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DISSECTING QUANTITATIVE TRAIT LOCI FOR AGRONOMIC TRAITS RESPONDING TO IRON DEFICEINCY IN MUNGBEAN [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Calcareous soil is prevalent in many areas causing substantial yield loss of crops. The research previously identified two quantitative trait locus (QTL) *qIDC3.1* and *qIDC2.1* controlling leaf chlorosis in mungbean grown in calcareous soil in 2010 and 2011 using visual score and SPAD measurement in a RIL population derived from KPS2 (susceptible) and NM10-12-1 (resistant). The two QTLs together accounted for 50% of the total leaf chlorosis variation and only *qIDC3.1* was confirmed, although heritability estimated for the traits was 91.96%. It detected QTLs associated with days to flowering, plant height, number of pods, number of seeds per pods, and seed yield in the same population grown under the same environment with the aim to identify additional QTLs controlling resistance to calcareous soil in mungbean. Single marker analysis revealed 18 simple sequence repeat markers, while composite interval mapping identified 33 QTLs on six linkage groups (1A, 2, 3, 4, 5 and 9) controlling the five agronomic traits. QTL cluster on LG 3 coincided with the position of *qIDC3.1*, while QTL cluster on LG 2 was not far from *qIDC2.1*. The results confirmed the importance of *qIDC3.1* and *qIDC2.1* and revealed four new QTLs for the resistance to calcareous soil.

Keywords: alkaline soil, calcareous soil, green gram, yield-related traits

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an important source of protein in South and Southeast Asia. It is mainly cultivated in Bangladesh, China, India, Indonesia, Myanmar, Pakistan, the Philippines, Sri Lanka, Thailand, and Viet Nam. In Thailand and the Philippines, planting area of mungbean is more than any other legume crops. Seed of mungbean is an inexpensive source of dietary proteins and amino acids for common people and vegetarians in the region. One of the key targets of mungbean breeding programs is to develop high yielding varieties. However, yield is the most complex trait in crops. It is directly determined by three yield-component traits (pod number, seed number per pod, and seed size) and is also indirectly influenced by other yield-related traits such as plant height, branch number, and resistance to biotic and abiotic stresses. Each of these traits is complex and quantitatively controlled by polygenes. Hence, it is difficult to accurately evaluate and select for high yield trait in conventional breeding programs, owing to the interplay between genotype and environment and the in all growth and development stages (Quarrie *et al*., 2006).

Thailand is a major producer and exporter of mungbean seeds and products with the main production in the lower north and upper central regions. In these regions, high pH soil appears in patches across several hundred thousand hectares. Yield loss due to iron deficiency has been observed in most high yielding mungbean varieties (Nopparat *et al.,* 1997). The susceptible varieties show significantly lower yield and yield-related traits as compared to the resistant ones. Recently, QTLs controlling resistance to iron deficiency in mungbean have been identified (Prathet *et al.,* 2012). The research reported one major and one minor QTLs for leaf chlorosis in a

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RIL population grown in iron deficient field in two years. The major QTL was located on linkage group 3, and was named as *qIDC3.1*. *qIDC3.1* was consistently detected in two years by visual scoring and greenness (soil-plant analysis development; SPAD) measurement of leaves, whereas the minor QTL (*qIDC2.1*) was detected in only one year by visual scoring. Although heritability of the resistance measured by SPAD values was as high as 91.96%, the detected QTLs explained less than 40% of trait variation. Thus it appears that additional QTL(s) conditioning the resistance undetectable in the study of Prathet *et al.* (2012). The nutrient use efficiency of a genotype is defined as the ability to produce higher yield in soils with limited nutrient supplies. A better understanding of genetic control of the macro- or micronutrient deficiency can be achieved by QTL mapping of yield and yield-related traits as demonstrated by Zhao *et al*. (2012) and Shi *et al*. (2013). In mungbean, the information of QTLs for yield and yield-related traits under iron deficiency is not known, although these traits can be employed as indicators for measuring resistance to iron deficiency.

The objective of this study was to identify QTLs for agronomic, yield and yield-related traits under iron deficiency using a RIL population.

MATERIALS AND METHODS

Plant Materials

The population used in this study was a recombinant inbred line (RIL) developed from a cross between resistant line "NM10-12-1" and susceptible cultivar "Kamphaeng Saen 2" (KPS2), using KPS2 as the female parent. It is the same population used by Prathet *et al*. (2012) and Krishnachandee *et al*. (2013) for identifying QTLs controlling leaf chlorosis grown under calcareous soil, and mungbean yellow mosaic India virus (MYMIV) disease, respectively. In brief, an F_1 seed from the cross was grown, self-fertilized and generations advanced as F_2-derived lines by single seed descent method. Finally, a population of 122 F_8 RILs was obtained.

Genomic DNA of the parents and RILs were extracted from young leaves following the method described by Lodhi *et al.* (1994). DNA was quantified against lambda DNA on 1.5% agarose gel stained with ethidium bromide.

Traits Measurement

Field experiments were carried out at Nakhon Sawan Field Crops Research Center, Nakhon Sawan province, Thailand in the dry season (March-May) of 2010 and 2011. The soil in this field had pH of 7.92, 2.1% organic matter, 17.83 mg/kg of available P, 97.01 mg/kg of exchangeable K, 15,883 mg/kg of exchangeable Ca, 499.63 mg/kg of exchangeable Mg, 0.99 mg/kg of extractable Fe, 13.62 mg/kg of extractable Mn, 1.63 mg/kg of extractable Cu, and 0.47 mg/kg of extractable Zn.

The 122 RILs and their parents were sown in a randomized complete block design (RCBD) with two and three replications in 2010 and 2011, respectively. In each block, each entry was sown in a single row of 2.5 m long with 12.5 cm intra-row spacing (ca. 20 plants/row) and 50 cm inter-row spacing. Irrigation was performed at 7-days interval. Pesticides were applied as per a standard recommendation for mungbean (Park 1978). In both experiments, five agronomic traits, namely days to flowering (DF), plant height (PH; cm), number of pods per plant (PN), number of seeds per pod (SN), and seed yield per plant (SY; g) were recorded. Five competitive plants of each row were randomly chosen and used for trait measurement. The average data from five plants was used for further analysis.

Linkage Map and QTL Analysis

A simple sequence repeat (SSR) based genetic linkage map previously developed for the same RIL population to locate QTLs controlling leaf chlorosis under calcareous soil (Prathet et al. (2012) was used in this study. In brief, the population was analyzed with 62 polymorphic SSR markers, screened from 1,203 SSR markers derived from mungbean (Somta *et al.,* 2008, 2009, Seehalak *et al.,* 2009, Tangphatsornruang *et al.,* 2009), azuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi) (Wang *et al.,* 2004), cowpea (*Vigna unguiculata* (L.) Walp.) (Li *et al.,* 2001, Kongjaimun *et al.,* 2012) and common bean (*Phaseolus vulgaris* L.) (Buso *et al.,* 2006, Blair *et al.,* 2003, Gaitán-Solís *et al.,* 2002, Guerra-Sanz 2004), together with 8 polymorphic AFLP markers generated from 4 primer combinations. The genetic linkage map was constructed using Join Map version 3.0 software with a minimum LOD threshold of 3.0 and maximum recombination frequency of

0.50. Map distance in centimorgan (cM) was calculated using Kosambi mapping function (Kosambi, 1944).

For QTL analysis, markers associated with a trait were each determined by single regression analysis at $P < 0.01$ using *R*-program version 2.10.0 (R Development Core Team 2010). Then the software WinQTL Cartographer version 2.5 (Wang *et al.,* 2012) was used to locate QTLs for the traits by composite interval mapping (CIM) with standard model (model 6), "window size" of 10 cM, and forward regression. Walking speed was set at 1 cM. Significant threshold for the QTLs of each trait was computed by 1,000 runs of a permutation test at $P = 0.01$.

RESULTS AND DISCUSSIONS

RESULTS

Variation in Agronomic Traits of Rils Grown Under Calcareous Soil

The mean value, range and broad-sense heritability (h^2) of the four traits of the 122-RIL population tested in 2010 and 2011, and combined data are presented in Table 1. A wide variation was observed for all the traits between the two parents and among the RILs in both years. In all cases, the trait values of NM10-12-1 were higher than those of KPS2 (Table 1). For all traits, frequency distributions were continuous and segregations were transgressive. These suggested that the traits are controlled by polygenes and one parent possessed alleles for increased values and the other for decreased value.

Correlation analysis among days to flowering, plant height, seed yield, and yield-related traits of the RIL population grown under iron deficiency revealed that all the traits were significantly correlated in both years 2010 and 2011, and combined data ($r > 0.42$, $P < 0.001$) (Table 2). DF showed negative correlation with the other traits,that showed positive correlation between each others. The correlation among the traits suggested that they may be controlled by some common genetic factors.

QTLs for Mungbean Yield and Yield-Related Traits Grown Under Calcareous Soil

Single marker analysis revealed markers on LGs 1A, 2, 3, 4, 5 and 9 associate with DF, PH, PP, SP and SY of the mungbean RILs (Table 3). Five markers on LG3, including E-ACT/M-CTA-175, CEDG159, CEDC031, CEDG084 and E-CAG/M-TAC-100, showed consistent association with all the traits. In general, these markers explained more than 30% of the trait variation. The rest of the significant markers accounted for less than 10% of the trait variation, with exception to a few of them.

Composite interval mapping was carried out to locate QTLs controlling yield and yield-related traits responded to calcareous soil onto the linkage map. In total, 33 QTLs were identified for the five traits (Table 2). In 2010, a major QTL with one or four minor QTLs were found for PH, PP, SP and SY but only one (major) QTL was identified for DF. Major QTLs for these traits were all overlapped on LG 3 (Fig. 2). The major QTLs explained between 44.29% and 60.50% of the trait variation, depending on traits. At all detected QTLs, except *qSP4.1_2010*, alleles from NM10-12-1 increased trait values. A cluster of minor QTLs for PH, PP, SP and SY was found on LG 2, while additional minor QTLs were identified two each on LG 4 and LG 9. In most cases, the minor QTLs accounted for less than 10% of trait variation.

In 2011, QTL analysis showed similar results to that in 2010. The results revealed that a major QTL with one to three minor QTLs control DF, PH, PP, SP and SY. The locations of major QTLs for five traits were similar to those found in 2010. The major QTLs explained 18.22% to 50.55% of the trait variation. Similar to 2010 data, in most cases, alleles from NM10-12-1 increased trait values. Again, minor QTLs for PH, PP and SY were identified on LG 2 and accounted for less than 10% of the trait variation.

Combined data from two years were also used for locating the QTLs. Composite interval mapping detected only one major QTL for DF and one major with two to four minor QTLs for PH, PP, SP and SY. All the major QTLs were on LG 3 with similar locations. The minor QTLs on LG2 for PH, PP, SP and SY showed similar QTL locations. The same was true for the minor QTLs on LG4 for PP and SP. The major and minor QTLs detected for each trait showed similar positions and effects with those detected in 2010 and/or 2011.

Table 1. Minimum, maximum, mean, and standard deviation (SD) values of days to flowering (DF), plant height (PH), number of pods per plant (PN), number of seeds per pod (SN), and seed yield per plant (SY) in the mungbean RIL population of KPS2 x NM10-12-1 grown in calcareous soil in Thailand in 2010 and 2011

	DF			PH			PP			SY			SP		
	2010	**2011**	**combined**	**2010**	**2011**	**combined**	**2010**	**2011**	**combined**	**2010**	**2011**	**combined**	**2010**	**2011**	**combined**
min	29.00	29.00	29.50	15.53	25.33	20.43	0	1.33	0.67	0	0.48	0.24	5.57	4.31	4.75
max	41.00	36.00	38.00	70.10	68.93	68.72	16.85	13.07	14.66	9.95	6.22	6.77	11.08	11.42	11.16
mean	33.90	31.68	32.79	45.62	48.37	47.00	8.65	7.56	8.10	3.58	3.56	3.57	8.94	9.01	8.93
SD	2.95	1.33	1.83	12.65	7.69	9.81	4.71	2.59	3.42	2.36	1.38	1.74	1.33	1.28	1.25

Table 2. Correlation among days to flowering (DF), plant height (PH), number of pods per plant (PP), number of seeds per pod (SP) and seed yield per plant (SY) in the mungbean RIL population (KPS2 x NM10-12-1) grown in calcareous soil in Thailand in 2010 and 2011

Year	Trait	DF	PH	PP	SP
2010	PH	-0.51***			
	PP	-0.73***	0.86***		
	SP	-0.61***	0.74***	0.79***	
	SY	-0.67***	0.82***	0.93***	0.75***
2011	PH	-0.40***			
	PP	-0.50***	0.71***		
	SP	-0.52***	0.64***	0.82***	
	SY	-0.52***	0.73***	0.96***	0.85***
Combined data	PH	-0.48***			
	PP	-0.68***	0.84***		
	SP	-0.59***	0.70***	0.81***	
	SY	-0.66***	0.84***	0.95***	0.81***

Remarks: *** Significant at $P = 0.001$

DISCUSSION

Iron (Fe) is one of the most important micronutrients which is required for the formation of chlorophyll in plant cells. Fe is taken up by plants as ferrous ions (Fe^{++}). In calcareous soils, iron-inefficient mungbean genotypes often display deficiency symptoms which affect seed yield and yield component in crops. In this study, field trials were conducted under iron deficiencyfor two years to identify QTLs for days to flowering (DF), seed yield (SY) and yield-related traits (SN, PN, and PH), using an F_8 RIL population of mungbean derived from a cross between two cultivars, 'KPS2' and 'NM10-12-1'. The parents showed significant differences in the seed yield and yield-related traits under iron deficiency with NM10-12-1 showed higher values of all the traits. In the RIL population, considerable transgressive segregation was observed for all the traits under iron deficiency chlorosis (IDC) except for SN (Fig. 1). Similar results were reported by Zhao et al. (2012) and Shi et al. (2013) who reported respectively that *Brassica napus* grown under boron and phosphorus deficiency showed highly variation in PH, SP, PP and especially SY.

In total, 24 QTLs associated with DF, PH, SP, PP and SY of the RIL population were detected in two years and combined data (Table 4). All except one of these QTLs were clustered onto four main genomic regions of LGs 1A, 2, 3, 4 and 9 (Fig. 2). Clustering of the QTLs for different traits confirmed high correlations among the traits (Table 2). The major QTLs of all traits were located in the same genomic region, being between marker CEDG159 and E-CAG/M-TAC-100 on LG 3. Prathet *et al*. (2012) reported that the major QTL, *qIDC3.1*, for iron deficiency determined by visual score and SPAD value of leaf chlorosis locates between markers CEDG159 and E-CAG/M-TAC-100. Thus the major QTLs detected for DF, PH, PN, SN and SY under iron deficiency in our study appear to be the same as the *qIDC3.1* reported by Prathet *et al.,* (2012). Since more resistance the RILs more the normal traits, the QTLs for iron deficiency resistance is likely located on the same position as the QTLs for yield and yield-related traits. In addition to the major QTL on the LG 3, our results also revealed minor QTLs on LGs 1A, 2, 4, 5 and 9 yield and yield-related traits under iron deficiency. Comparison on locations of the QTLs in this study with those detected under normal growing conditions reported by Isemura *et al*. (2012) and Kajonphol *et al*., (2012) showed that only *qSP9.1_2010* and *qSP9.1_combined* were common to a QTL for seed number per pod, *Sdnppd5.9.1*, identified by Isemura et al. (2012), suggesting that the QTLs identified for DF, PH, SP, PP and SY in our study are possibly specific to the resistance to iron deficiency.

Table 3. Markers associated with days to flowering (DF), plant height (PH), number of pods per plant (PP), number of seeds per pod (SP) and seed yield per plant (SY) determined by regressions analysis in the mungbean RIL population of KPS2 x NM10-12-1 grown in calcareous soil in Thailand in 2010 and 2011

Traits	LG[1]	Marker	2010		2011		combined	
			P value	R^2	*P value*	R^2	*P value*	R^2
DF	3	E-ACT/M-CTA-175	0.0000	28.6	0.0000	16.0	0.0000	29.3
		CEDG159	0.0000	29.7	0.0000	14.5	0.0000	29.6
		CEDC031	0.0000	43.2	0.0000	24.8	0.0000	43.9
		CEDG084	0.0000	42.0	0.0000	24.9	0.0000	43.2
		E-CAG/M-TAC-100	0.0000	24.1	0.0002	10.2	0.0000	23.4
	5	DMB-SSR080	Ns[2]	Na[3]	0.0009	7.8	0.0060	5.2
PH	1A	CLM637	0.0057	5.7	0.0000	12.2	0.0006	9.2
	2	cp03715	0.0000	18.1	0.0012	8.0	0.0000	15.9
		CEDG100	0.0000	14.6	0.0066	5.4	0.0000	12.2
		cp02662	0.0002	12.3	Ns	Na	Ns	Na
		CEDG225	0.0040	6.1	Ns	Na	Ns	Na
		CEDG108	0.0031	6.4	Ns	Na	Ns	Na
		CEDG244	0.0010	8.65	Ns	Na	Ns	Na
	3	E-ACT/M-CTA-175	0.0000	19.4	0.0005	9.0	0.0000	16.7
		CEDG159	0.0000	24.2	0.0000	13.8	0.0000	22.8
		CEDC031	0.0000	36.6	0.0000	14.1	0.0000	30.8
		CEDG084	0.0000	33.8	0.0000	13.2	0.0000	28.6
		E-CAG/M-TAC-100	0.0000	18.6	0.0002	11.2	0.0000	16.9
	4	CEDC055	Ns	Na	0.0007	8.5	0.0005	5.7
		VR113	Ns	Na	0.0012	7.6	0.0033	6.2
		DMB-SSR199	Ns	Na	0.0019	7.7	0.0067	5.8
PP	2	BM220	0.0036	6.2	Ns	Na	Ns	Na
		cp03715	0.0000	12.1	Ns	Na	0.0003	10.1
		CEDG100	0.0007	8.7	Ns	Na	0.0021	7.5
		cp02662	0.0032	7.4	Ns	Na	0.0099	5.5
	3	VR169	0.0046	6.0	0.0066	5.5	0.0028	6.8
		E-ACT/M-CTA-175	0.0000	30.6	0.0000	22.8	0.0000	31.2
		CEDG159	0.0000	34.5	0.0000	31.3	0.0000	38.1
		CEDC031	0.0000	52.7	0.0000	40.7	0.0000	55.1
		CEDG084	0.0000	49.5	0.0000	37.7	0.0000	51.5
		E-CAG/M-TAC-100	0.0000	25.9	0.0000	24.4	0.0000	29.2
SP	3	VR169	0.0014	9.6	Ns	Na	Ns	Na
		E-ACT/M-CTA-175	0.0000	33.0	0.0000	20.3	0.0000	30.0
		CEDG159	0.0000	28.8	0.0000	28.8	0.0000	32.6
		CEDC031	0.0000	47.9	0.0000	35.9	0.0000	44.9
		CEDG084	0.0000	45.5	0.0000	37.2	0.0000	50.0
		E-CAG/M-TAC-100	0.0000	20.6	0.0000	27.1	0.0000	27.9
	4	DMB-SSR199	0.0051	7.4	Ns	Na	Ns	Na
SY	1A	CLM637	0.0054	5.8	Ns	Na	Ns	Na
	2	cp03715	0.0013	8.0	Ns	Na	Ns	Na
		CEDG100	0.0063	0.5	Ns	Na	Ns	Na
		cp02662	0.0074	6.0	Ns	Na	Ns	Na
	3	VR169	0.0026	6.8	0.0086	5.1	0.0021	7.2
		E-ACT/M-CTA-175	0.0000	29.0	0.0000	23.1	0.0000	31.0
		CEDG159	0.0000	31.3	0.0000	32.1	0.0000	36.2
		CEDC031	0.0000	49.6	0.0000	41.6	0.0000	53.2
		CEDG084	0.0000	46.4	0.0000	40.7	0.0000	50.9
		E-CAG/M-TAC-100	0.0000	24.6	0.0000	28.6	0.0000	30.0

Remarks: [1] Linkage group, [2] Not significant at $P = 0.01$, [3] Not available

Table 4. Quantitative trait loci for five agronomic traits detected by composite interval mapping in the RIL population of KPS2 x NM10-12-1 grown in calcareous soil in Thailand in 2010 and 2011

Trait	Year	QTL name	LG[1]	Marker interval	Position[2]	LOD score	PVE[3] (%)	Additive effect
Days to flowering (DF)	2010	*qDF3.1_2010*	3	CEDC031 – CEDG084	127.9	18.53	51.01	2.18
	2011	*qDF3.1_2011*	3	CEDG084 – E-CAG/M-TAC100	128.5	12.12	31.24	0.79
		qDF5.1_2011	5	DMB-SSR080 – CEDG014	0.1	3.40	7.54	0.37
	Combined	*qDF3.1_combined*	3	CEDG084 – E-CAG/M-TAC100	128.5	20.45	47.58	1.29
Plant height (PH)	2010	*qPH2.1_2010*	2	BM220 – cp03715	24.00	12.31	22.46	-6.10
		qPH3.1_2010	3	CEDC031 – CEDG084	127.9	21.51	44.29	-8.57
	2011	*qPH1.1_2011*	1A	VR194 – CLM637	53.0	3.36	13.82	2.95
		qPH2.1_2011	2	BM220 – cp03715	20.00	4.40	12.75	-2.76
		qPH3.1_2011	3	CEDG084 – E-CAG/M-TAC100	133.50	6.79	18.22	-3.37
	Combined	*qPH11.1_combined*	1A	VR194 – CLM637	45.0	2.98	9.64	3.02
		qPH2.1_combined	2	BM220 – cp03715	21.01	3.68	21.56	-3.16
		qPH3.1_combined	3	CEDC031 – CEDG084	126.90	17.0	32.74	-5.42
		qPH4.1_combined	4	VR113 – DMB-SSR199	79.15	2.72	4.42	2.09
	2010	*qPP2.1_2010*	2	BM220 – cp03715	23.0	3.34	5.58	-1.49
Number of pods per plant (PP)		*qPP3.1_2010*	3	CEDG159 – CEDDC031	123.5	27.40	60.50	-3.71
	2011	*qPP2.1_2011*	2	BM220 – cp03715	26.10	3.33	6.40	-0.66
		qPP3.1_2011	3	CEDC031 – CEDG084	125.9	11.99	45.32	-1.77
	Combined	*qPP2.1_combined*	2	BM220 – cp03715	23.00	10.31	16.89	-1.42
		qPP3.1_combined	3	CEDG159 – CEDDC031	124.50	28.51	60.93	-2.69
	2010	*qSP2.1_2010*	2	CEDG108 – CEDG244	69.8	2.79	5.42	-0.34
Number of seeds per pod (SP)		*qSP3.1_2010*	3	CEDC031 – CEDG084	126.9	20.35	54.73	-1.05
		qSP4.1_2010	4	VR113 – DMB-SSR199	79.15	3.31	5.44	2.32
		qSP5.1_2010	9	CEDG304 – CEDG172	69.4	2.60	6.33	-0.34
	2011	*qSP2.1_2011*	2	CEDG108 – CEDG244	62.8	2.65	5.42	-0.31
		qSP3.1_2011	3	CEDC031 – CEDG084	127.9	15.25	40.91	-0.83
	Combined	*qSP2.1_combined*	2	CEDG108 – CEDG244	69.80	2.84	5.32	-0.34
		qSP3.1_combined	3	CEDC031 – CEDG084	126.90	3.29	40.10	-1.05
		qSP4.1_combined	4	VR113 – DMB-SSR199	79.15	2.92	5.98	2.32
		qSP9.1_ combined	9	CEDG304 – CEDG172	69.40	19.53	6.54	-0.34
Seed yield (SY)	2010	*qSY2.1_2010*	2	CEDG100 – cp02662	35.7	6.13	10.92	-0.79
		qSY3.1_2010	3	CEDG159 – CEDDC031	123.5	25.30	59.90	-1.85
	2011	*qSY2.1_2011*	2	CEDG108 – CEDG244	62.8	3.61	6.50	-0.36
		qSY3.1_2011	3	CEDG159 – CEDDC031	124.5	19.92	50.55	-1.00
		qSY9.1_ 2011	9	VR098 – cp01225	132.7	3.12	5.66	0.41
	Combined	*qSY2.1_combined*	2	cp02662– CEDG225	47.7	6.94	11.64	-0.60
		qSY3.1_combined	3	CEDC031 – CEDG084	126.9	26.12	57.91	-1.35
		qSY9.1_combined	9	CEDG304 – CEDG172	67.8	3.50	4.87	-0.39

Remarks: [1] Linkage group, [2] Position of detected QTL on the linkage group, [3] Phenotypic variance explained by the QTL

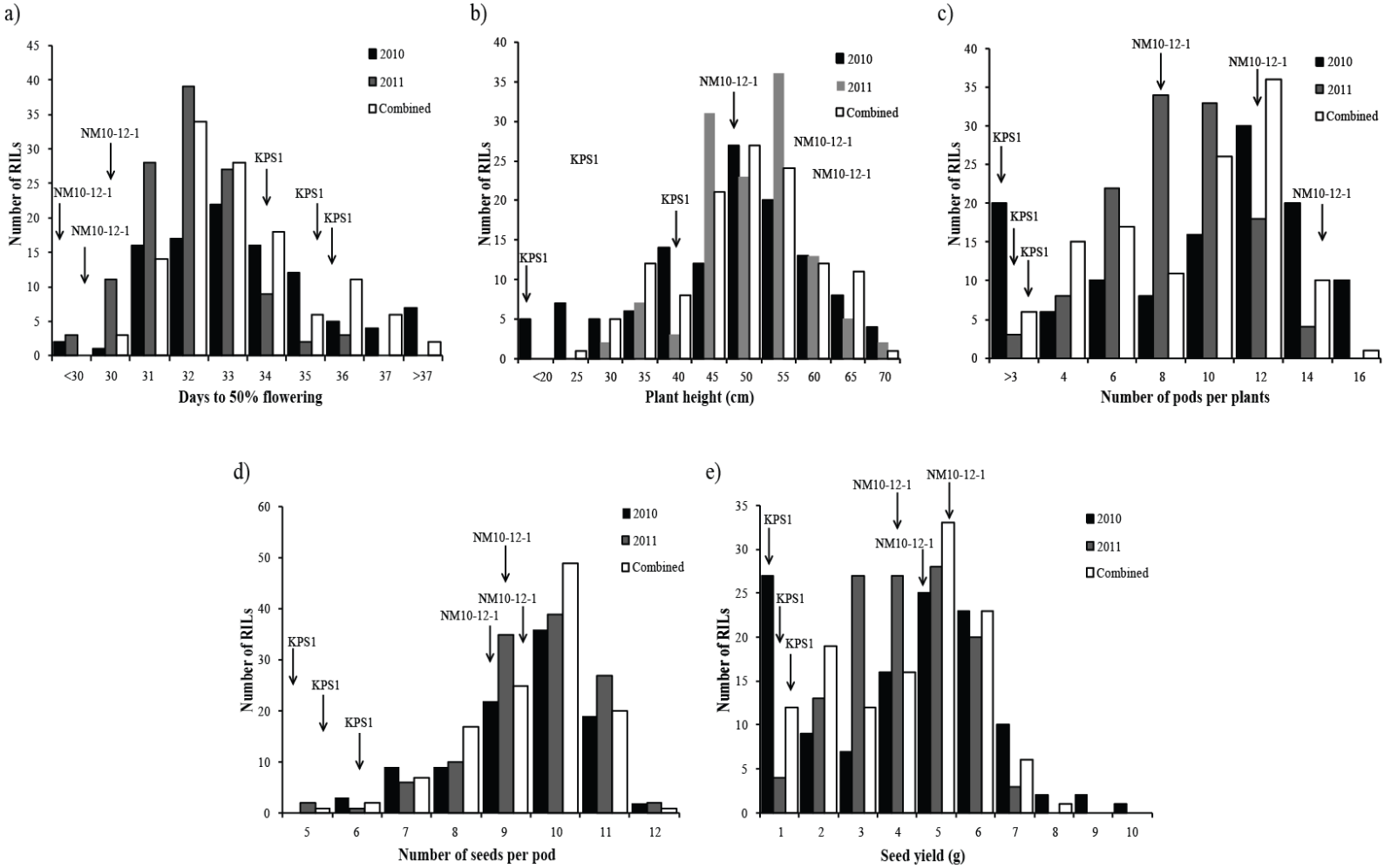

Figure 1. Frequency distribution of days to 50% flowering (a), plant height (b), number of pods per plant (c) , number of seeds per pod (d), and seed yield per plant (e) of the RIL population of KPS2 x NM10-12-1 grown in calcareous soil in Thailand in 2010 and 201

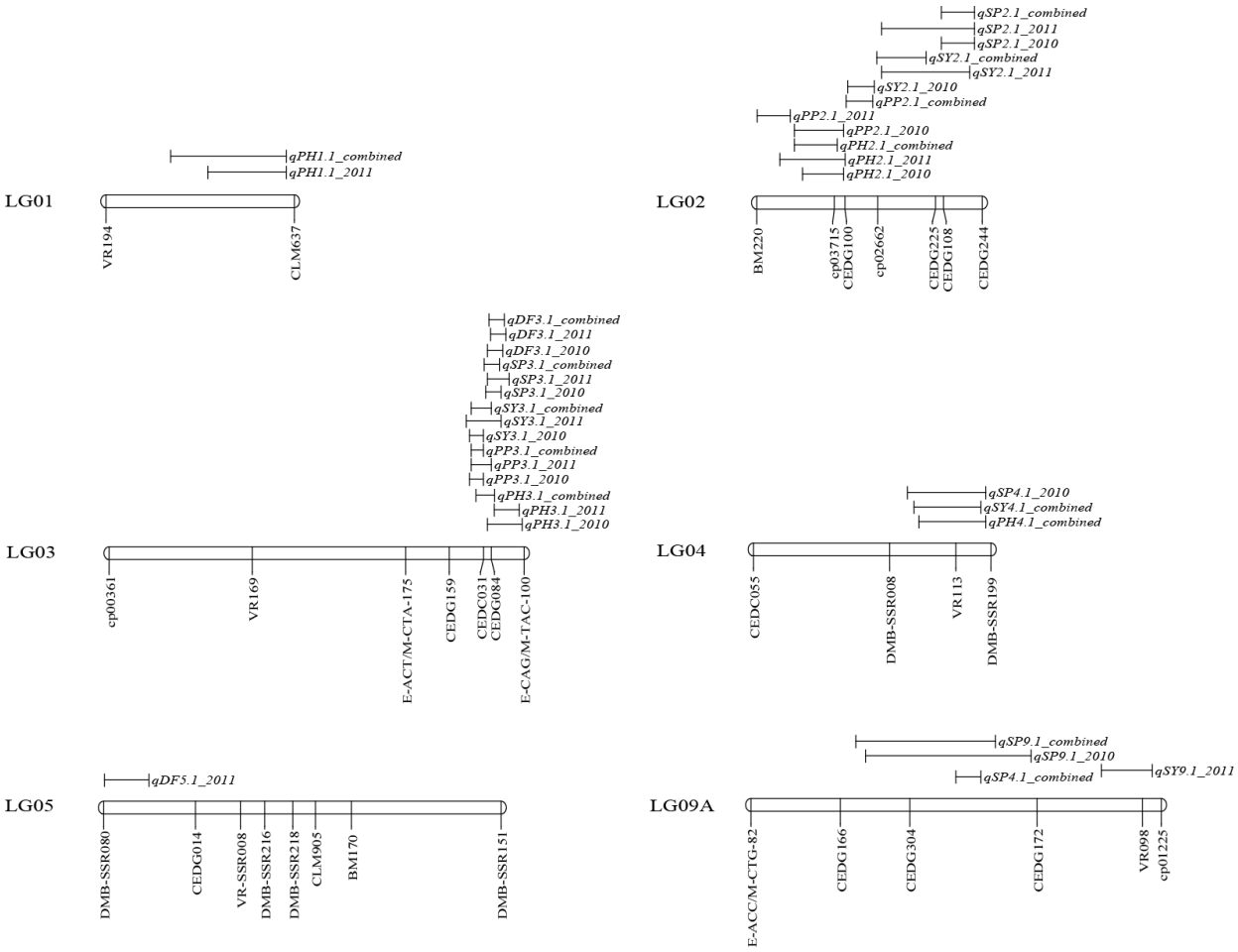

Figure 2. Positions of QTLs for days to flowering, plant height, number of pods per plant, number of seeds per pod, and seed yield per plant on linkage groups of the RIL population of KPS2 x NM10-12-1 grown in calcareous soil in Thailand in 2010 and 2011

Although Prathet *et al*., (2012) found that a QTL on LG 2, *qIDC2.1*, also involved in the leaf chlorosis in 2010, the authors failed to confirm its existence in the following year. However, this study showed and confirmed the association between *qIDC2.1* and resistance to iron deficiency, and also demonstrated the involvement of four additional minor QTLs, one each on LG 1 (*qIDC1.1*), LG4 (*qIDC4.1*), LG5 (*qIDC5.1*) and LG9 (*qIDC9.1*) for the resistance. Altogether, the results reported by Prathet *et al*., (2012) and from our study showed that as high as six (one major and five minor) QTLs control resistance to iron deficiency in mungbean. This is in agreement with the studies in soybean that the resistance to iron deficiency is a complex trait being controlled by a major QTL with three to four minor QTLs (Lin *et al*., 1997; Lin *et al*., 2000). However, it is noteworthy that *qIDC1.1* was only found in 2010 using days to flowering.

CONCLUSIONS AND SUGGESTIONS

CONCLUSSIONS

QTL mapping of days to flowering, plant height, number of pods per plant, number of seeds per pod and seed yield of the mungbean RIL population descended from a cross between Kamphaeng Saen 2 and NM10-12-1 grown in calcareous soil for two years demonstrated that resistance to iron deficiency in mungbean is controlled by one major and five minor QTLs. The results confirmed the QTLs *qIDC3.1* and *qIDC2.1* for the resistance as identified

previously using leaf chlorosis score and SPAD values in the same population, and revealed four new QTLs for the resistance as measured from yield and yield-related traits.

SUGGESTIONS

A major QTL controlling resistance to iron deficiency was located on the linkage group 3 franking by SSR markers CEDC031 and CEDG084. These two markers can be used for marker-assisted selection of mungbean resistance to iron deficiency. Four additional QTLs can also considered if the breeder wants to accumulate more resistance genes with yield benefit in the selected mungbean genotypes.

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REFERENCES

Blair, M.W., F. Pedraza, H.F. Buendia, E. Gaitán-Solís, S.E. Beebe, P. Gepts and J. Thome. 2003. Development of a genome-wide anchored microsatellite map of common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 107: 1362–1374.

Buso, G.S.C., Z.P.S. Amaral, R.P.V. Brondani and M.E. Ferreira. 2006. Microsatellite markers for the common bean *Phaseolus vulgaris*. Mol. Ecol. Notes. 6: 252–254.

Chankaew, S., P. Somta, W. Sorajjapinun and P. Srinives. 2011. Quantitative trait loci mapping of Cercospora leaf spot resistance in mungbean, *Vigna radiata* (L.) Wilczek. Mol. Breed. 28:255–264.

Guerra-Sanz, J.M. 2004. New SSR markers of *Phaseolus vulgaris* from sequence databases.Plant Breed. 123:87–89.

Isemura, T., A. Kaga, S. Tabata, P. Somta, P. Srinives, T. Shimizu, U. Jo, D.A. Vaughan and N. Tomooka. 201 2. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS ONE 7(8): e41304. doi: 10.1371/jou rnal.pone.0041304.

Kajonphol, T., C. Sangsiri, P. Somta, T. Toojinda and P. Srinives. 2012. SSR map construction and quantitative trait loci (QTL) identification of major agronomic traits in mungbean (*Vigna radiata* (L.) Wilczek). SABRAO J. Breed. Genet. 44: 71–86.

Kitsanachandee, R., P. Somta, O. Chatcha-wankanphanich, K.P. Akhtar, T.M. Shah, R.M. Nair, T.S. Bains, A. Sirari, L. Kaur and P. Srinives. 2013. Detection of quantitative trait loci for mungbean yellow mosaic India virus (MYMIV) resistance in mungbean (*Vigna radiata* (L.) Wilczek) in India and Pakistan. Breed. Sci. 63: 367–373.

Kongjaimun, A., A. Kaga, N. Tomooka, P. Somta, T. Shimizu, Y. Shu, T. Isemura, D.A. Vaughan and P. Srinives. 2012. An SSR-based linkage map of yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* Sesquipedalis Group) and QTL analysis of pod length. Genome 55: 81–92.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12: 172–175.

Li, C.D., C.A. Fatokun, B. Ubi, B.B. Singh and G.J. Scoles. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci. 41: 189–197.

Lin, S., S. Cianzio and R. Shoemaker. 1997. Mapping genetics loci for iron deficiency chlorosis in soybean. Mol. Breed. 3: 219–229.

Lin, S., S. Cianzio and R. Shoemaker. 2000. Molecular characterization of iron deficiency chlorosis in soybean. J. Plant Nutr. 23: 1929–1939.

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Rep. 12: 6-13

Nopparat, S., P. Srinives, R. Kaveeta and S. Jintakanon. 1997. An inheritance of mungbean tolerance to microessential element deficiency in Takhli soil series. P. 78–82. *In* Romkaew J. *et al.* (Editors) Proceedings of National Mungbean Research Conference VII, Phitsanulok, Thailand.

Park, H.G. 1978. Suggested cultural practices for mungbean. Asian Vegetable Research and Development Center, Tainan, Taiwan.

Prathet, P., P. Somta and P. Srinives. 2012. Mapping QTL conferring resistance to iron deficiency chlorosis in mungbean [*Vigna radiata* (L.) Wilczek]. Field Crops Res 137:230–236.

Quarrie, S., S.P. Quarrie, R. Radosevic, D. Rancic, A. Kaminska, J.D. Barnes, M. Leverington, C. Ceoloni and D. Dodig. 2006. Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. J. Exp. Bot. 57: 2627–2637

R Development Core Team. 2010. *R*: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Seehalak, W., P. Somta, W. Musch and P. Srinives. 2009. Microsatellite markers for mungbean developed from sequence database. Mol.Ecol. Resour.9: 862–864.

Shi, T., R. Li, Z. Zhao, G. Ding, Y. Long, J. Meng, F. Xu and L. Shi. 2013. QTL for yield traits and their association with functional genes in response to phosphorus deficiency in *Brassica napus*. PLoS ONE 8(1): e54559. doi:10.1371/journal.pone. 0054559

Somta, P., W. Musch, B. Kongsamai, S. Chanprame, S. Nakasathien, T. Toojinda, W. Sorajjapinun, W. Seehaluk, S. Tragoonrung and P. Srinives. 2008. New microsatellite markers isolated from mungbean (*Vigna radiata* (L.) Wilczek). Mol. Ecol. Resour. 8: 1155–1157

Somta, P., W. Seehalak and P. Srinives. 2009. Development, characterization and cross-species amplification of mungbean (*Vigna radiata*) genic microsatellite markers. Conserv. Genet. 10: 1939–1943.

Tangphatsornruang, S., P. Somta, P. Uthaipaisanwong, J. Chanprasert, D. Sangsrakru, W. Seehalak, W. Sommanas, S. Tragoonrung and P. Srinives. 2009. Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol. 9:137. doi:10.1186/ 1471-2229-9-137

Wang, S., C.J. Basten and Z.B., Zeng. 2012. Window QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA.

Wang, X.W., A. Kaga, N. Tomooka, D.A. Vaughan. 2004. The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theor. Appl. Genet. 109:352–360.

Zhao Z., L. Wu, F. Nian, G. Ding, T. Shi, D. Zhang, L. Shi, F. Xu and J. Meng. 2012 Dissecting quantitative trait loci for boron efficiency across multiple environments in *Brassica napus*. PLoS ONE 7(9): e45215. doi:10.1371/ journal. pone.0045215