THE ASSOCIATION BETWEEN AFLP MARKERS AND GENE CONTROLLING TOLERANCE TO IRON DEFICIENCY IN MUNGBEAN (*Vigna radiata* (L.) Wilczek)

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important food legumes in Asia. It has a long history in production and utilization. Mungbean seed contains 20 - 25% protein and 1.0 - 1.2% fat. It is rich in vitamins (A, B_1, B_2 and C and niacin), and minerals (K, P and Ca) (Potter and Hotchkiss, 1997). Mungbean can be consumed as boiled seed, sprout, noodle, starch, cookies, snack and high protein foods (Prabavat 1986). The major production area of mungbean in Thailand is in the central and lower northern parts where the calcareous high pH soil scatters in the area of several hundred thousand hectares. Most recommended mungbean cultivars grown in this soil showed chlorosis induced by iron deficiency. Iron deficiency reduces the function of ferredoxin, which is required for the formation of chlorophyll in plant cells. Thus iron is essential to growth and development of all plants and serves as an activator for respiration and photosynthesis. Ohwaki *et al.* (1996), Ohwaki *et al.* (1997) and Oonkasem and Thavarasook (1991) reported that the two most popular mungbean cultivars in Thailand, Kamphaeng Saen 1 (KPS1) and Kamphaeng Saen 2 (KPS2) grown on calcareous soils (iron-deficient soils) showed high chlorosis symptoms and died prematurely or giving extremely low yield. KPS2 is more sensitive than KPS1. However, information on field grown mungbean cultivars susceptible/tolerance to iron deficiency has been poorly documented. Yet soil heterogeneity and heavy rains can hinder the expression of this character. Rain water replacing air in soil pores creates reduction state in the soil and induces temporary iron availability to the mungbean. This is a major obstacle in field screening of germplasm and selecting segregating progenies. The use of DNA makers to help screening and selection in the laboratory can reduce time and money required for field grow out as well as preventing false identification of the tolerant-susceptible genotypes.

So far, there were only a few studies available on the inheritance of tolerance to iron deficiency in field crops. Cianzio and Fehr (1980) and Cianzio (1999) reported that a major gene and several modifying genes controlling iron deficiency tolerance in soybean. In chickpea, tolerance to iron deficiency chlorosis is controlled by a single dominant gene over susceptibility (Saxena *et al*., 1990). Iron deficiency chlorosis tolerance in oats has been reported to be controlled by a major dominant gene with modifiers (McDaniel and Brown, 1982). Most breeders working on iron deficiency always faced problems in screening and phenotyping of this trait. Soil heterogeneity and rainfalls are major problems causing low repeatability of the trait in field screening. The use of molecular markers can help saving time and money in screening and selecting for the tolerance. The AFLP method developed by Vos *et al*. (1995) combines features of RFLP with PCR and is a highly reproducible marker system (Vuylsteke *et al*., 1999). The evidence on usefulness of simplified AFLP systems based on *Eco*RI and *Mse*I was further given in self and cross-pollinated plant species (Ranamukhaarachchi *et al*., 2000). Powell *et al*. (1996) and Pejic *et al*. (1998) convinced that AFLP is 10-fold higher efficiency than RFLP, RAPD and SSR. Sommanus (2000) worked on 199 F_8 recombinant inbred lines from the cross between KPS1 (susceptible) and NM10-12 (tolerance) and found two AFLP makers linked to the gene controlling susceptible to iron deficiency, E-CAG/M-TAC and E-CGT/M-CTG locating at 2.9 and 3.0 cM away, respectively. Chalee (2005) worked on 190 F_2 plants from the cross between KPS1 (susceptible) and NM10-12 (tolerance) and found two additional AFLP makers linking to gene controlling tolerance iron deficiency, E-ACC/M-CTG and E-ACT/M-CTA.

The objective of this study is to detect the presence of the four AFLP markers in 241 mungbean germplasm from various countries.

LITERATURE REVIEWS

Taxonomy of mungbean (*Vigna radiata* (L.) Wilczek)

Mungbean is classified into the family Fabaceae, sub-family Papilionoideae, tribe Phaseoleae, sub-tribe Phaseolinae (Arora *et al.*, 1973). The genus *Vigna* is divided into seven subgenera: *Vigna*, *Plectotropis*, *Ceratotropis*, *Lasiospron*, *Sigmoidotropis*, *Haydonia* and *Macrorhynchus* which each subgenus comes from different origin. The subgenus *Ceratotropis* is related with mungbean and originated in the Asiatic. It consists of mungbean/green gram (*Vigna radiata* (L.) Wilczek), black gram/urd bean (*Vigna mungo* (L.) Hepper), moth bean (*Vigna aconitifolia* (Jacq.) Maréchal), adzuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi) and rice bean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi). This group is homogeneous with highly specialized floral structures and is treated as the subgenus *Ceratotropis* of the genus *Vigna* (Verdcourt, 1970; Maréchal *et al.*, 1981; Ohashi, 1980; Tateishi and Ohashi, 1990). The subgenus is characterized in having peltate stipule, standard petal with a protuberance, incurved keel-petals with pocket and style-beak. From these features the subgenus is considered to be monophyletic (Maréchal *et al.*, 1981; Tateishi and Ohashi, 1992). Chromosome number of *Ceratotropis* is 2n=22, 2x (Chandel *et al.*, 1984; Morton *et al.*, 1982; Sarbhoy, 1977 and 1978), except for *V. reflexo-pilosa* and *V. glabrescens* which are tetraploid (2n=44, 4x). Moreover, mungbean has a small genome ($5x10^8$ bp) with 40% repetitive DNA (Hughes, 1996). Poehlman (1982) classified *V. radiata* into 3 subspecies viz. *radiata*, *sublobata* and *glaba*. Subspecies *sublobata* is the wild ancestral form of cultivated sub species *radiata* (Verdcourt, 1970).

Origin of mungbean (*Vigna radiata* (L.) Wilczek)

The mungbean (*Vigna radiata* (L.) Wilczek) is the most widely cultivated species (Oplinger *et al.*, 1997). It arose from the wild variety *sublobata* (Roxb.) Verdc., which was first domesticated in India. So India is believed to be the center of origin of mungbean where morphological diversity, existence of wild and weedy types and archaeological remains of mungbean were found (Arora, 1985; Singh *et al.*, 1974). A large number of var. *sublobata* are found in India, of which two distinct wild types, viz. *Vigna radiata* var. *sublobata* and *Vigna radiata* var. *setulos*. Wild forms of mungbean, *Vigna radiata* var. *sublobata* show a wide area of distribution, covering Central and East Africa, Madagascar, Southeast Asia, Northern-South America, New Guinea, and North and East Australia (IBPGR, 1985; Jain and Mehra, 1980).

Characteristics of mungbean (*Vigna radiata* (L.) Wilczek)

Mungbean is an erect or sub-erect herb, being about 60 to 75 cm. tall, having trifoliate leaves and highly branched (Oplinger *et al.*, 1997). The pale yellow flowers are borne in cluster of 12 to 15 near the top of the plant. Pods are 3 to 4 inches long, each having 10 to 15 seeds. There are several pods clustering at a leaf axil, with typically totaling 30 to 40 pods per plant (Thomas Jefferson Agricultural Institute, 1998). Mature pods are variable in color varying from yellowish-brown, pale gray, brown, or black. Mature seed color exhibits a wide range of variation from yellow, greenish yellow, light green, shiny green, dark green, dull green, black, brown, and green with black mottle. Mungbeans are self-pollinated crop. Germination is epigeal with the cotyledons and stem emerging from the seedbed.

Environmental requirements of mungbean

Climate Mungbeans are warm season crop requiring 60 to 100 days from planting to maturity. Sufficient rainfalls are required from flowering to late pod filling in order to secure good yield. Mungbeans that are flowering during high temperature and low rain fall period normally give low yield (Dept. of Agricultural Extension, 2000).

Soil Mungbeans do best on fertile sandy, loamy soil with good drainage. Performance is best on soils with a pH between 6.2 and 7.2. Plants can show severe iron chlorosis symptoms and certain micronutrient deficiencies when grown in calcareous soils (Dept. of Agricultural Extension, 2000).

Mungbean utilization and nutritative value

The nutritative value of mungbean contained 20-25% protein, 1.0-1.2% fat, vitamins and minerals that are necessary for human body. Mungbean seeds are rich in vitamins (A, B_1, B_2, C and niacin) and minerals (K, P and Ca). Mungbeans are raw materials of several food products, such as mungbean noodle, starch, low-cost high-protein foods, protein supplemented foods, bean sprouts, and several kinds of desserts and main dishes. Furthermore, protein isolated from the left-over of mungbean noodle production can be used to make textured protein for human consumption at low cost (Prabhavat, 1987).

Mungbean and calcareous soils

Calcareous soils are alkaline soil having limestone (calcite, $CaCO_3$) parent material. This caused excessive accumulation of HCO_3^- causing the increase in soil pH of 7.3 - 8.5 (Lindsay, 1979). As the pH increases, CO_2 and HCO_3^- are disassociated and release CO_3^{2-} and H^+ in which the CO_3^{2-} may react with Ca^{2+} ions and precipitate as solid calcium carbonate. The latter is less dissolved in water and accumulates in the soil. The problem of calcareous soils is associated with less dissolve of micronutrients, such as iron, manganese, zinc, and copper. Specifically, iron will decrease 10^3 fold per each unit of pH that increases. Consequently, soluble irons (Fe^{2+} and Fe^{3+}) are precipitated as ironoxide (Fe_2O_3), which resulted in accumulation of $CaCO_3$. Thus, the plants grown in calcareous soils may show the symptoms of iron deficiency (Loeppert and Hossner, 1984).

Calcareous soils in Thailand

Calcareous soil is found in cultivated areas. The major calcareous soil series in Thailand are Lop Buri soil series and Takhli soil series. Calcareous soils are found mostly in the central region of Thailand as the result of accumulation of limestone. Calcareous soils can be classified into 2 orders, vertisols and molisols, which are found in

Takhli soil series and Lop Buri soil series. A part of calcareous soils can be found in Nakhon Sawan Field Crops Research Center. The dominant characteristic of the soil in order vertisols is clayey and highly elastic. Dry soil is deeply broken on surface and causes soil inversion. This order may have grumusols as main soil group and basic parental materials. Furthermore, the vertisols can be found in the semi-humid or semi-arid regions. In Thailand, soil series in vertisols group is the Lop Buri soil series, which is found in the central region.

Iron deficiency in calcareous soils

The plants cultivated in calcareous soil may show chlorosis symptom since the limestone causes high HCO_3^- in soil solute. The HCO_3^- impairs absorption, mobility, and availability of iron to plant (Marschner, 1995). The influence of HCO_3^- in calcareous soil is as follows:

1. The dissolvable of iron in soil is reduced when pH of soils is increased.
2. The mechanism of H^+ - efflux pumps (releasing of proton) is impaired because the proton (H^+) interacts with HCO_3^- and thus could not decrease the pH in soil solution.

3. The release of phenol in root plants is reduced.
4. The reduction of ferric is occurred at only on the plasma membrane.
5. In highly HCO_3^- solution, the roots increase organic acid synthesis which form into iron chelate accumulated in root vacuoles.

6. The collection of iron in root vacuoles causes the reduction in iron transport to leaves.

7. Root extension and osmotic pressure are decreased.
8. Growth development of chloroplasts and leaves are stopped because the plants cannot move cytokinin from roots.

Calcareous soils with calcium carbonate and high pH can cause iron deficiency in peanut, soybean and mungbean and causes reduction in nodule mass, hemoglobin content, and crop yield (Food and Fertilizer Technology Center, 2001). Corn and sorghum grown in calcareous soils with iron deficiency may have less number of grana and lamella. Iron deficiency in soybean crops grown in black calcareous soils resulted in chlorosis symptoms (Goos and Johnson, 2003). Ohwaki *et al*. (1996) and Oonkasem and Thavarasook (1991) reported that the two most popular mungbean cultivars in Thailand, Kamphaeng Saen 1 (KPS1) and Kamphaeng Saen 2 (KPS2) when grown on calcareous soils (iron-deficient soils) showed high chlorosis symptoms and died prematurely or gave very low yield. Ohwaki *et al*. (1997) demonstrated the symptoms of iron deficiency in 10 mungbean cultivars based on chlorotic symptoms and found that KPS2 was highly susceptible; KPS1, PSU1 and Pag-asa1 were susceptible; VC1163B was moderately tolerant; CN36, CN60, UT1 and CNM-I were tolerant; and CNM8509B was highly tolerant when cultivated in calcareous soils field in Nakhon Sawan Field Crops Research Center.

Iron deficiency in plants

Iron deficiency can occur at both extremes of the pH range of agricultural soils. Many factors may contribute either singly or in combination with the development of chlorosis, low iron supply, the ratio of calcium carbonate to bicarbonate in soil, irrigation water (over irrigation or waterlogged conditions), high phosphate levels, high level of heavy metals, low or high temperatures, high light intensities, high level of nitrate nitrogen, imbalance in cation retios, poor soil aeration, amount of organic matter in soil, viruses, root damage by nematodes and other organisms.

The symptoms of iron deficiency

Iron deficiency is usually manifested as an interveinal chlorosis of young leaves while the veins remain green, hence termed as iron deficiency chlorosis. Iron chlorosis is found where the soils are calcareous (Rawe, 2003). The expression of the symptoms in young leaves is due to the inability to redistribute iron within the plant. If the deficiency is severe, the entire leaf may become yellow. Finally, the leaf edges turn brown. Iron deficiency can affect chloroplast formation as followed:

1. In leaves of iron deficient sugar beet the quantity of thylakoid membrane per chloroplast is decreased (Platt *et al.*, 1983), galactolipids are reduced.

2. Reduction of pigments responsible as photoreceptor, such as chlorophyll a, chlorophyll b and carotenoids.

3. Electron transport system in thylakoid membrane is interfered, because of less quantity of electron carriers, such as cytochrome F and ferredoxin (Terry and Abadia, 1986).

4. Decrease in chlorophyll synthesis and the amount of enzyme associating with δ - amino levulinic acid synthesis (Huang *et al.*, 1984).

5. Plants stop thylakoid formation. Number of thylakoids per chloroplast is reduced along with its essential compounds (Terry and Abadia, 1986).

6. The activity of RuBP carboxylase decreases, resulting in less CO_2 fixation (Terry and Abadia, 1986).

In addition, hormones in iron deficient plants can impair plant growth. For example, ethylene and auxin involving in root development and morphogenesis were found response to iron deficiency. When iron-deficient cucumber plantlets are treated with an inhibitor of ethylene synthesis, the Fe-deficiency induced ferric reductase activity is inhibited, whereas addition of ACC increases the response (Romera and Alcantara, 1994). In *Arabidopsis* mutants, the alteration of ethylene metabolism caused inhibition in formation of ectopic root hairs in response to iron starvation (Schmidt *et al.*, 2000; Schmidt and Schikora, 2001). On the contrary, mutants affected in hormone signaling show a normal increase in Fe^{3+} chelate reductase acitivity upon exposure to iron deficiency.

Inheritance of gene controlling tolerance to iron deficiency in field legumes

The inheritance of tolerance to iron deficiency chlorosis in field legumes vary depending on the genotypes of the crop parents used in developing the population and the test condition. In dry bean, iron deficiency tolerance is controlled by two complementary, dominant genes (Zaiter *et al.*, 1987). While in soybean, response to iron deficiency was controlled by a major gene, with a recessive allele conditioning inefficiency (Weiss, 1943). Cianzio and Fehr (1980) and Cianzio (1999) reported that a major gene and several modifying genes controlling iron deficiency tolerance in soybean. In chickpea, tolerance to iron deficiency chlorosis is dominant over susceptibility, and is controlled by a single gene (Saxena *et al.*, 1990). Iron deficiency chlorosis tolerance in oats was reported to be controlled by a major dominant gene with modifiers (McDaniel and Brown, 1982). Dasgan *et al.* (2004) reported that iron deficiency tolerance in tomato is controlled by polygenic loci with a relatively high additive effect.

AFLP (Amplified Fragment Length Polymorphism) molecular marker

AFLP markers are generated by a combination of restriction digestion and PCR amplification. First step, target DNA was digested with restriction enzyme, depending on size of target DNA. Second step, the number of DNA fragments with increased by combining adapters at the end of DNA fragments, next to recognition site. Adapters are short double-stranded DNAs with one end is easily bound with one end of the DNA fragments. Third step, the number of DNA markers are increased by PCR amplification based on the primers that are specific with adapter at the end regions of DNA fragments (Vrieling *et al.*, 1997). Finally DNA polymorphism was detected by gel electrophoresis in acrylamide gel, which can separate size of DNA fragments (Melcher, 2000). The mechanism of gel electrophoresis is based on migration of DNA in an electric field passing through acrylamide gel at different concentration (Ogden and Adams, 1987). While the DNA is negatively charged, the samples are loaded near the negative pole, and migrate toward the positive pole. Separation of the molecules is strictly based on size, the smaller fragments move farther in the gel because they can be navigated through the small pores in the gel better than larger molecules (McClean, 2000).

AFLP markers for detecting the gene controlling tolerance to iron deficiency

The AFLP technique, originally known as selective restriction fragment amplification (SRFA), produces highly complex DNA profiles by arbitrary amplification of restriction fragments ligated to double-stranded adaptors with hemi-specific primers harboring adaptor-complementary 5´ termini (Vos *et al.*, 1995). The technique has been widely used in the construction of genetic maps containing high densities of DNA marker loci. The AFLP protocol amplifies restriction fragments obtained by endonuclease digestion of target DNA using "universal" AFLP primers complementary to the restriction site and adapter sequence. However, not all restriction fragments are amplified because AFLP primers also contain selective nucleotides at the 3´ termini that extend into the amplified restriction fragments. These arbitrary terminal sequences result in the amplification of only a small subset of possible restriction fragments. Generally, the abundant restriction fragments produced from complex genomes require AFLP primers with longer selective regions. Conversely, analysis of small genomes require only few arbitrary nucleotides added at the primer 3´ termini. The resulting AFLP fingerprints are usually a rich source of DNA polymorphism that can be used in mapping and fingerprinting endeavors.

The investigation on gene controlling iron deficiency in mungbean was firstly done by Somanus (2000). She used bulked-segregant analysis on 199 recombinant inbred lines (RILs) and found AFLP markers E-CGT/M-CTG and E-CAG/M-TAC that stayed close to the gene controlling iron deficiency in mungbean. Chalee (2005) worked on 160 F_2 plants from the cross between NM10-12 (tolerant parent) and KPS1 (susceptible parent) and found two markers (E-ACC/M-CTG and E-ACT/M-CTA) linking to gene controlling tolerance to iron deficiency. In soybean, Charlson *et al.* (2005) found molecular marker Satt481 associated with iron-deficiency chlorosis resistance in a soybean breeding population. Lin and Shoemaker (1997) mapped genes controlling iron deficiency chlorosis in intraspecific soybean (*Glycine max* (L.) Merrill) populations. A benefit in obtaining information on molecular markers would be to increase the efficiency in selection of parents for producing a population to map a gene or QTLs for a particular trait and initial screening method for identification of the genes.

MATERIALS AND METHODS

Plant Materials

Two hundred and fourty-one mungbean (*Vigna radiata* (L.) Wilczek) germplasm accessions (Table1) were randomly chosen from mungbean germplasm obtained from Asian Vegetable Research and Development Center (AVRDC-The World Vegetable Center). They were divided into two sets. Set I consisted of materials from Thailand and set II was from other mungbean growing countries, especially Afghanistan, India, Iran, Pakistan, Philippines, Taiwan, United States of America, etc.

Field experiment

A field experiment was conducted at Nakhon Sawan Field Crops Research Center, Thailand. The mungbean accessions were planted in rows of 1 m long. NM10-12 and KPS1 were alternately planted as resistant and susceptible check genotypes every 10 rows. In each row, the seeds were sown at the spacing of 10 cm between plants and 50 cm between rows. Chlorosis symptom in each accession was evaluated at one month after planting, which was about the day to first flowing of most entries. Visual observations were made using the scale 1 for no chlorosis, 2 for slight chlorosis, 3 for mild chlorosis, 4 for moderate chlorosis, and 5 for severe chlorosis.

Table 1 List of 241 accessions of mungbean germplasm used in this study.

Entry no.	Accession no.	Seed origin	Name
1	V1658BBR	Afghanistan	EC15095
2	V1658BG	Afghanistan	EC15095
3	V1660BG	Afghanistan	EC15097
4	V1661BG	Afghanistan	EC15100
5	V1670AG	Afghanistan	EC15146
6	V1670BG	Afghanistan	EC15146
7	V1671BBR	Afghanistan	EC15149
8	V1671BG	Afghanistan	EC15149
9	V1672AG	Afghanistan	EC15151
10	V1672BG	Afghanistan	EC15151
11	V1673BG	Afghanistan	EC15152
12	V1675BG	Afghanistan	EC15157
13	V1676BBR	Afghanistan	EC15161
14	V1676BG	Afghanistan	EC15161
15	V1687ABLM	Afghanistan	EC15188
16	V1687AG	Afghanistan	EC15188
17	V2190BG	Afghanistan	PI 211067
18	V2467BG	Afghanistan	EC15125
19	V2478BG	Afghanistan	EC15157
20	V2479AG	Afghanistan	EC15159
21	V2494AG	Afghanistan	EC15187
22	V2495AG	Afghanistan	EC15188
23	V3215AG	Afghanistan	001-M
24	V3215BG	Afghanistan	001-M
25	V3218AG	Afghanistan	008-M
26	V3220AG	Afghanistan	049-M
27	V3221AG	Afghanistan	060-M
28	V3222AG	Afghanistan	062-M

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
29	V3222BBR	Afghanistan	062-M
30	V3223AG	Afghanistan	063-M
31	V3224AG	Afghanistan	081-M
32	V3226BBR	Afghanistan	090-M
33	V3227AG	Afghanistan	109-M
34	V3230AG	Afghanistan	120-M
35	V3235AG	Afghanistan	161-M
36	V3237AG	Afghanistan	174-M
37	V3241AG	Afghanistan	181-M
38	V3242AG	Afghanistan	182-M
39	V3243AG	Afghanistan	185-M
40	V3244AG	Afghanistan	186-M
41	V3245AG	Afghanistan	188-M
42	V3246AG	Afghanistan	189-M
43	V3246BBR	Afghanistan	189-M
44	V3247AG	Afghanistan	195-M
45	V3248AG	Afghanistan	200-M
46	V3248BG	Afghanistan	200-M
47	V3249BBR	Afghanistan	209-M
48	V3250AG	Afghanistan	224-M
49	V3250BG	Afghanistan	224-M
50	V3251ABLM	Afghanistan	226-M
51	V3251BBR	Afghanistan	226-M
52	V3252AG	Afghanistan	227-M
53	V3253AG	Afghanistan	230-M
54	V3253ABLM	Afghanistan	230-M
55	V3255BG	Afghanistan	242-M
56	V3256ABLM	Afghanistan	245-M

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
57	V3257AG	Afghanistan	262-M
58	V3258AG	Afghanistan	264-M
59	V3260AG	Afghanistan	268-M
60	V3263ABLM	Afghanistan	278-M
61	V3264AG	Afghanistan	302-M
62	V3264BG	Afghanistan	302-M
63	V3267AG	Afghanistan	320-M
64	V3271AG	Afghanistan	364-M
65	V3277AG	Afghanistan	399-M
66	V3278AG	Afghanistan	418-M
67	V3278BBR	Afghanistan	418-M
68	V3280AG	Afghanistan	430-M
69	V3281BBR	Afghanistan	437-B-M
70	V3282AG	Afghanistan	438-M
71	V3282BBR	Afghanistan	438-M
72	V3283AG	Afghanistan	441-M
73	V2065AG	Australia	Mt. Tyson
74	V2075BY	Australia	Golden Mung
75	V2085BG	Australia	Hermitagers
76	V2010BG	China	PI 298915
77	V2915BG	England	NGG29
78	V2062AG	Guam	Guxpaguma 1
79	V2062BG	Guam	Guxpaguma 1
80	V1990BG	Guatemala	PI164889
81	V1278AG	India	PI288593
82	V1279 AG	India	PI288594
83	V1280 AG	India	PI288595
84	V1287 AG	India	PI288822

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
85	V1289 AG	India	PI288824
86	V1292 AG	India	PI288827
87	V1296 AG	India	PI288831
88	V1298 AG	India	PI288833
89	V1457 BG	India	CPI12106
90	V1488 BG	India	CPI12105
91	V1532 AG	India	PI376952
92	V1532BG	India	PI376952
93	V1533BG	India	NP-03
94	V 1536BG	India	NP-05-1
95	V1539AG	India	NP-07
96	V1544AG	India	NP-15-1
97	V1546AG	India	NP-16-1
98	V1547AG	India	NP-16-2
99	V1548AG	India	NP-16-3
100	V1549AG	India	NP-17-1
101	V1553AG	India	NP-20
102	V1553BG	India	NP-20
103	V1554AG	India	NP-21
104	V1555AG	India	NP-22
105	V1555BG	India	NP-22
106	V1556AG	India	NP-23
107	V1557AG	India	NP-26
108	V1573BG	India	Moong-6
109	V1631BG	India	EC15049
110	V1649AG	India	EC15077
111	V1667BG	India	EC15133
112	V1730ABLM	India	M.S.9721

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
113	V1735AG	India	1791
114	V1735BG	India	1791
115	V1776BG	India	PI377196
116	V1837AG	India	OB4-2(T1X444ID)
117	V1837BG	India	OB4-2(T1X444ID)
118	V1844ABLM	India	OB41(T1X4449-8)
119	V1857ABLM	India	OB76-2/4(T1X4425-2)
120	V1867AG	India	C35-2(T1X4448-2)
121	V1969AG	India	PI223711
119	V1857ABLM	India	OB76-2/4(T1X4425-2)
120	V1867AG	India	C35-2(T1X4448-2)
121	V1969AG	India	PI223711
122	V1984BG	India	Jalagaon 781
123	V2268BG	India	PI377902
124	V2273AG	India	PI378039
125	V2273BG	India	PI378039
126	V2365BG	India	Krishna-11
127	V2396BG	India	NP-33
128	V2565AG	India	P401
129	V2565BG	India	P401
130	V2773AG	India	NA*
131	V2773BG	India	NA*
132	V2774AG	India	NA*
133	V3017BBR	India	Bnalili 3-4
134	V3017BG	India	Bnalili 3-4
135	V3372AG	India	Shining Moong
136	V3372BG	India	Shining Moong
137	V3495ABLM	India	Jhain Mung 1-4

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
138	V1471 BG	Indonesia	PI425082
139	V1653AG	Iran	EC15085
140	V1655AG	Iran	EC15090
141	V1655BG	Iran	EC15090
142	V1656AG	Iran	EC15091
143	V1656BG	Iran	EC15091
144	V1709AG	Iran	EC15227
145	V1709BG	Iran	EC15227
146	V1710AG	Iran	EC15229
147	V1710BG	Iran	EC15229
148	V1711BG	Iran	EC15230
149	V1714AG	Iran	EC15233
150	V1715BG	Iran	EC15236
151	V1716AG	Iran	EC15238
152	V1717BG	Iran	EC15240
153	V1718AG	Iran	EC15242
154	V1718BG	Iran	EC15242
155	V1719BG	Iran	EC15243
156	V1720AG	Iran	EC15245
157	V1720BG	Iran	EC15245
158	V1721BG	Iran	EC15248
159	V1722BG	Iran	EC15250
160	V1723AG	Iran	EC15252
161	V1724AG	Iran	EC15255
163	V1725AG	Iran	EC15256
164	V1725BG	Iran	EC15256
165	V1726BG	Iran	EC15257
166	V1998AG	Iran	EC15085

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
167	V1998BG	Iran	EC15085
168	V1999AG	Iran	EC15087
169	V1999BG	Iran	EC15087
170	V2000AG	Iran	PI201874
171	V2209BG	Iran	EC15092
172	V2329AG	Iran	PI385301
173	V2490AG	Iran	EC15181
174	V2523BG	Iran	EC15234
175	V2525AG	Iran	EC15246
176	V3088BBR	Iran	PI201866
177	V3170AG	Iran	PI227248
178	V3171AG	Iran	PI249552
179	V3171BG	Iran	PI249552
180	V3178AG	Iran	PI385299
181	V3179AG	Iran	PI385300
182	V3180AG	Iran	PI385301
183	V3732AG	Iran	PI425861
184	V3734AG	Iran	PI425863
185	V3736AG	Iran	PI425865
186	V3737AG	Iran	PI425866
187	V3738AG	Iran	PI425867
188	V3739AG	Iran	PI425868
189	V3740AG	Iran	PI425869
190	V3741AG	Iran	PI425870
191	V3743ABR	Iran	PI425872
192	V3743BG	Iran	PI425872
193	V3746AG	Iran	PI425875
194	V3748AG	Iran	PI425877

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
195	V3748BG	Iran	PI425877
196	V3749AG	Iran	PI425878
197	V3750BBR	Iran	PI425879
198	V3751ABBR	Iran	PI425880
199	V3751BBR	Iran	PI425880
200	V3752BG	Iran	PI425881
201	V3754ABR	Iran	PI425883
202	V3756AG	Iran	PI425885
203	V3757AG	Iran	PI425886
204	V3757BBR	Iran	PI425886
205	V3758BBR	Iran	PI425887
206	V3759BG	Iran	PI425888
207	V3760ABR	Iran	PI425889
208	V3760BG	Iran	PI425889
209	V 1311 AG	Iraq	PI305413
210	V 1311 BG	Iraq	PI305413
211	V2949BG	Korea	Jaerae-Jong #7
212	V3096AG	Korea	Myungnokdu
213	V2815AY	Nigeria	Acc.65001
221	V3484AG	Pakistan	6601
214	NM20-11	Pakistan	NA*
215	NM10-12	Pakistan	NA*
216	NM45-24-1	Pakistan	NA*
217	NM63-15-8	Pakistan	NA*
218	NM92	Pakistan	NA*
219	NM98	Pakistan	NA*
220	NM3960-88	Pakistan	NA*
221	Kabulimung	Pakistan	NA*

Table 1 (Cont'd)

Entry no.	Accession no.	Seed origin	Name
222	V 1410 AG	Philippines	MD15-2
223	V1944BY	Philippines	MG 50-10A (Yellow)
224	V1946AY	Philippines	Yellow Mungo
225	V1947AG	Philippines	PI413521
226	V1948AG	Philippines	PI413522
227	V2184AG	Philippines	PI413539
228	V2191BG	Philippines	PI413544
229	V3476AG	Philippines	CES87 X ML -4
230	V 1323 BG	Taiwan	TN-B-19
231	V 1326 AG	Taiwan	CY-B-2
232	V 1327 AY	Taiwan	CY-B-6
233	V3404AG	Thailand	M-7-A
234	V3523AG	Thailand	PI376846
235	VC1628AG	Thailand	Chai Nat 60
236	VC19734AG	Thailand	KPS1
237	VC2278AG	Thailand	KPS2
239	V1745AG	USA	M-325
240	V1968AG	USA	Okla.1215002
241	V3387AG	USA	Lincoln

* = Not available

Chlorophyll extraction

Total chlorophyll content was isolated from leaves of individual germplasm accessions that were grown in the field by using the method of Moran (1982). Chlorophyll of three leaf sections of 0.198 cm^2 was extracted with 4 ml of DMF (N,N-dimethyformamide) for 24 to 48 h at $4^{\circ}C$ in dark room. The solution was measured with spectrophotometer at the wave length of 664 and 647 nm. The chlorophyll content values (g/cm^{-2}) were calculated from the formulas:

Chlorophyll *a* content = $(-2.99A_{647} + 12.64A_{664})$ Vol/ (X*Area*100)
Chlorophyll *b* content = $(23.26\ A_{647} - 5.60\ A_{664})$ Vol/ (X*Area*100)
Total Chlorophyll content = $(20.27\ A_{647} + 7.04\ A_{664})$ Vol/ (X*Area*100)

where
Area = the area of leaves (cm^2)
Vol = the volume of DMF used (ml)
X = dilution ratio of DMF
A_{647} = the value of solution measured with spectrophotometer at the wave length of 647 nm
A_{664} = the value of solution measured with spectrophotometer at the wave length of 664 nm

DNA extraction

Total genomic DNA was isolated from leaf tissues of individual plants using the method suggested by Doyle and Doyle (1990) with a slight modification. Approximately 300 mg of primary leaves were collected from one-week-old seedlings, ground in liquid nitrogen, and suspended in 1 ml of 2X CTAB buffer containing 1.4% sodium metabisulfite, 0.3% beta-mercaptoethanol, and 1% polyvinylpyrrolidone K-30 (PVP K-30) and immersed in a water bath at 60°C for 1 h. The solution was mixed with chloroform/isoamylalcohol (24:1) and centrifuged. The supernatant was mixed with 50 µl of 10% CTAB solution and an equal volume of precipitation buffer. After being allowed to stand for 30 min, and after centrifugation, the residue was dissolved in 500 µl of high salt TE buffer with about 1 µl of Rnase for 1 h. at 50°C. After the addition of an equal volume of isopropanol and centrifugation, the residue was washed in 70% ethanol and dissolved in 20 to 100 µl of 0.1X TE buffer.

Detection the concentration of DNA by gel electrophoresis

Prepare 0.8% agarose gel (0.8% agarose gel, 0.5X TBE buffer), pour onto a tray, wait for 30 min until the gel becomes solid, pull out the comb and soak gel in electrophoresis containing 0.5X TBE buffer. The λDNA was used as the marker at the concentration of 50 ng/μl and loaded together with 10 μl sample consisting of 1 μl sample and 9 μl 3X loading dye. The samples were passed through 100 V electric current for 20 min. The gel was stained with ethidium bromide for 30 min, rinse with dH_2O for 30 min, taken photo with a gel document and compared each band with the marker.

AFLP (Amplification Fragment Length Polymorphism) analysis

The genomic DNA template was prepared at the concentration of 50 ng/μl for reaction of AFLP technique. The reaction process can be divided according to Vos *et al.* (1995) as followed.

Digest each genomic DNA (50 ng/μl) with two restriction enzymes, one that cuts frequently (*Mse*I, 4 bp recognition site) and the other cuts less frequently (*Eco*RI, 6 bp recognition site). The reaction has total volume of 20 μl, including 2 μl of DNA, 2 μl 10X buffer A, 1 unit *Eco*RI, 1 unit *Mse*I and 14 μl dH_2O. Vortex and briefly centrifuge the mixture incubated at 37°C for 3 h., then stop activity of the enzymes at 70°C for 15 min.

Ligate adapters to each digested genomic DNA by adding 2 μl *Eco*RI adapter, 2.5 μl *Mse*I adapter, 1.5 μl 10 mM ATP, 1 μl 10X buffer A, 0.5 μl T4 DNA ligase and 2.5 μl dH_2O (total volume of 10 μl) into 20 μl digestion mixture. Vortex and briefly centrifuge mixture, incubate at room temperature for 3 h.

Dilute the digestion-ligation reaction mixture 10 times with dH_2O (5 μl DNA in 50 μl dH_2O) and used as template DNA for AFLP reaction The mixture can be stored at -20°C for further use.

Pre-amplification reaction was performed at a total volume of 20 µl with the following components: 2 µl ligated DNA, 1 µl *Eco*RI-primer +N oligo (50 ng/µl), 1 µl *Mse*I-primer +N oligo (50 ng/µl), 4 µl 5 mM dNTPs, 1.2 µl $MgCl_2$, 0.4 µl Taq DNA polymerase (1 unit), 2 µl 10X PCR buffer and 8.4 µl dH_2O. Pre-amplify the mixture for 20 cycles, beginning by denaturing for 30 sec at 94°C, annealing for 60 sec at 65°C and extension for 60 sec at 72°C.

Twenty microliters of the mixture from pre-amplification reaction was diluted with dH_2O (5 µl DNA in 50 µl dH_2O) and stored at -20°C for further use.

Assemble the reaction (total volume of 10 µl) by adding 1 µl pre-ampified ligated DNA, (0.5 µl *Eco*RI +NNN oligo (50 ng/µl), 0.5 µl *Mse*I +NNN oligo (50 ng/µl), 2 µl 5 mM dNTPs, 1 µl 10X PCR buffer, 0.6 µl $MgCl_2$, 0.2 µl Taq DNA polymerase and 4.2 µl dH_2O). Tubes are amplified in a thermocycler as followed: the first cycle comprised denaturing at 94°C for 30 sec, annealing at 65°C for 30 sec, extension at 72°C for 60 sec, followed by 12 cycles with a stepwise decrease of the annealing temperature in each subsequent cycle by 0.7°C, and 23 cycles at 94°C for 30 sec, at 56°C for 30 sec and at 72°C for 60 sec.

This study utilized four AFLP primers. Two primers, E-ACC/M-CTG and E-ACT/M-CTA were reported by Chalee (2005) as linked to tolerance to iron deficiency in NM10-12 when crossed with KPS1. Both markers were reported to locate on the same linkage group at the distance of 1 cM. The other two primers, E-CGT/M-CTG and E-CAG/M-TAC were reported earlier by Sommanus (2000) as linked to susceptible to iron deficiency in KPS1. The latter two markers are located on the same linkage group with the gene controlling susceptibility to iron deficiency at the distance of 2.9 and 3.0 cM, respectively. The sequence of above AFLP primers used in this study are given as followed:

*Eco*RI + 3 primers (E-ACC) : 5′-GACTGCGTACCAATTCACC- 3′
*Eco*RI + 3 primers (E-ACT) : 5′-GACTGCGTACCAATTCACT- 3′
*Eco*RI + 3 primers (E-CAG) : 5′-GACTGCGTACCAATTCCAG- 3′

*Eco*RI + 3 primers (E-CGT) : 5′-GACTGCGTACCAATTCCGT- 3′
*Mse*I + 3 primesr (M-CTA) : 5′-GATGAGTCCTGAGTAACTA- 3′
*Mse*I + 3 primers (M-CTG) : 5′-GATGAGTCCTGAGTAACTG- 3′
*Mse*I + 3 primers (M-TAC) : 5′-GATGAGTCCTGAGTAATAC- 3′

Polyacrylamide gel electrophoresis of AFLP products

Amplification products were analyzed on 4.5% denaturing polyacrylamide sequencing gel. The process of analysis began from preparation 55 ml 4.5% polyacrylamide gel and combined 300 µl 10% APS, 20 µl TEMED in beaker and mixed well. The solution was transferred into a syringe. The glass was prepared by wiping the chamber with 95% ethanol 3 times and with clearview 1 time. Wipe long glass with 95% ethanol 3 times and with 0.5% acetic acid in 95% ethanol 1 time. The glass was laied horizontally, placed spacers on long glass and put the chamber on top of the long glass, erected the whole unit vertically and clamped side braces. The space between glasses was adjusted, until the comb could be inserted by adjusting and leaning clamps on top of the tube racks. The gel solution was injected, the comb was inserted and the unit was set in horizontal position. The gels should be cased at least 2 h. before use, then pre-run for 30 min before loading the samples using 5X TBE buffer as running buffer.

Gel loading

Each AFLP reaction products was mixed with an equal volume (10 µl) of loading dye. The samples were heated for 3 min at 94°C, then quickly cool on ice. Rinse the gel wells with running buffer. The gel slots were made by carefully pushing sharktooth combs about 0.5 mm. into the gel surface. Rinse the gel slots with TBE buffer and loaded 3.5 µl of each sample per well.

Silver staining

The plates were separated while keeping the gel attached to the glass. Fix the gel by placing it in a tray, cover with cold fix/stop solution and agitate well for 20 min, pour the fix/stop solution. The solution can be reused. Wash the gel with dH_2O 3 times for 2-3 min and agitate, lift gel from solution and allow to drain for 10-20 sec The gel was transferred to staining solution and agitated well for 30 min. Pour the staining solution to beaker and rinse gel for 5-10 sec with dH_2O, fix gel with 1 l of the developing solution into a tray, agitate in developing solution until DNA bands appear. Discard the developing solution and fix the gel by adding 1 l of fix/stop solution directly to developing solution and agitate for 2- 3 min. Rinse gel twice for 2 min each in dH_2O and dry gel on glass.

Gel scoring and scanning

Bands of DNA from the reaction of each pair of primers were recorded. When the primers E-ACT/M-CTA and E-ACC/M-CTG were used to screen the germplasm, the genotypes showing band (presumably liked to tolerance as in NM10-12) were scored “1” while those without band were scored “0”. On the contrary, when the primers CGT/M-CTG and E-CAG/M-TAC were screened across the same mungbean germplasm, the genotypes with band (presumably linked to susceptibility as KPS1) were scored as “1” while those without bands were scored as “0”.

Multiple regression analysis

A multiple regression analysis was used to detect the association between presence/absence of four AFLP markers and iron deficiency chlorosis from visual score and chlorophyll concentration in the germplasm

RESULTS

Reaction of mungbean germplasm to iron deficiency in the field

A total of 241 mungbean accessions were grown in calcareous soils at Nakhon Sawan Field Crops Research Center, Thailand. They exhibited various degrees of chlorosis that could be visually classified in two groups: no chlorosis versus some degrees of chlorosis. Among the chlorotic germplasm, 51 accessions showed slight chlorosis, 38 showed mild chlorosis, 8 showed moderate chlorosis, and 3 showed severe chlorosis (Figure 1). No chlorosis was observed in 141 accessions, which were considered highly tolerant. The symptoms shown by each accession was compared with NM10-12 which is tolerance to iron deficiency, and KPS2 which are highly susceptible to iron deficiency, respectively.

The phenotypes observed from the field test agreed well with those displayed by chlorophyll content determination method (Moran, 1982). From which chlorophyll concentration was found corresponding to the concentration of iron nutrient because the enzymes involved in formation of chlorophyll contain molecules of iron. Jacobson and Oertli (1995) determined chlorophyll content to investigate the relationship between iron and chlorophyll contents in chlorotic sunflower leaves and found that chlorosis correlated with iron concentration and chlorophyll contents. The leaf without chlorosis symptom had higher iron concentration and chlorophyll content than the chlorotic ones. However, iron in a leaf is not all in the same chemical forms and does not all perform the same function. Iron is still necessary for non-green plants and thus there is the presence of iron containing enzymes in all plants (Hill and Lehmann, 1941). Marsh *et al*. (1963) found that the effect of iron deficiency on the formation of porphyrin compounds in cowpea leaves depend on the concentration of either heme or chlorophyll and the level of iron in the nutrient solution. They also found that iron deficiency results in limited formation of δ-aminolevulinic acid and that blocking of the enzymes involved is one rate-limiting step

Figure 1 Symptoms of iron deficiency: a = no chlorosis, b = slight chlorosis, c = mild chlorosis, d = moderate chlorosis and e = severe chlorosis, compared with NM10-12 (tolerance to iron deficiency, f) and KPS1 and KPS2 (susceptible to iron deficiency,g and h).

in chlorophyll synthesis. Consequently, the leaf iron is closely related to chlorophyll formation, since each mungbean accession contained certain amounts of chlorophyll *a*, chlorophyll *b* and total chlorophyll, which were associated with iron deficiency symptoms (Figure 2). Total chlorophyll contents of NM10-12 (tolerant) and KPS2 (susceptible) were 1.70 and 0.39 g/cm^{-2}, respectively. Their average value (1.05 g/cm^{-2}) was used to mark the difference between tolerant and susceptible germplasm after which 196 and 45 accessions were observed (Figure 3).

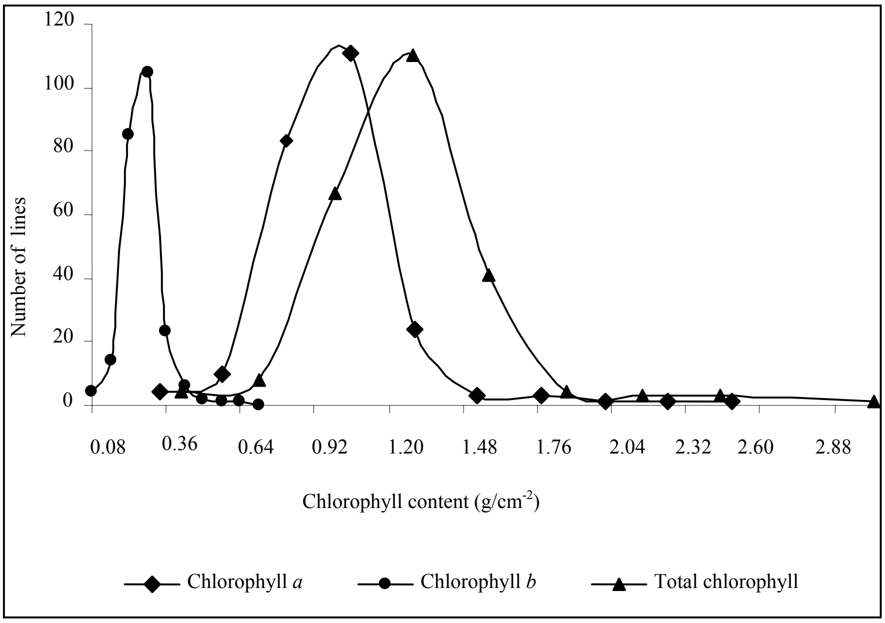

Figure 2 Distribution of chlorophyll *a*, chlorophyll *b* and total chlorophyll in 241 mungbean accessions.

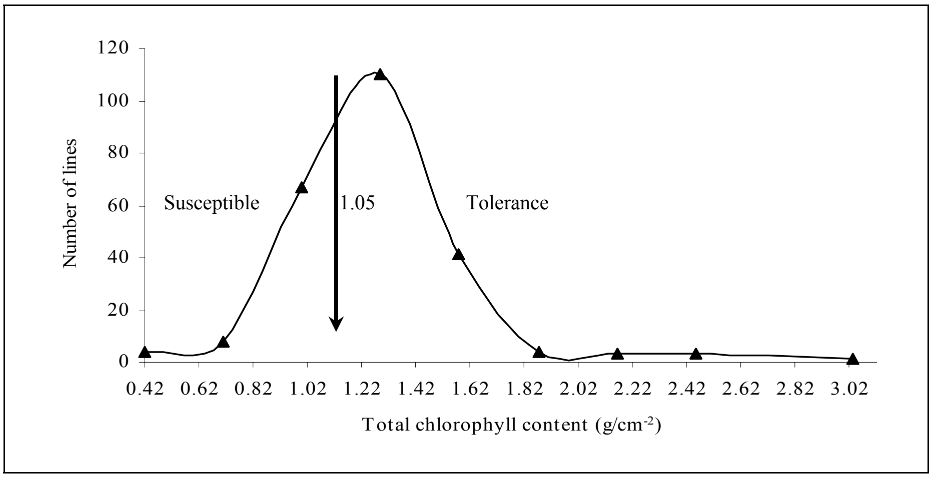

Figure 3 Distribution of mungbean accessions were classified into tolerant and susceptible groups at the score of 1.05, which is the mid-point between mean total chlorophyll contents of KPS2 (0.39) and NM10-12 (1.70).

Correlation between visual score and chlorophyll content

The relationship between visual score and chlorophyll concentration was determined by calculating Pearson linear correlation coefficient (r) (Table 2). Visual score was negatively correlated with chlorophyll contents, i.e. the greener plants gave lower score. Total chlorophyll was positively correlated with chlorophyll *a* and chlorophyll *b*. The other correlations, although significant, were rather low in magnitude.

Chlorophyll content and visual score were used to classify the degree of tolerance to iron deficiency in groundnut by Samdur *et al.* (2000). The genotypes were grouped into three categories, viz. severe chlorosis with visual score of 5 and total chlorophyll content of 0.28 – 0.39 g/cm^{-2}, moderate chlorosis with visual score of 2-4 and total chlorophyll content of 0.52–1.20 g/cm^{-2} and no chlorosis with visual score of 1 and total chlorophyll content of 1.21-3.12 g/cm^{-2} (Appendix Table 1).

Table 2 Pearson linear correlation coefficient (r) between visual score and chlorophyll content in 241 mungbean germplasm

	Chl *b*[1]	Total chl[1]	Chl *a*: Chl *b*[1]	Visual score
Chl *a*[1]	0.97**	0.99**	0.21**	-0.72**
Chl *b*[1]		0.98**	0.41**	-0.71**
Total chl[1]			0.25**	-0.72**
Chl *a*: Chl *b*[1]				-0.25*

[1] Chl *a* = Chlorophyll *a* content, Chl *b* = Chlorophyll *b* content, Total chl = Total chlorophyll content, Chl *a*:Chl *b* = Chlorophyll *a* : Chlorophyll *b* content

*, ** Significant at P = .05 and .01, respectively

Association between AFLP markers and iron deficiency chlorosis in mungbean germplasm

Among 196 tolerant accessions, the E-ACT/M-CTA and E-ACC/M-CTG markers were used to amplify the corresponding DNA segment linking to the gene controlling tolerance to iron deficiency. The former marker was found present in 148 accessions (76%) (Figure 4 and Appendix Table 2) while the latter marker was found in 143 accessions (73%) (Figure 5 and Appendix Table 2). Both markers identified the same tolerant accessions from Pakistan, china, England and Indonesia. Specially, E-ACT/M-CTA was able to select all tolerant accessions from Australia, Iraq and Taiwan, thus it is considered a more useful marker than E-ACC/M-CTG. However, E-ACT/M-CTA marker could not completely identify all tolerant accessions in Afghanistan, India, Iran, Philippines and Thailand and selected 41, 33, 49, 3 and 2 from 64, 40, 59, 6 and 4 tolerant accessions, respectively. Among 45 susceptible germplasm, 3 of them showed the marker ACT/M-CTA. Two accessions were from India and one from Iran. The marker E-ACC/M-CTG, was found in 11 out of 45 susceptible accessions. These accessions were from Afghanistan, Australia, China, India, Iran and Philippines. The germplasm from the latter two countries may have higher genetic diversity than those from the other countries, which could be preliminarily observed from the variation found in seed coat color (comprising green, yellow, brown and mosaic black). E-ACT/M-CTA marker was able to explain 45.4% of the variation in chlorophyll content while E-ACC/M-CTG marker explains only 22.6% of the variation. Thus E-ACT/M-CTA marker may be used to preliminarily screen for the iron deficiency tolerant germplasm.

On the other hand, E-CGT/M-CTG and E-CAG/M-TAC markers selected 30 (67%) and 19 (42.2%) out of 45 susceptible accessions, respectively (Figure 6 and 7 and Appendix Table 2). Yet, these markers were present in 105 and 123 out of 196 tolerance accessions, respectively. So, they were considered not useful for distinguishing tolerant and susceptible accessions.

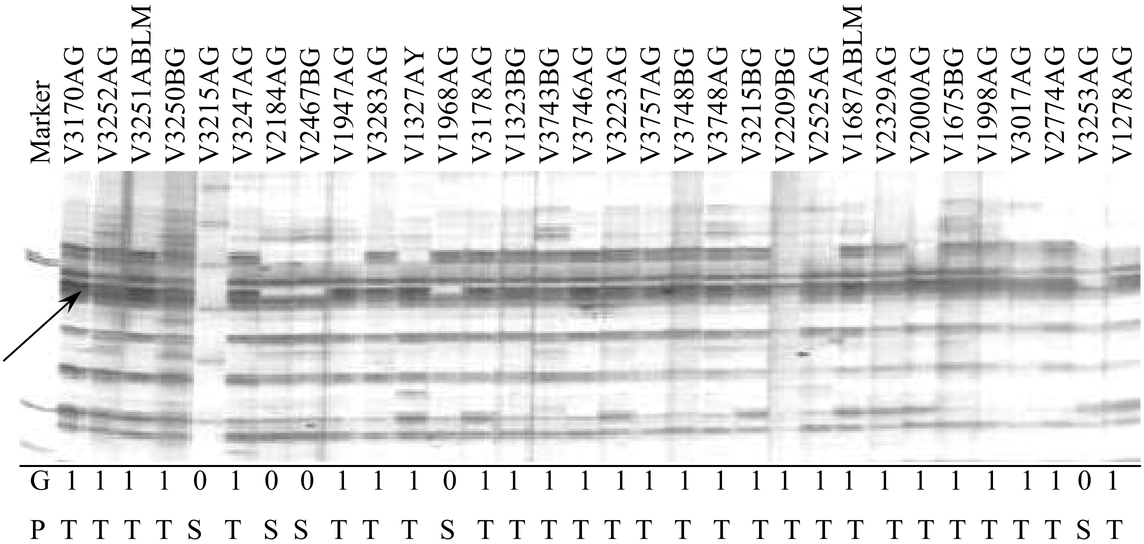

Figure 4 AFLP analysis by E-ACT/M-CTA marker on mungbean accessions showing tolerant and susceptible to iron deficiency. The polymorphic marker associated with tolerance to iron deficiency is marked by arrow.

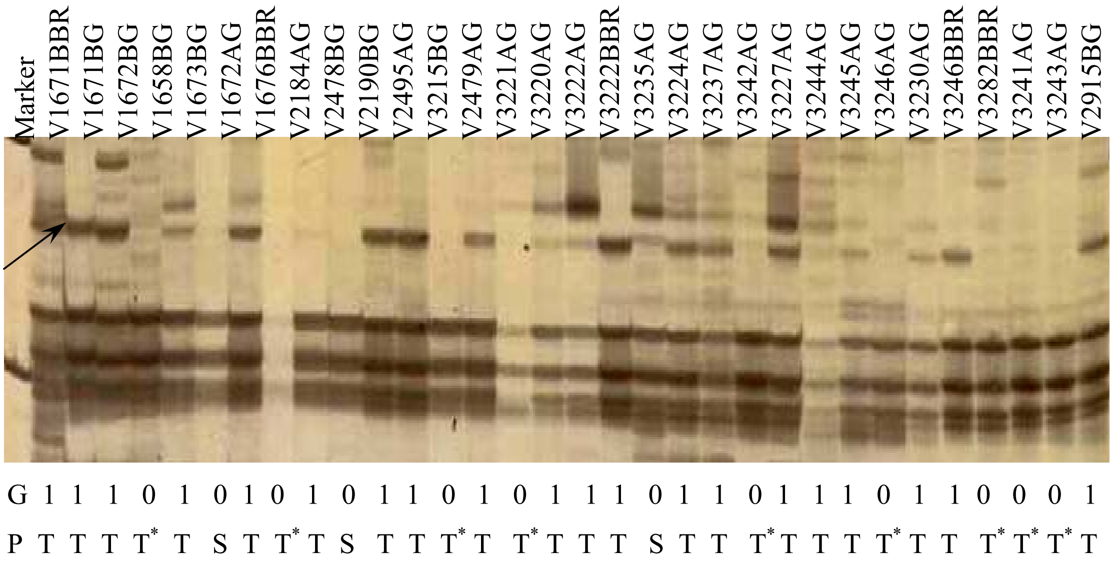

Figure 5 AFLP analysis by E-ACC/M-CTG marker on mungbean accessions showing tolerant and susceptible to iron deficiency. The polymorphic marker associated with tolerance to iron deficiency is marked by arrow. (* = tolerant marker absent in tolerant).

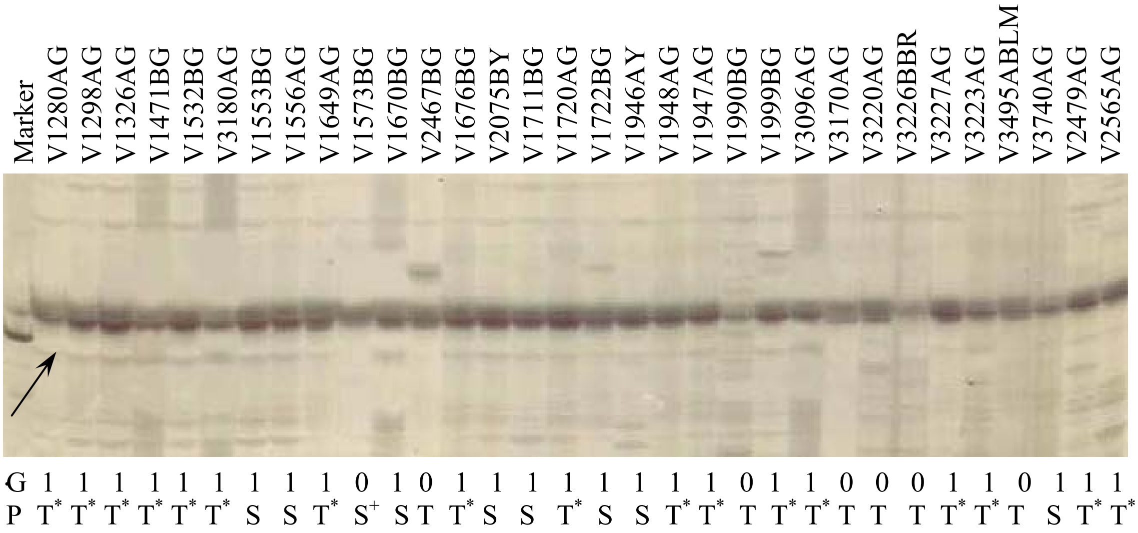

Figure 6 AFLP analysis by E-CGT/M-CTG marker on mungbean accessions showing tolerant and susceptible to iron deficiency. The polymorphic marker associated with susceptible to iron deficiency is marked by arrow. (* = Susceptible marker found in tolerance accessions, $^+$ = Susceptible marker absent in susceptible accessions).

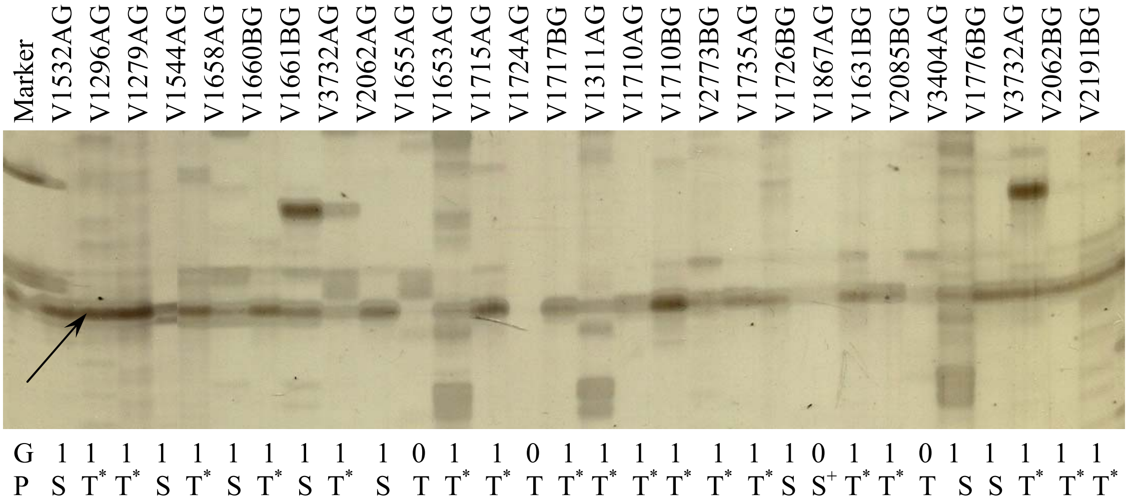

Figure 7 AFLP analysis by E-CAG/M-TAC marker on mungbean accessions showing tolerant and susceptible to iron deficiency. The polymorphic marker associated with susceptible to iron deficiency is marked by arrow. (* = Susceptible marker found in tolerance accessions, $^+$ = Susceptible marker absent in susceptible accessions).

DISCUSSION

Evaluation of iron deficiency symptoms by spectrometric chlorophyll determination is more sensitive than by visual scoring. Cianzio *et al.* (1979) showed a negative correlation between chlorophyll content and visual score for iron deficiency chlorosis in soybean. Lin and Shoemaker (1997) reported that the visual score data of two soybean populations grown in two years were not difference, while the chlorophyll concentration of both populations were different between years due to sensitivity of this character to the environment in different years as compare to visual scores. However, the latter method can be jointly used with chlorophyll meter. Azia and Stewart (2001) investigated the relationship between extractable chlorophyll and SPAD value in muskmelon leaves and found that SPAD reading was significantly related to extracted chlorophyll, both on a fresh weight and leaf area basis. In addition, chlorophyll content can be used to investigate the relationship between iron concentration change and iron deficiency symptoms. Jacobson and Oertli (1995) determined chlorophyll content to observe the relationship between iron and chlorophyll contents in chlorotic sunflower leaves and found that chlorosis correlated with iron concentration and chlorophyll content. The leaf without chlorosis symptom had higher iron concentration and chlorophyll content than the chlorotic leaves. Moreover, the change in iron concentration can be examined physiologically and morphologically in deciduous fruit tree plants (Cinelli *et al*., 2003), tomato (Dansgan *et al*., 2004) and chickpea (Ohwaki and Sugahara, 1997). Physiological root response to iron deficiency was reported in tomato that the tolerance “Roza” had higher number of lateral roots, Fe^{+3} reduction capacity, uptake rate and translocation of iron, and thicker root tips than the susceptible “227/1 line”, including the reduction of pH of the nutrient solution from 6.0 to 4.6-5.3 in the tolerance line (Dansgan *et al*., 2002). Ohwaki and Sugahara (1997) reported in chickpea that the period of acidification induced by iron deficiency and the rate of extrusion of protons was much higher than the rate of exudation of carboxylic acids from the roots of the tolerance K-850.

However, field detection by chlorophyll meter or chlorophyll content determination in a large segregating population is not practical. Yet a false positive may occur from deficiency of the other micronutrients. In addition, the symptoms of iron deficiency can sometimes be confound with the soil environment. So the use marker-assisted selection (MAS) can solve this problem and help saving time and money required in field screening. The AFLP technique is appropriate for MAS because it does not require data on genetic background of the mungbean and can be detected in highly polymorphic bands. Powell *et al.* (1996) stated that the AFLP markers showed the highest effective multiplex ratio with high correlation in genetic similarity matrices revealed in soybean germplasm. If the comparison was involved only the cultivated genotypes, similarity estimates by AFLP and RAPD are more closely correlated than those involving other marker systems. However, correlations of RAPD marker data with other marker systems were lower, because RAPDs produce highly estimates of interspecific similarities. In this study AFLP markers, viz. E-ACC/M-CTG and E-ACT/M-CTA markers showed close association with gene conditioning tolerance to iron deficiency. This relationship can potentially be used to detect the response to iron deficiency within a mungbean germplasm collection as an initial screening of parental lines as well as in marker-assisted selection for the tolerance progenies. E-ACT/M-CTA marker is more effective than E-ACC/M-CTG in identifying the tolerant germplasm from any countries. While E-CAG/M-TAC and E-CGT/M-CTG markers are linked to most of susceptible accessions. However, the latter two markers were also present in high frequency in tolerant accessions and thus made them less useful than the tolerant-linked marker E-ACT/M-CTA.

The mungbean germplasm used in this study was obtained from different sources with high genetic variation, as a result the gene controlling tolerance to iron deficiency may or may not link to the marker. Association between the markers and the gene in some accessions might be lost during the generations of selfing and occasional out-crossing and thus could not be detected in the germplasm. A multiple regression analysis in germplasm showed that E-ACT/M-CTA and E-ACC/M-CTG together could significantly explain the variation in chlorophyll contents. In contrast, E-CGT/M-CTG and E-CAG/M-TAC markers did not show significant association with iron deficiency chlorosis gene, probably the distance between the markers and the gene was far enough to allow a high frequency of double crossing over. Thus, E-CGT/M-CTG and E-CAG/M-TAC markers were not as useful as E-ACT/M-CTA and E-ACC/M-CTG markers in sceening this set of germplasm. Chowdhury *et al*. (2002) found an RAPD marker OPH-02_{1250} linking with resistance to downy mildew disease in soybean population and the marker could be detected in 13 out of 16 resistant soybean cultivars and absent in all susceptible cultivars. Charlson *et al*. (2005) found that the SSR marker Satt481 is associated with iron-deficiency chlorosis resistance in a soybean breeding population. However, SSR marker technology in mungbean is still at the beginning state, thus the mungbean breeding program can directly use the E-ACT/M-CTA AFLP marker to screen for tolerant and susceptible genotypes or convert them into SCAR markers to increase the precision in locating the gene target in a germplasm or a large mungbean population.

CONCLUSION

1. A diverse germplasm of 241 mungbean accessions were grown in Takhli soil filed in Nakhon Sawan province during November 2004, February 2005 and May 2005. They were compared with the mungbean cultivars KPS2 (susceptible to iron deficiency) and the line NM10-12 (tolerance to iron deficiency). The germplasm can be divided based on their iron deficiency symptom that there are 51 accessions with slight chlorosis, 38 accessions with mild chlorosis, 8 accessions with moderate chlorosis, 3 accessions with severe chlorosis and 141 accessions with no chlorosis.

2. Chlorophyll concentration determination method was found to agree well with the phenotype observed. The average chlorophyll contents in KPS2 and NM10-12 were 0.39 and 1.70 g/cm^{-2}, respectively. The average value (1.05 g/cm^{-2}) was used to mark the difference between the tolerant and susceptible germplasm after which 196 and 45 accessions were respectively observed. Correlation between chlorophyll content and visual score was negative.

3. E-ACT/M-CTA and E-ACC/M-CTG markers were used to classify the tolerant and susceptible germplasm and found to present in 148 (76%) and 143 (73%) of 196 tolerant accessions, respectively. Both markers were also present in 3 and 11 of 45 susceptible accessions, respectively. On the other hand, E-CGT/M-CTG and E-CAG/M-TAC markers were present in 30 (67%) and 19 (42.2%) out of 45 susceptible accessions, respectively. However, the latter markers were also present in 105 and 123 out of 196 tolerant accessions.

4. Using a multiple regression analysis, E-ACC/M-CTG marker explained 45.37% and E-ACT/M-CTA marker explained 22.59% of the variation in chlorophyll content among mungbean germplasm grown in iron deficient soil.

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APPENDIX

Appendix Table1 Visual score, chlorophyll *a*, chlorophyll *b* total chlorophyll contents (g/cm^{-2}) and the ratio of chlorophyll *a*: chlorophyll *b* content in 241 mungbean germplasm.

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V1658BBR	Afghanistan	2	0.92	0.26	1.17	3.59
V1658BG	Afghanistan	1	0.97	0.25	1.22	3.79
V1660BG	Afghanistan	3	0.72	0.22	0.94	3.21
V1661BG	Afghanistan	2	0.89	0.27	1.16	3.26
V1670AG	Afghanistan	1	0.95	0.26	1.21	3.61
V1670BG	Afghanistan	3	0.77	0.20	0.97	3.93
V1671BBR	Afghanistan	1	1.20	0.34	1.54	3.55
V1671BG	Afghanistan	1	1.08	0.28	1.36	3.87
V1672AG	Afghanistan	3	0.72	0.19	0.91	3.84
V1672BG	Afghanistan	1	1.12	0.30	1.41	3.75
V1673BG	Afghanistan	1	1.20	0.33	1.52	3.65
V1675BG	Afghanistan	1	1.09	0.29	1.39	3.71
V1676BBR	Afghanistan	1	1.16	0.34	1.50	3.42
V1676BG	Afghanistan	1	0.98	0.27	1.24	3.66
V1687ABLM	Afghanistan	1	1.25	0.36	1.61	3.53
V1687AG	Afghanistan	3	0.84	0.22	1.06	3.79
V2190BG	Afghanistan	3	0.70	0.18	0.87	3.96
V2467BG	Afghanistan	2	0.88	0.23	1.12	3.76
V2478BG	Afghanistan	1	1.15	0.33	1.49	3.45
V2479AG	Afghanistan	3	0.85	0.23	1.07	3.71
V2494AG	Afghanistan	1	1.03	0.27	1.29	3.85
V2495AG	Afghanistan	1	1.15	0.30	1.45	3.79
V3215AG	Afghanistan	1	0.97	0.27	1.23	3.60
V3215BG	Afghanistan	1	1.06	0.28	1.34	3.85

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V3218AG	Afghanistan	3	0.84	0.24	1.08	3.42
V3220AG	Afghanistan	3	0.88	0.22	1.09	4.07
V3221AG	Afghanistan	1	1.00	0.27	1.27	3.64
V3222AG	Afghanistan	1	1.05	0.28	1.33	3.79
V3222BBR	Afghanistan	1	1.05	0.28	1.33	3.72
V3223AG	Afghanistan	1	0.98	0.27	1.25	3.58
V3224AG	Afghanistan	3	0.76	0.21	0.97	3.65
V3226BBR	Afghanistan	2	0.94	0.24	1.19	3.87
V3227AG	Afghanistan	2	0.93	0.27	1.20	3.49
V3230AG	Afghanistan	3	0.85	0.24	1.09	3.58
V3235AG	Afghanistan	1	1.18	0.32	1.49	3.69
V3237AG	Afghanistan	1	1.13	0.32	1.44	3.55
V3241AG	Afghanistan	2	0.90	0.25	1.15	3.66
V3242AG	Afghanistan	1	1.11	0.30	1.41	3.74
V3243AG	Afghanistan	2	0.91	0.24	1.15	3.72
V3244AG	Afghanistan	1	1.15	0.31	1.45	3.73
V3245AG	Afghanistan	1	1.09	0.29	1.38	3.70
V3246AG	Afghanistan	1	1.00	0.27	1.27	3.69
V3246BBR	Afghanistan	1	1.08	0.28	1.36	3.78
V3247AG	Afghanistan	1	1.01	0.30	1.30	3.39
V3248AG	Afghanistan	1	0.94	0.27	1.21	3.55
V3248BG	Afghanistan	2	0.89	0.25	1.14	3.55
V3249BBR	Afghanistan	2	0.88	0.25	1.14	3.46
V3250AG	Afghanistan	1	1.07	0.31	1.38	3.50
V3250BG	Afghanistan	1	1.11	0.31	1.42	3.52
V3251ABLM	Afghanistan	1	1.05	0.28	1.34	3.70

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V3251BBR	Afghanistan	1	0.96	0.25	1.21	3.80
V3252AG	Afghanistan	1	1.08	0.29	1.38	3.70
V3253AG	Afghanistan	3	0.75	0.19	0.95	3.86
V3253ABLM	Afghanistan	1	1.21	0.33	1.54	3.65
V3255BG	Afghanistan	2	0.93	0.26	1.18	3.61
V3256ABLM	Afghanistan	1	0.97	0.25	1.22	3.79
V3257AG	Afghanistan	2	0.92	0.25	1.17	3.75
V3258AG	Afghanistan	2	0.90	0.25	1.15	3.58
V3260AG	Afghanistan	3	0.83	0.24	1.07	3.43
V3263ABLM	Afghanistan	2	0.89	0.26	1.15	3.46
V3264AG	Afghanistan	1	1.10	0.18	1.28	6.11
V3264BG	Afghanistan	3	0.85	0.23	1.08	3.61
V3267AG	Afghanistan	2	0.85	0.24	1.09	3.61
V3271AG	Afghanistan	1	1.89	0.46	2.35	4.13
V3277AG	Afghanistan	3	0.78	0.21	0.98	3.79
V3278AG	Afghanistan	3	0.86	0.21	1.07	4.08
V3278BBR	Afghanistan	2	0.95	0.24	1.19	3.90
V3280AG	Afghanistan	3	0.72	0.19	0.91	3.83
V3281BBR	Afghanistan	1	0.97	0.26	1.23	3.66
V3282AG	Afghanistan	1	1.50	0.41	1.91	3.65
V3282BBR	Afghanistan	1	1.15	0.33	1.48	3.50
V3283AG	Afghanistan	1	1.10	0.31	1.41	3.57
V2065AG	Australia	3	0.81	0.23	1.04	3.59
V2075BY	Australia	3	0.82	0.22	1.04	3.79
V2085BG	Australia	1	1.05	0.28	1.34	3.71
V2010BG	China	2	0.88	0.24	1.12	3.71

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V2915BG	England	1	1.00	0.28	1.29	3.55
V2062AG	Guam	1	0.96	0.26	1.22	3.65
V2062BG	Guam	3	0.67	0.18	0.85	3.82
V1990BG	Guatemala	2	0.88	0.25	1.13	3.52
V1278AG	India	1	0.95	0.26	1.21	3.68
V1279 AG	India	1	1.68	0.43	2.10	3.92
V1280 AG	India	1	1.25	0.30	1.55	4.13
V1287 AG	India	1	1.66	0.59	2.25	2.81
V 1289 AG	India	1	2.47	0.65	3.12	3.79
V 1292 AG	India	3	0.82	0.22	1.04	3.77
V 1296 AG	India	1	2.00	0.52	2.52	3.87
V 1298 AG	India	1	1.98	0.49	2.47	4.04
V 1457 BG	India	2	0.90	0.23	1.13	3.92
V 1488 BG	India	1	1.25	0.34	1.59	3.71
V 1532 AG	India	3	0.78	0.21	0.99	3.79
V 1532BG	India	1	1.02	0.28	1.31	3.60
V1533BG	India	3	0.70	0.17	0.87	4.07
V 1536BG	India	2	0.94	0.24	1.18	3.90
V1539AG	India	3	0.79	0.21	1.00	3.76
V 1544AG	India	2	0.89	0.24	1.00	3.73
V1546AG	India	1	1.01	0.27	1.28	3.74
V1547AG	India	3	0.70	0.18	0.87	3.93
V1548AG	India	1	1.04	0.29	1.33	3.60
V1549AG	India	2	0.94	0.25	1.19	3.74
V1553AG	India	2	0.93	0.23	1.17	3.97
V1553BG	India	3	0.67	0.18	0.85	3.76

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V1554AG	India	3	0.82	0.21	1.03	3.84
V1555AG	India	1	1.00	0.25	1.25	3.94
V1555BG	India	1	1.02	0.28	1.31	3.60
V1556AG	India	3	0.76	0.21	0.97	3.61
V1557AG	India	1	1.11	0.28	1.39	3.96
V1573BG	India	4	0.49	0.12	0.61	3.93
V1631BG	India	3	0.83	0.22	1.04	3.84
V1649AG	India	1	1.00	0.28	1.28	3.57
V1667BG	India	1	1.12	0.29	1.41	3.87
V1730ABLM	India	2	0.93	0.27	1.20	3.46
V1735AG	India	2	0.89	0.23	1.12	3.81
V1735BG	India	1	1.05	0.28	1.33	3.74
V1776BG	India	2	0.96	0.24	1.20	3.93
V1837AG	India	1	1.11	0.30	1.42	3.67
V1837BG	India	3	0.76	0.21	0.97	3.60
V1844ABLM	India	1	1.16	0.32	1.48	3.68
V1857ABLM	India	1	1.05	0.28	1.33	3.81
V1867AG	India	3	0.74	0.18	0.92	4.02
V1969AG	India	2	0.90	0.25	1.15	3.68
V1984BG	India	1	1.19	0.31	1.50	3.79
V2268BG	India	4	0.55	0.13	0.68	4.12
V2273AG	India	1	1.00	0.25	1.25	3.94
V2273BG	India	3	0.78	0.21	0.99	3.78
V2365BG	India	2	0.92	0.25	1.17	3.76
V2396BG	India	1	1.09	0.31	1.39	3.57
V2565AG	India	2	0.92	0.25	1.17	3.67

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V2565BG	India	4	0.60	0.16	0.75	3.81
V2773AG	India	1	1.19	0.32	1.51	3.70
V2773BG	India	1	1.02	0.28	1.30	3.57
V2774AG	India	1	0.97	0.26	1.23	3.75
V3017BBR	India	2	0.93	0.25	1.18	3.75
V3017BG	India	1	1.32	0.36	1.68	3.61
V3372AG	India	1	0.97	0.26	1.22	3.80
V3372BG	India	3	0.70	0.19	0.89	3.69
V3495ABLM	India	2	0.85	0.23	1.07	3.73
V1471 BG	Indonesia	1	1.11	0.29	1.40	3.86
V1653AG	Iran	2	0.91	0.25	1.16	3.65
V1655AG	Iran	3	0.78	0.21	0.99	3.83
V1655BG	Iran	1	1.00	0.27	1.27	3.68
V1656AG	Iran	1	1.09	0.29	1.38	3.76
V1656BG	Iran	1	1.17	0.34	1.51	3.47
V1709AG	Iran	1	0.96	0.26	1.22	3.72
V1709BG	Iran	1	0.96	0.26	1.22	3.71
V1710AG	Iran	3	0.85	0.23	1.08	3.68
V1710BG	Iran	1	1.14	0.32	1.46	3.59
V1711BG	Iran	3	0.72	0.20	0.91	3.66
V1714AG	Iran	1	0.98	0.26	1.23	3.83
V1715BG	Iran	2	0.85	0.23	1.08	3.75
V1716AG	Iran	1	1.01	0.27	1.28	3.76
V1717BG	Iran	1	0.95	0.26	1.21	3.68
V1718AG	Iran	3	0.84	0.22	1.06	3.79
V1718BG	Iran	3	0.81	0.22	1.03	3.78

<u>Appendix Table 1</u> (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V1718BG	Iran	3	0.81	0.22	1.03	3.78
V1719BG	Iran	1	1.08	0.29	1.37	3.70
V1720AG	Iran	3	0.90	0.23	1.12	3.94
V1720BG	Iran	2	0.86	0.22	1.08	3.93
V1721BG	Iran	1	0.97	0.26	1.23	3.74
V1722BG	Iran	4	0.51	0.13	0.63	4.04
V1723AG	Iran	1	0.96	0.25	1.20	3.89
V1724AG	Iran	1	1.05	0.30	1.34	3.53
V1725AG	Iran	2	0.90	0.25	1.15	3.65
V1725BG	Iran	1	1.02	0.28	1.30	3.64
V1726BG	Iran	2	0.89	0.23	1.12	3.80
V1998AG	Iran	1	1.22	0.33	1.55	3.65
V1998BG	Iran	3	0.80	0.21	1.01	3.73
V1999AG	Iran	1	1.03	0.28	1.31	3.67
V1999BG	Iran	1	1.14	0.32	1.46	3.50
V2000AG	Iran	1	1.01	0.28	1.29	3.61
V2209BG	Iran	1	1.12	0.30	1.42	3.70
V2329AG	Iran	1	1.13	0.31	1.44	3.67
V2490AG	Iran	3	0.68	0.18	0.86	3.86
V2523BG	Iran	1	1.02	0.29	1.31	3.57
V2525AG	Iran	1	1.37	0.40	1.77	3.42
V3088BBR	Iran	1	1.22	0.36	1.57	3.43
V3170AG	Iran	1	1.08	0.29	1.37	3.73
V3171AG	Iran	4	0.43	0.08	0.52	5.10
V3171BG	Iran	3	0.88	0.23	1.11	3.91
V3178AG	Iran	1	1.07	0.31	1.37	3.50

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V3179AG	Iran	1	1.04	0.30	1.34	3.45
V3180AG	Iran	1	0.99	0.28	1.27	3.55
V3732AG	Iran	4	0.65	0.19	0.84	3.44
V3734AG	Iran	3	0.82	0.23	1.05	3.59
V3736AG	Iran	1	1.37	0.36	1.73	3.77
V3737AG	Iran	2	0.87	0.24	1.12	3.59
V3738AG	Iran	1	1.07	0.30	1.37	3.56
V3739AG	Iran	1	0.99	0.27	1.27	3.66
V3740AG	Iran	4	0.67	0.17	0.84	3.92
V3741AG	Iran	3	0.76	0.21	0.97	3.67
V3743ABR	Iran	1	1.06	0.31	1.37	3.47
V3743BG	Iran	1	1.02	0.27	1.29	3.71
V3746AG	Iran	1	1.03	0.29	1.32	3.54
V3748AG	Iran	1	1.21	0.32	1.53	3.77
V3748BG	Iran	1	1.18	0.32	1.50	3.66
V3749AG	Iran	1	1.00	0.27	1.27	3.75
V3750BBR	Iran	1	1.14	0.32	1.46	3.60
V3751ABBR	Iran	1	1.04	0.28	1.33	3.67
V3751BBR	Iran	1	1.24	0.34	1.58	3.63
V3752BG	Iran	1	1.13	0.31	1.44	3.59
V3754ABR	Iran	1	1.21	0.33	1.54	3.61
V3756AG	Iran	1	0.97	0.27	1.24	3.61
V3757AG	Iran	1	1.05	0.28	1.33	3.75
V3757BBR	Iran	1	1.12	0.31	1.43	3.62
V3758BBR	Iran	3	0.83	0.22	1.05	3.75
V3759BG	Iran	1	1.17	0.32	1.49	3.72

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V3760ABR	Iran	3	0.84	0.22	1.06	3.87
V3760BG	Iran	1	1.24	0.34	1.59	3.61
V1311AG	Iraq	1	1.51	0.45	1.96	3.39
V1311BG	Iraq	1	1.31	0.38	1.69	3.44
V2949BG	Korea	1	1.09	0.29	1.38	3.73
V3096AG	Korea	3	0.90	0.24	1.14	3.83
V2815AY	Nigeria	5	0.22	0.05	0.28	4.18
V3484AG	Pakistan	1	0.76	0.20	0.95	3.85
NM10-12	Pakistan	1	1.33	0.38	1.70	3.51
NM20-11	Pakistan	1	1.09	0.30	1.39	3.69
NM45-24-1	Pakistan	1	1.36	0.36	1.72	3.78
NM63-15-8	Pakistan	1	1.19	0.36	1.55	3.35
NM92	Pakistan	1	0.87	0.25	1.12	3.56
NM98	Pakistan	1	1.18	0.33	1.51	3.61
NM3960-88	Pakistan	1	1.11	0.30	1.41	3.74
Kabulimung	Pakistan	1	0.98	0.27	1.25	3.61
V 1410 AG	Philippines	1	1.10	0.28	1.38	3.85
V1944BY	Philippines	1	0.99	0.26	1.25	3.73
V1946AY	Philippines	3	0.79	0.20	0.99	4.04
V1948AG	Philippines	3	1.23	0.33	1.56	3.74
V1947AG	Philippines	1	1.12	0.32	1.44	3.48
V2184AG	Philippines	5	0.22	0.06	0.28	3.75
V2191BG	Philippines	2	0.88	0.25	1.13	3.50
V3476AG	Philippines	1	1.00	0.27	1.27	3.68
V 1323 BG	Taiwan	1	1.64	0.43	2.07	3.81
V 1326 AG	Taiwan	1	1.18	0.30	1.49	3.88

Appendix Table 1 (Cont'd)

Accession no.	Seed origin	Chlorosis rating	Chl *a*	Chl *b*	Total chl	Chl *a*: Chl *b*
V 1327 AY	Taiwan	1	1.11	0.30	1.41	3.69
V3404AG	Thailand	3	0.85	0.21	1.06	3.97
V3523AG	Thailand	1	1.18	0.33	1.50	3.60
VC1628AG	Thailand	1	0.90	0.23	1.13	3.90
VC19734AG	Thailand	4	0.62	0.16	0.78	3.87
VC2278AG	Thailand	5	0.32	0.07	0.39	4.55
VC2768AG	Thailand	1	1.10	0.29	1.39	3.77
V1745AG	USA	2	0.91	0.25	1.16	3.68
V1968AG	USA	3	0.79	0.23	1.02	3.47
V3387AG	USA	3	0.83	0.23	1.06	3.58

Appendix Table 2 The presence/absence of the AFLP markers E-ACC/M-CTG, E-ACT/M-CTA, E-CGT/M-CTG and E-CAG/M-TAC in 241 mungbean germplasm.

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V1658BBR	Afghanistan	T	+	-	+	-
V1658BG	Afghanistan	T	-	-	+	+
V1660BG	Afghanistan	S	+	-	+	+
V1661BG	Afghanistan	T	+	+	+	+
V1670AG	Afghanistan	T	+	+	+	-
V1670BG	Afghanistan	S	-	-	+	+
V1671BBR	Afghanistan	T	+	+	+	-
V1671BG	Afghanistan	T	+	+	+	+
V1672AG	Afghanistan	S	-	-	+	-
V1672BG	Afghanistan	T	+	+	+	-
V1673BG	Afghanistan	T	+	+	+	+
V1675BG	Afghanistan	T	+	+	+	+
V1676BBR	Afghanistan	T	+	+	+	+
V1676BG	Afghanistan	T	+	+	-	+
V1687ABLM	Afghanistan	T	+	+	+	-
V1687AG	Afghanistan	T	-	-	+	+
V2190BG	Afghanistan	S	-	-	-	+
V2467BG	Afghanistan	T	+	-	-	-
V2478BG	Afghanistan	T	+	+	-	+
V2479AG	Afghanistan	T	-	-	+	+
V2494AG	Afghanistan	T	+	-	+	-
V2495AG	Afghanistan	T	+	+	+	+
V3215AG	Afghanistan	T	+	+	-	-
V3215BG	Afghanistan	T	+	+	-	-
V3218AG	Afghanistan	T	+	-	+	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V3220AG	Afghanistan	T	-	-	+	-
V3221AG	Afghanistan	T	+	+	+	+
V3222AG	Afghanistan	T	+	+	-	+
V3222BBR	Afghanistan	T	+	+	+	-
V3223AG	Afghanistan	T	-	+	-	+
V3224AG	Afghanistan	S	-	-	+	+
V3226BBR	Afghanistan	T	+	+	+	-
V3227AG	Afghanistan	T	-	-	+	+
V3230AG	Afghanistan	T	-	-	+	+
V3235AG	Afghanistan	T	+	+	-	-
V3237AG	Afghanistan	T	+	+	+	+
V3241AG	Afghanistan	T	-	-	+	-
V3242AG	Afghanistan	T	+	+	-	-
V3243AG	Afghanistan	T	-	-	+	+
V3244AG	Afghanistan	T	+	+	+	+
V3245AG	Afghanistan	T	+	+	-	-
V3246AG	Afghanistan	T	+	+	+	-
V3246BBR	Afghanistan	T	+	+	-	+
V3247AG	Afghanistan	T	+	+	+	-
V3248AG	Afghanistan	T	-	-	-	-
V3248BG	Afghanistan	T	-	+	+	+
V3249BBR	Afghanistan	T	+	-	+	+
V3250AG	Afghanistan	T	+	+	-	-
V3250BG	Afghanistan	T	+	+	+	-
V3251ABLM	Afghanistan	T	+	+	+	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V3251BBR	Afghanistan	T	+	-	+	-
V3252AG	Afghanistan	T	-	+	+	-
V3253AG	Afghanistan	S	-	-	-	-
V3253ABLM	Afghanistan	T	+	+	+	+
V3255BG	Afghanistan	T	-	+	-	-
V3256ABLM	Afghanistan	T	+	-	-	-
V3257AG	Afghanistan	T	+	-	-	-
V3258AG	Afghanistan	T	-	+	+	-
V3260AG	Afghanistan	T	+	-	-	-
V3263ABLM	Afghanistan	T	+	-	-	+
V3264AG	Afghanistan	T	+	+	+	-
V3264BG	Afghanistan	T	-	-	-	+
V3267AG	Afghanistan	T	+	-	-	+
V3271AG	Afghanistan	T	+	+	-	+
V3277AG	Afghanistan	S	-	-	-	+
V3278AG	Afghanistan	T	-	-	+	-
V3278BBR	Afghanistan	T	-	+	+	-
V3280AG	Afghanistan	S	-	-	-	+
V3281BBR	Afghanistan	T	-	-	-	-
V3282AG	Afghanistan	T	-	+	+	+
V3282BBR	Afghanistan	T	-	+	+	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V3283AG	Afghanistan	T	-	+	+	-
V2065AG	Australia	S	-	-	-	-
V2075BY	Australia	S	+	-	-	+
V2085BG	Australia	T	-	+	+	+
V2010BG	China	T	+	+	-	-
V2915BG	England	T	+	+	+	+
V2062AG	Guam	T	-	-	+	+
V2062BG	Guam	S	-	-	+	+
V1990BG	Guatemala	T	+	-	+	-
V1278AG	India	T	-	+	-	-
V1279 AG	India	T	-	+	+	-
V1280 AG	India	T	+	+	-	+
V1287 AG	India	T	+	+	+	+
V 1289 AG	India	T	+	+	+	+
V 1292 AG	India	S	+	-	-	-
V 1296 AG	India	T	+	+	+	-
V 1298 AG	India	T	+	+	+	+
V 1457 BG	India	T	-	-	+	-
V 1488 BG	India	T	+	+	+	+
V 1532 AG	India	S	-	-	+	-
V 1532BG	India	T	+	+	+	+
V1533BG	India	S	-	-	+	-
V 1536BG	India	T	+	-	-	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V1539AG	India	S	-	-	-	+
V 1544AG	India	S	+	-	+	+
V1546AG	India	T	+	+	-	+
V1547AG	India	S	-	-	-	+
V1548AG	India	T	+	+	-	+
V1549AG	India	T	-	+	-	-
V1553AG	India	T	-	+	-	+
V1553BG	India	S	-	-	-	+
V1554AG	India	S	-	+	-	+
V1555AG	India	T	-	+	-	+
V1555BG	India	T	-	+	+	-
V1556AG	India	S	-	-	-	+
V1557AG	India	T	+	+	-	+
V1573BG	India	S	-	-	-	-
V1631BG	India	S	-	+	-	+
V1649AG	India	T	+	+	-	+
V1667BG	India	T	+	+	+	+
V1730ABLM	India	T	-	+	-	+
V1735AG	India	T	+	+	+	-
V1735BG	India	T	+	+	+	+
V1776BG	India	T	+	+	+	-
V1837AG	India	T	+	+	+	+
V1837BG	India	S	-	-	-	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V1844ABLM	India	T	+	+	+	+
V1857ABLM	India	T	+	+	+	+
V1867AG	India	S	-	-	+	+
V1969AG	India	T	+	-	-	-
V1984BG	India	T	+	+	-	+
V2268BG	India	S	-	-	-	+
V2273AG	India	T	-	+	+	-
V2273BG	India	S	-	-	-	+
V2365BG	India	T	-	+	+	+
V2396BG	India	T	+	+	+	+
V2565AG	India	T	+	-	-	+
V2565BG	India	S	-	-	-	+
V2773AG	India	T	-	+	+	-
V2773BG	India	T	+	+	+	-
V2774AG	India	T	+	-	-	+
V3017BBR	India	T	+	-	+	+
V3017BG	India	T	+	+	+	+
V3372AG	India	T	-	+	+	-
V3372BG	India	S	+	-	+	-
V3495ABLM	India	T	-	-	-	-
V1471 BG	Indonesia	T	+	+	+	+
V1653AG	Iran	T	+	-	-	-
V1655AG	Iran	S	-	-	+	+

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V1655BG	Iran	T	-	+	+	+
V1656AG	Iran	T	+	+	+	+
V1656BG	Iran	T	+	+	+	+
V1709AG	Iran	T	-	+	+	-
V1709BG	Iran	T	+	+	+	+
V1710AG	Iran	T	-	+	+	-
V1710BG	Iran	T	+	+	+	+
V1711BG	Iran	S	+	-	+	+
V1714AG	Iran	T	-	+	-	+
V1715BG	Iran	T	+	+	+	-
V1716AG	Iran	T	+	+	-	+
V1717BG	Iran	T	+	+	-	-
V1718AG	Iran	T	+	-	-	+
V1718BG	Iran	S	+	-	-	+
V1719BG	Iran	T	+	+	+	+
V1720AG	Iran	T	+	-	+	+
V1720BG	Iran	T	+	+	+	-
V1721BG	Iran	T	+	-	+	-
V1722BG	Iran	S	-	-	+	+
V1723AG	Iran	T	+	-	+	-
V1724AG	Iran	T	+	+	+	-
V1725AG	Iran	T	-	-	-	-
V1725BG	Iran	T	+	+	+	+

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V1726BG	Iran	T	+	+	+	+
V1998AG	Iran	T	+	+	+	-
V1998BG	Iran	S	+	-	+	-
V1999AG	Iran	T	-	+	-	+
V1999BG	Iran	T	+	+	+	+
V2000AG	Iran	T	+	+	+	-
V2209BG	Iran	T	+	+	+	+
V2329AG	Iran	T	+	+	+	-
V2490AG	Iran	S	+	-	-	-
V2523BG	Iran	T	+	-	-	+
V2525AG	Iran	T	+	+	+	+
V3088BBR	Iran	T	+	+	+	-
V3170AG	Iran	T	+	+	-	-
V3171AG	Iran	S	-	-	-	-
V3171BG	Iran	T	+	-	-	+
V3178AG	Iran	T	+	+	-	-
V3179AG	Iran	T	+	+	+	+
V3180AG	Iran	T	+	+	+	+
V3732AG	Iran	S	-	-	+	-
V3734AG	Iran	T	-	+	-	-
V3736AG	Iran	T	+	+	+	+
V3737AG	Iran	T	-	+	-	-
V3738AG	Iran	T	+	+	+	+

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptibility	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-CAG/ M-TAG	E-CGT/ M-CTG
V3739AG	Iran	T	+	-	-	-
V3740AG	Iran	S	-	-	-	+
V3741AG	Iran	S	+	+	-	-
V3743ABR	Iran	T	-	+	+	-
V3743BG	Iran	T	+	+	-	+
V3746AG	Iran	T	-	+	-	+
V3748AG	Iran	T	+	+	+	+
V3748BG	Iran	T	+	+	-	+
V3749AG	Iran	T	+	+	+	-
V3750BBR	Iran	T	+	+	-	+
V3751ABBR	Iran	T	+	+	+	-
V3751BBR	Iran	T	+	+	+	-
V3752BG	Iran	T	+	+	+	+
V3754ABR	Iran	T	+	+	-	+
V3756AG	Iran	T	-	+	+	+
V3757AG	Iran	T	+	+	+	+
V3757BBR	Iran	T	+	+	-	-
V3758BBR	Iran	T	+	+	+	-
V3759BG	Iran	T	-	+	-	+
V3760ABR	Iran	T	+	+	+	-
V3760BG	Iran	T	+	+	+	-
V1311AG	Iraq	T	-	+	+	+
V1311BG	Iraq	T	+	+	+	-

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptible	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-ACC/ M-CTG	E-ACT/ M-CTA
V2949BG	Korea	T	+	-	-	+
V3096AG	Korea	T	-	-	+	+
V2815AY	Nigeria	S	+	+	-	+
V3484AG	Pakistan	T	+	+	-	-
NM10-12	Pakistan	T	+	+	-	-
NM20-11	Pakistan	T	+	+	-	-
NM45-24-1	Pakistan	T	+	+	+	+
NM63-15-8	Pakistan	T	+	+	+	+
NM92	Pakistan	T	+	+	-	+
NM98	Pakistan	T	+	+	+	+
NM3960-88	Pakistan	T	+	+	-	-
Kabulimung	Pakistan	T	+	+	-	-
V 1410 AG	Philippines	T	+	-	-	-
V1944BY	Philippines	T	+	-	+	+
V1946AY	Philippines	S	+	+	-	+
V1948AG	Philippines	T	+	+	+	+
V1947AG	Philippines	T	-	-	+	+
V2184AG	Philippines	S	-	-	+	+
V2191BG	Philippines	T	+	-	+	-
V3476AG	Philippines	T	+	+	+	+
V 1323 BG	Taiwan	T	-	+	+	-
V 1326 AG	Taiwan	T	+	+	+	+
V 1327 AY	Taiwan	T	+	-	-	+
V3404AG	Thailand	T	-	+	+	+

Appendix Table 2 (Cont'd)

Accession no.	Seed origin	Phenotype	Markers of Tolerant		Markers of Susceptible	
			E-ACC/ M-CTG	E-ACT/ M-CTA	E-ACC/ M-CTG	E-ACT/ M-CTA
V3523AG	Thailand	T	-	-	-	+
VC1628AG	Thailand	T	-	-	+	+
VC19734AG	Thailand	S	-	-	+	+
VC2278AG	Thailand	S	+	+	+	+
VC2768AG	Thailand	T	-	+	+	-
V1745AG	USA	T	-	-	+	+
V1968AG	USA	S	+	-	+	+
V3387AG	USA	T	+	+	-	-

T, S = Tolerant and susceptible to iron deficiency

+ , - = Presence and absence of AFLP markers