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Mapping QTL conferring resistance to iron deficiency chlorosis in mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Foliar chlorosis caused by iron deficiency of plants grown on calcareous soil results in substantial crop yield loss and is an important in crop production problem. The objective of this study was to identify the quantitative trait locus (QTL) controlling resistance to iron deficiency chlorosis (IDC) in mungbean. An RIL population of 122 F_8 lines developed from the cross between a susceptible cultivar, "Kamphaeng Saen 2" and a resistant line, "NM10-12" was genetically analyzed with simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. The population was evaluated for IDC resistance in an iron deficient field by visually scoring and SPAD measurements in 2010 and 2011. Segregation of the visual scores and SPAD values of the RILs in both years suggested quantitative inheritance of the resistance to IDC. Visual score and SPAD value from each year and combined data were used for QTL analysis. Single marker analysis revealed that 12 DNA markers from 3 linkage groups (LG) associated with the resistance. Four SSR and two AFLP markers on LG 3 associated with the resistance in all cases. Composite interval mapping identified two QTLs, *qIDC3.1* and *qIDC2.1*, controlling the resistance. *qIDC3.1* on LG 3 was identified from visual scores and SPAD values to account for 12.12% and 41.67% of the total variation depending on traits measured and years. *qIDC2.1* on LG 2 was detected only from visual score data in 2010 and explained 45.66% of the total variation. The *qIDC3.1* was the same as *qIR* which was the major QTL previously reported for IDC in mungbean grown in hydroponic conditions. The SSR markers CEDG084 and CEDC031 flanked and closely linked to the *qIDC* are useful for marker-assisted selection for mungbean resistance to IDC.

1. Introduction

It is estimated that about 800 million ha of the World's land is salt-affected and salt constitutes a major portion of the problem soils that affect agricultural production (FAO, 2005). Calcareous soil is characterized by its having a pH between 7.5 and 8.5, calcium carbonate concentration of 10–14 g kg^{-1} in the soil surface to a depth of 15 cm, and DTPA-Fe concentration between 10 and 20 mg/kg. In the calcareous soil, the availability of Fe^{3+} is decreased substantially as the results of precipitation of inorganic Fe-III. The soil causes iron deficiency chlorosis (IDC) where interveinal leaf tissue is yellowish or necrotic while the veins remain green, resulting in limited growth, development and production. Breeding for IDC resistance is an important objective in breeding program of several crops.

Mungbean [*Vigna radiata* (L.) Wilczek] is a socio-economically important legume in South and Southeast Asia with the annual planting area of about 6 million ha. It is mainly cultivated in China, Bangladesh, India, Myanmar, Pakistan, Sri Lanka, Thailand, the Philippines, and Viet Nam. In Thailand and the Philippines, planting area of mungbean is greater 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. Sprout produced from mungbean seed is popularly consumed in Eastern and Western cuisines as a vegetable for minerals and vitamins. 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 losses due to IDC have been observed in most high yielding mungbean varieties (Nopparat et al., 1997). As a result, average yield of mungbean in Thailand is only about 750 kg/ha, although yield potential of the cultivars released in Thailand is between 1.8 and 2.5 ton/ha. Although foliar application of iron chelate on mungbean can alleviate IDC, it is not practical or economic for small-farm holders.

There are only a few reports on genetic control of IDC in mungbean. Nopparat et al. (1997) reported that resistance to IDC is controlled by the inhibitory action of two genes, but a single

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dominant gene action is also possible. By using bulk segregant analysis technique in a recombinant inbred line population, Sommanus (2000) identified two amplified fragment length polymorphism (AFLP) makers, E-CAG/M-TAC and E-CGT/M-CTG, associated with the IDC resistance. Later, by studying in a hydroponic condition Srinives et al. (2010) reported that resistance to IDC is controlled by a single dominant gene, designated as *IR*, with modifying genes. The authors confirmed an association between the AFLP markers reported by Sommanus (2000) and the *IR* gene, and also identified two new AFLP markers, E-ACT/M-CTA and E-ACC/M-CTG, associated with the *IR* gene. The QTL controlling the resistance, designated as *qIR*, was located between the two markers and explained as high as 76% of the variation in IDC. However, these markers are not highly effective for marker-assisted selection (MAS) because they are relatively far from the *qIR* (3.1 and 10 cM, respectively) and are a dominant (Srinives et al., 2010).

In legume crops, genetics of the resistance to IDC has been well studied in soybean (*Glycine max* (L.) Merr.) (Charlson et al., 2005; Cianzio and Fehr, 1980, 1982; Lin et al., 1997, 2000; O'Rourke et al., 2007, 2009; Wang et al., 2008). Cianzio and Fehr (1980) showed that resistance to IDC is controlled by a single major gene with modifiers. Later, Lin et al. (1997) associated located a major QTL on chromosome 3 of soybean for the IDC resistance. Although several candidate genes were found associated with IDC (Mamidi et al., 2012; O'Rourke et al., 2007, 2009), Peiffer et al. (2012) identified two genes, *Glyma03g28610* and *Glyma03g28630*, with the positions coincide with the QTL region for IDC resistance on chromosome 3 identified by Lin et al. (1997), and found that *Glyma03g28610* has a 12-bp deletion within a predicted dimerization domain. The authors hypothesized that the deletion may hinder the FIT/bHLH heterodimer which induce other iron acquisition genes.

The objectives of this study were to locate the QTL(s) controlling resistance to IDC under field condition and to determine if results confirm the QTL identified earlier by Srinives et al. (2010). DNA markers developed from sequences within and/or nearby the *bHLH* gene from common bean [*Phaseolus vulgaris* (L.)] and soybean were also analyzed to test for their association with the IDC resistance in mungbean.

2. Materials and methods

2.1. Plant materials

A recombinant inbred line (RIL) population was used in this study. The population was developed from a cross between resistant line "NM10-12-1" and susceptible cultivar "Kamphaeng Saen 2" (KPS2), using KPS2 as the female parent. The parental mungbeans used in this study are the same as those used by Nopparat et al. (1997), Sommanus (2000) and Srinives et al. (2010), although the population or generation used is different. 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.

2.2. Evaluation for resistance to iron deficiency chlorosis

Field evaluation for resistance to IDC was carried out in an experimental field of Nakhon Sawan Field Crops Research Center, Nakhon Sawan province, Thailand where alkaline soil is uniformly prevailing (Srinives et al., 2010). 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.

In the dry season (March) of 2010 and 2011, the 122 RILs and their parents were sown in a randomized complete block design (RCBD) with two and three replications, respectively. Dry season plantings helped avoid interference from rains that often causes reduction in soil pH and increasing iron availability during RIL evaluation. In each block, each entry was sown in a single row 2.5 m long with 12.5 cm intra-row spacing (ca. 20 plants/row) and 50 cm inter-row spacing. In both experiments, IDC was evaluated by two methods, viz., visual scoring and soil-plant analysis development (SPAD) measurement at 3 weeks after planting. For visual score, plants were evaluated in rows based on leaf chlorosis symptoms. Scoring system was the same as reported by Srinives et al. (2010), viz., 1 (no yellowing), 2 (slight yellowing), 3 (moderate yellowing), 4 (intense yellowing coverage), and 5 (severe yellowing with some necrosis). Scoring was conducted by a panel of two trained staff. For SPAD measurement, 5 plants from each RIL were determined using a MINOLTA SPAD-502 meter (MINOLTA, Tokyo). Each plant was measured on 4 spots (2 each on the left and right of the midrib) on the terminal leaflet of the youngest fully expanded trifoliate leaf of the plant. The measurement was done immediately after visual scoring. The average SPAD reading from 5 plants was used to characterize IDC symptom of a RIL.

2.3. Estimation of heritability of IDC

Narrow-sense heritability (h^2) was estimated based on the property that RILs are theoretically a population of pure lines. Thus only additive genetic variation (σ_a^2) and interaction between the additive genes contribute to total genetic variation (σ_g^2), while dominant genetic variation (σ_d^2) can be negligible. Analysis of variance for SPAD value and visual score were performed on the RIL data using *R*-program version 2.10.0 (R Development Core Team, 2008). The heritability for both traits were estimated from the formula $h^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_e^2/r)]$; where σ_e^2 is the experimental error variance and r is the number of replications in each season.

2.4. SSR marker analysis

A total of 1191 SSR primer pairs from various legume crops were screened for polymorphism between the parents. Among them, 530 were from mungbean (Seehalak et al., 2009; Somta et al., 2008, 2009; Tangphatsornruang et al., 2009), 332 from azuki bean [*Vigna angularis* (Ohwi) Ohwi & Ohashi] (Wang et al., 2004), 152 from common bean (*Phaseolus vulgaris* L.) (Blair et al., 2003; Buso et al., 2006; Gaitán-Solís et al., 2002; Guerra-Sanz, 2004), and 177 from cowpea [*Vigna unguiculata* (L.) Walp.] (Kongjaimun et al., 2012; Li et al., 2001; Xu et al., 2010). Polymerase chain reaction (PCR) amplification was performed in a 10 μl reaction volumes containing 2 ng genomic DNA, 1× *Taq* buffer with $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 2 mM dNTPs, 5 pmol of each forward and reverse primers and 1 U *Taq* DNA polymerase (Fermentas). The DNA was amplified in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems). Thermal cycling was programmed as follow: 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 47–60 °C (depending on primers) for 30 s, 72 °C for 1 min, and 72 °C for 10 min. The PCR products were mixed with 10 μl of formamide containing 10 mM EDTA, 0.02% bromophenol blue and xylene-cyanol, denatured at 94 °C for 2 min. Then 1.5–2 μl of the final product was loaded onto 5% polyacrylamide gel and visualized by silver staining.