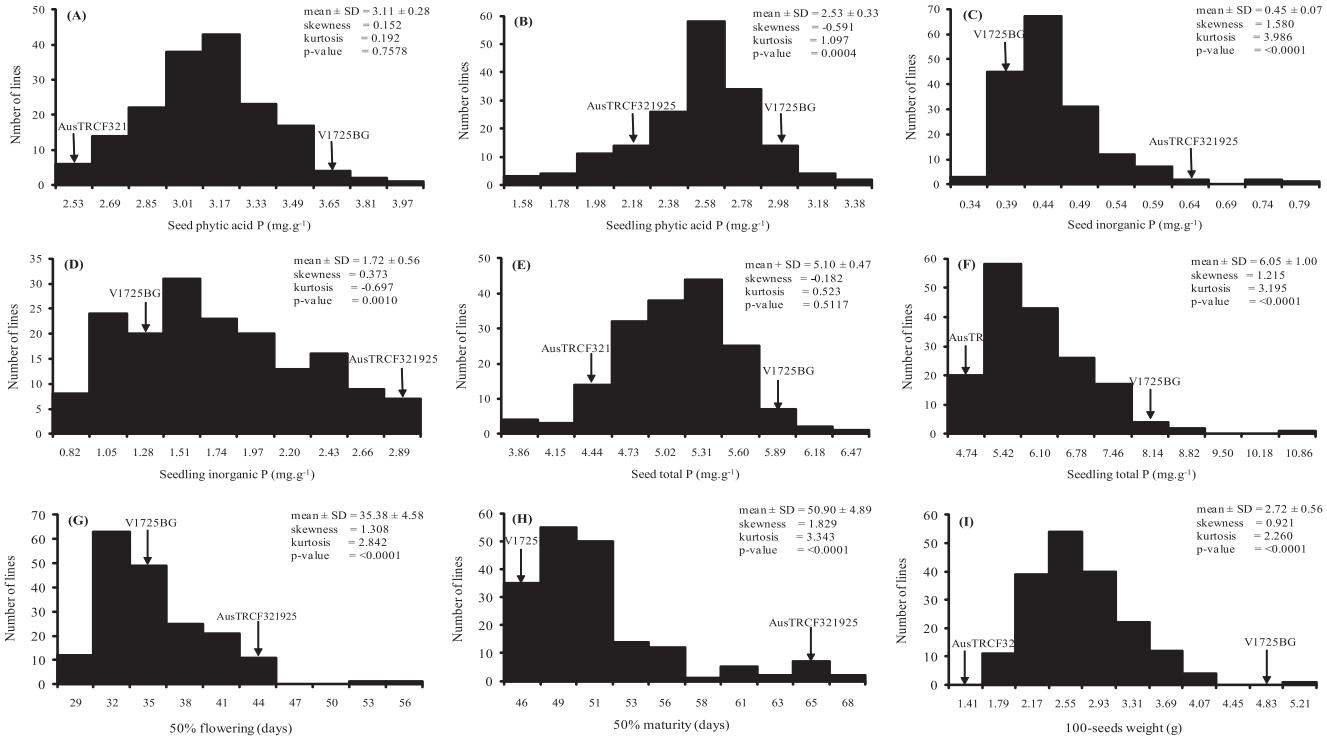

Fig. 1. Frequency distribution in $F_{2:3}$ population from the cross V1725BG × AusTRCF321925, for (a) phytic acid P in seed, (b) phytic acid P in seedling, (c) inorganic P in seed, (d) inorganic P in seedling, (e) total P in seed, (f) total P in seedling, (g) days to 50% flowering, (h) days to 50% maturity and (i) 100-seed weight.

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

	SDPAP	SLPAP	SDIP	SLIP	SDTP	SLTP	DFL	DMT
SLPAP	0.321***							
SDIP	0.0802	0.0021						
SLIP	0.199**	–0.0301	0.0515					
SDTP	0.534***	0.273***	0.181*	0.386***				
SLTP	0.303***	0.298***	0.188*	0.647***	0.518***			
DFL	0.279**	0.0345	0.0525	0.210**	0.219**	0.300**		
DMT	0.297**	0.0395	0.0751	0.184*	0.230**	0.282**	0.762**	
SD100WT	–0.0533	0.201**	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.

QTL identification

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 seedling P fractions, only one (*SLPAP8.1*) 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%, wherase 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.

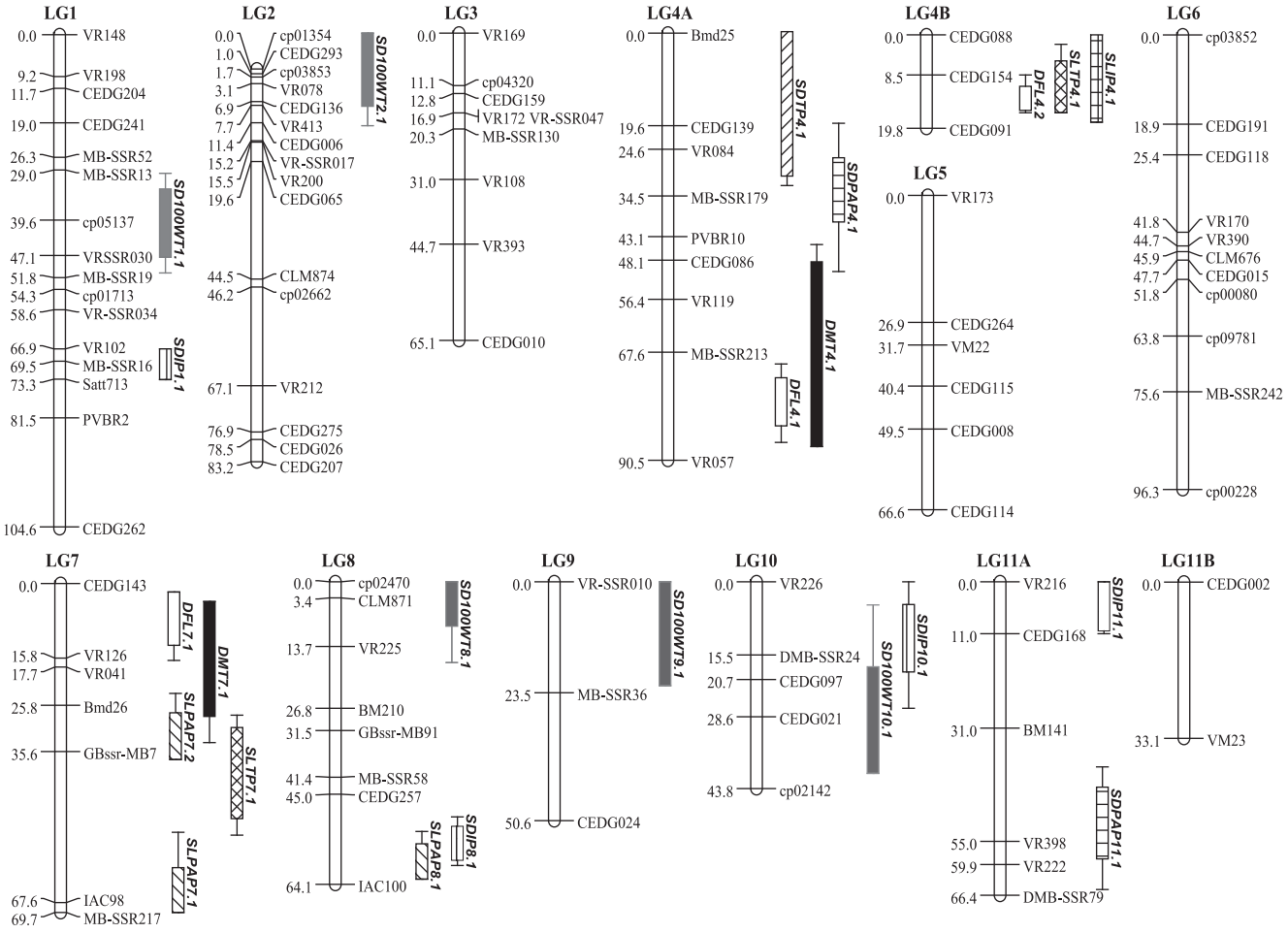

Fig. 2. An SSR-based linkage map constructed from an F_2 population from the cross V1725BG × AusTRCF321925 and genomic regions associated with phytic acid P (PAP), inorganic P (IP) and total P (TP) in seed (SD) and seedling (SL), days to 50% flowering (DFL), days to 50% maturity (DMT) and seed weight (SD100WT).

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 TP and PA 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*

Table 2. QTLs detected for 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). The mapping population is $F_{2:3}$ families derived from V1725BG × AusTRCF321925

Trait	QTL	Linkage group	Interval markers	LOD	%PVE[a] from regression		Additive effect[b]
					Single	Multiple	
Seed PAP	*SDPAP4.1*	4A	CEDG139-MB-SSR179	3.78	11.24	14.39	0.0406
	SDPAP11.1	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-CEDG097	3.45	5.59		–0.0006
	sSDIP11.1	11A	VR216-CEDG168	2.96	5.54		0.0285
Seed TP	*SDTP4.1*	4A	Bmd25-MB-SSR179	2.64	10.58		0.1349
Seedling PAP	*SLPAP7.1*	7	MB-SSR217-GBssr-MB7	5.35	7.56	8.11	–0.3350
	SLPAP7.2	7	IAC98-Bmd26	4.13	7.57		0.1458
	SLPAP8.1	8	MB58-IAC100	3.66	1.38		–0.5518
Seedling IP	*SLPAP4.1*	4B	CEDG088-CEDG091	2.65	7.49		–0.2413
Seedling TP	*SLTP4.1*	4B	CEDG088-CEDG091	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-CEDG091	8.35	25.70		–1.3533
	DFL7.1	7	VR126-CEDG143	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-CEDG143	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-CEDG136	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

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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