1943

THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Agricultural Biotechnology)
DEGREE

Agricultural Biotechnology
FIELD

Interdisciplinary Graduate Program
PROGRAM

TITLE: Inheritance and Molecular Marker Analysis of Grain Quality and Days to Flowering in Vegetable Soybean (*Glycine max* (L.) Merrill)

NAME: Mr. Pornpan Pooprompan

THIS THESIS HAS BEEN ACCEPTED BY

Peerasak Srinives **THESIS ADVISOR**
(Professor Peerasak Srinives, Ph.D.)

Sontichai Chan **COMMITTEE MEMBER**
(Assistant Professor Sontichai Chanprame, Ph.D.)

T. Toojinda **COMMITTEE MEMBER**
(Mr. Theerayut Toojinda, Ph.D.)

Sirikul Wasee **COMMITTEE MEMBER**
(Miss Sirikul Wasee, Ph.D.)

Pongthep A. **GRADUATE COMMITTEE CHAIRMAN**
(Associate Professor Pongthep Akratanakul, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON 16 May 2006

Vinai Artkongharn **DEAN**
(Associate Professor Vinai Artkongharn, M.A.)

THESIS

INHERITANCE AND MOLECULAR MARKER ANALYSIS OF GRAIN QUALITY AND DAYS TO FLOWERING IN VEGETABLE SOYBEAN (*Glycine max* (L.) Merrill)

PORNPAN POOPROMPAN

A Thesis Submitted in Partial Fulfilment of
the Requirements for the Degree of
Doctor of Philosophy (Agricultural Biotechnology)
Graduate School, Kasetsart University
2006

ISBN 974-16-1667-8

Pornpan Pooprompan 2006: Inheritance and Molecular Marker Analysis of Grain Quality and Days to Flowering in Vegetable Soybean (*Glycine max* (L.) Merrill). Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Professor Peerasak Srinives, Ph.D. 84 pages.

ISBN 974-16-1667-8

Grain quality and days to flowering are major characters to be considered in vegetable soybean improvement. The objectives of this study were to investigate the inheritance and identify simple sequence repeat (SSR) markers associated with quantitative trait loci (QTL) for seed weight (SW), total soluble solid (TSS) content of green seed, and days to flowering (DTF) in recombinant inbred lines (RILs) derived from a cross between the vegetable soybean cultivar 'AGS292' and the grain soybean line (G8891xG7945)-31-3-5-5 (or 'K3') grown in two environments. A significant positive transgressive segregation of TSS content was observed. The narrow-sense heritability of SW, TSS and DTF in 2 environments were 0.754 vs 0.905, 0.582 vs 0.652, and 0.916 vs 0.942 while the combined estimates across two environments were 0.620, 0.455, and 0.291, respectively. This indicated that the heritability of DTF and SW were not consistent across the environments. Negative correlations between DTF with TSS and SW were also observed.

The SSR allele size profiles of the parents were analyzed with 161 SSR markers to identify their polymorphism. Molecular marker analysis of 63 polymorphic marker loci in a selective genotyping population of TSS content revealed that at least seven QTL were involved in controlling SW, three QTL controlling TSS content, whereas four QTL controlling DTF. The QTL near an SSR marker (Satt166) in molecular linkage group (MLG) L had the greatest effect on SW, Satt236 in MLG A1 for TSS content and Satt431 in MLG J for DTF. The marker loci associated with SW and TSS content were highly consistent across environments. The QTL found in this study can facilitate vegetable soybean breeders in using marker-assisted selection (MAS) to improve grain quality of vegetable soybean.

Pornpan Pooprompan
Student's signature

Peerasak Srinives 1 / 05 /2006
Thesis Advisor's signature

ACKNOWLEDGEMENTS

I am very grateful to my advisor, Prof. Dr. Peerasak Srinives for his valuable suggestion, helpful guidance and encouragement throughout my studies. I am also very grateful to Assoc. Prof. Dr. Jun Abe for his advice on the laboratory work and supporting. I also would like to acknowledge the guidance and assistance received from Assist. Prof. Dr. Sontichai Chanprame, Dr. Theerayut Toojinda and Dr. Sirikul Wasee.

I would like to thank Dr. Panie Tongpamnak for providing facilities and equipment. I also thank farm staffs of the Tropical Vegetable Research Center of Kasetsart University for all field work during conducting the experiments. Thank also to my best friend Rudy Soehendi, who shared both sad and joyful moments.

This research was supported by the Center for Agricultural Biotechnology, through the fund from Subproject Graduate Study and Research in Agricultural Biotechnology under the Higher Education Development Project, Commission on Higher Education, The Ministry of Education. Thank are also due to Thailand Research Fund for the financial support as well as Maejo University for granting me the study leave and providing scholarship for my study.

Finally, I am grateful to my wife Aoun, son Benz and daughter Bean for their help and understanding throughout my studies. I also wish to dedicate this thesis to my father who had given me so much when he was with us.

Pornpan Pooprompan
May, 2006

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (cont’d)

LIST OF TABLES

LIST OF TABLES (cont'd)

LIST OF TABLES (cont'd)

Appendix Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

SW = 100 seed weight
TSS = Total soluble solids
DTF = Days to flowering
QTL = Quantitative trait loci
MAS = Marker-assisted selection
RIL = Recombinant inbred line
SF-ANOVA = Single factor analysis of variance
MLG = Molecular linkage groups
SSR = Simple sequence repeat

INHERITANCE AND MOLECULAR MARKER ANALYSIS OF GRAIN QUALITY AND DAYS TO FLOWERING IN VEGETABLE SOYBEAN (*Glycine max* (L.) Merrill)

INTRODUCTION

Vegetable soybean, *Glycine max* (L.) Merr. is a large-seeded, slightly sweet type of soybean harvested while pods are still green, usually at the R_6 growth stage. Japan, China, Korea, and Taiwan have historically been major producers and consumers of vegetable soybean (Shanmugasundaram and Yan, 2004). Grain soybean of medium seed size type has been used as vegetable soybean for local markets in Thailand. However, the quality requirements for export markets are entirely different from those for traditional domestic markets (Srisombun *et al*., 2004). The quality of vegetable soybean depends on several characteristics such as seed size, sweetness, taste, texture, flavor and nutritive value (Takahashi, 1991). Seed qualities are important quantitative traits controlled by multiple genes having small or large effects. There have been studies that used recombinant inbred lines (RILs) population to investigate the inheritance of quantitative traits (Openshaw and Hadley, 1981) and constructed genetic linkage map (Keim *et al*., 1990; Maughan *et al.,* 2000; Hyten *et al*., 2004; Zhang *et al*., 2004). Since vegetable soybean breeding projects are largely belonged to private companies, publications on inheritance study in this crop are rather limited as compared to in field soybean.

Seed weight (SW) is a major yield component of grain soybean and vegetable soybean. SW is polygenically controlled and can range from 6 to 55 grams per 100 seeds. Soybean cultivars with either very small (<8 g/100 seeds) or very large (>20 g/100 seeds) seed sizes are used especially critical for various soybean food-types (Maughan *et al.,* 1996). For example, soybean seed used for sprouts should possess small SW, whereas soybean seed used for vegetable soybean should have large SW (Hoeck *et al*., 2003). The demand for vegetable-type soybean is increasing in the international market. Thus, the need for development of productive cultivars with desirable seed size is increasingly important (Mian *et al*., 1996).

Soybean seed traits such as seed protein and oil content are important quantitative traits. The major use of soybean is for soybean meal, a primary component that provides protein in livestock feed. An important by-product of meal production is soybean oil, extracted from crushed meal. Soybean oil is vegetable oil used worldwide in cooking and frying fats (Hyten *et al.*, 2004). Simultaneous increases in protein and oil concentration can proceed only a limited extent, and most experimental evidence shows protein and oil contents to be negatively correlated (Tajuddin *et al.*, 2003). However, improving levels of one trait at the expense of the other is possible, thus leading to the development of value-added soybeans with prescribes levels of protein or oil for specialty markets (Brummer *et al*., 1997).

Total sugar content of soybean seed has an influence on the quality characteristics of some soybean products and vegetable soybean (Masuda, 1991; Chandler and Fehr, 2000). In addition, taste of vegetable soybean seeds is mainly determined by sucrose (Masuda, 1991). Moreover, sucrose is the predominant sugar in vegetable soybean seed followed by glucose and fructose (Tsou and Hong, 1991). However, Krober and Cartter (1962) reported that increases in protein were associated with decreases in total soluble sugar. Moreover, total sugar is not routinely measured in a soybean breeding program because the current methods of analysis are too time consuming for assessment of a large number of genotypes (Chandler and Fehr, 2000). Since brix reading is the sum of total soluble solid (sugar, salts, protein, acids, etc.) dissolved in the extracted juice, it should reflect the total sugar content of vegetable soybean seed. A popular vegetable soybean cultivar ‘AGS292’ (a pure line selected from the Japanese cultivar ‘Taishoshiroge’ by AVRDC, the World Vegetable Center, Taiwan.) is high in total soluble solid (TSS) content and showed less sensitivity to photoperiod and temperature (Wasee, 1997; Shanmugasundaram and Yan, 2004).

Days to flowering (DTF) in soybean is a character that effect directly plant height, number of nodes, maturity time, lowest pod insertion, leaf area, lodging degree, and mainly grain yield, and other important agronomic characters of that crop. As well as, days to flowering is also a major trait to be considered by the farmers growing vegetable soybean. The cultivars with too short flowering date do not accumulate sufficient photosynthates to produce high quality seed, especially in a large-seeded cultivar. However, the cultivars with too long flowering dates require more days until harvesting and thus they are not suitable for farming systems in tropical countries. Tasma *et al.* (2001) reported that number of days to flowering and maturity in field soybean are useful for developing soybean cultivars with wider geographical adaptation.

The traditional method of soybean breeding involves artificial hybridization to develop genetic variability followed by self-fertilization and phenotypic selection for traits of interest among the offspring (Hoeck *et al.*, 2003). Molecular markers may improve traditional methods of breeding for traits of interest of vegetable soybean by increasing the reliability which desirable progeny are selected.

There have been many studies that have used molecular markers to mapped quantitative trait loci conditioning traits of interest, the regions of the chromosome responsible for quantitative traits such as seed weight, protein, oil and total sugar content. Hoeck *et al.* (2003) identified SSR markers associated with quantitative traits loci for seed size among individual F_2 plants in three populations derived from small-seeded x normal-size crosses. They found 12 markers that individually accounted for 8.1 to 14.9% of the variation for seed weight combined across environments in Population I , Pop II had 16 markers that individually accounted for 7.8 to 36.5% of the variation, and Pop III had 22 markers that individually accounted for 8.6 to 28.8% of the variation. Maughan *et al*. (1996) and Mian *et al.* (1996) identified and characterized several putative QTL that explained significant levels for seed weight. Mansur *et al.* (1996) observed three molecular markers that explained 23.1% of the variation for seed weight among $F_{2:7}$ lines developed from the cross between ‘Minsoy’ and ‘Noir 1’. Orf *et al.* (1999) found seven marker loci that accounted for 50% of the variation for seed weight among $F_{2:7}$ lines in the cross of ‘Noir 1’ x ‘Archer’, seven in a ‘Minsoy’ x ‘Noir 1’ population that accounted for 50%, and two in a ‘Minsoy’ x ‘Archer’ population that accounted for 12% of variation.

Brummer *et al.* (1997) identified QTL for soybean seed protein and oil contents using eight distinct populations and evaluated the coincidence of QTL across genetic backgrounds and different environments. Environmentally stable and environmentally sensitive QTL were identified for both protein and oil content in all eight populations. The identified QTL were sensitive to both environments and genetic background although some common QTL were identified in multiple populations across several years. Hyten *et al.* (2004) identified SSR markers representative protein, oil and seed size QTL in RIL population derived from a cross between two prominent ancestors of North American soybean (‘Essex’ x ‘Williams’). The RILs were grown in six environments. They found a total of four protein, six oil, and seven seed size QTL in this population.

Maughan *et al.* (2000) used molecular markers to identify and characterize QTL controlling sucrose content in an interspecific F_2 population soybean. They found that seventeen marker loci on seven different genomic regions were significantly associated with sucrose content. These markers explained from 6.1 to 12.4% of the total phenotypic variation. In a combined analysis these genomic regions explained 53% of total phenotypic variation.

Several QTL associated with flowering time and maturity have been previously mapped in soybean. Keim *et al.* (1990) reported that five markers on MLGs C1, C2 and D1were found to be associated with maturity and time of flowering in an F_2 population derived from a cross between *G.max* and *G. soja*. The observed QTL explained 17 to 23% of total phenotypic variation. Using other F_2 population of ‘Minsoy’ x ‘Noir’, Mansur *et al.* (1996) also reported a major QTL for flowering time on MLG C2 and minor QTL for maturity on MLG L and M. Lee *et al.* (1996 a) reported that QTL for maturity trait and days to flowering were found on MLG K. The study was based on an F_2 population derived from a cross between PI 97100 and ‘Coker 237’. The observed QTL explained 26.2 to 31.2% of phenotypic variance. In addition, Tasma *et al.* (2001) and Zhang *et al.* (2004) reported that major QTL controlling DTF was located on MLG C2 in independent populations.

The objectives of this study are

1. to investigate the inheritance of seed weight, total soluble solid content, and days to flowering in vegetable soybean.

2. to evaluate total soluble solid content in green seed for improvement of grain quality in vegetable soybean.

3. to identify SSR markers associating with QTL for seed weight, total soluble solid content in green seed, and days to flowering of a RIL population derived from the cross between 2 contrasting parents, viz. vegetable soybean cultivars ‘AGS292’ and the grain soybean experimental line ‘K3’.

LITERATURE REVIEWS

Vegetable soybean

Soybean (*Glycine max* (L.) Merrill) can be classified into grain soybean and vegetable soybean depending on its form of utilization and nutritional value. The primary constituents of soybean seed, on a dry matter basis, contained about 40% protein, 21% oil, and 11% soluble carbohydrates (Openshaw and Hadley, 1981). Hymowitz and Collins (1974) reported that sucrose is the primary carbohydrate in mature soybean seed followed by less amounts of stachyose and raffinose and also assumed that total sugar content was the sum of sucrose, raffinose and stachyose content. However, the oligosaccharides, raffinose and stachyose, have been implicated as causative factors for the flatulence and uncomfortable feeling often experienced after consuming products containing soybean meal. In this case, because the human digestive tract lacks α galactosidase activity, both raffinose and stachyose pass into the large intestine where they are anaerobically fermented to produce gas. Low molecular weight sugars such as sucrose and glucose are normally ingested along the lining of the small intestine (Hymowitz *et al*., 1972).

The grain soybean normally has small seed size and consumed as processed products. However, vegetable soybean is a large-seeded type of soybean harvested at the $R_{6.5}$ growth stage while pods and plants are still green (Fehr *et al*., 1971). The detached pods and shelled green beans are used as fresh vegetable in Japan, China, and Korea and to a less extent in Thailand and Nepal. The pods and shelled green bean are frozen and marketed. It is rich in protein (18%), cholesterol-free fat (5.7%), phosphorus (158 mg/100 g), calcium (78 mg/100 g.), vitamin B1, (0.4 mg/100 g) and B2 (0.17 mg/100 g), isoflavone, and tocopherol. There is a great potential to use vegetable soybean seed as a substitute for meat protein in developing countries. (Shanmugasundaram and Yan, 2004). It is also popular as a snack food because of its high sucrose, glutamic acid and alanine content (Lumpkin and Konovsky, 1991).

Vegetable soybean or sweet bean is a traditional Japanese vegetable and called ‘Edamame’ (pronounced “eh-dah-mah-may”). Edamame do not have edible pods but only beans are eaten. It can substitute for green peas or lima bean in any recipe. In China, the shelled beans are stir-fried with other ingredients. In Japan, pods are boiled in salted water and the beans are squeezed from the pod directly into the mouth, and the pod are discarded (Masuda, 1991). Vegetable soybean has been consumed for more than a hundred years in Thailand. The medium seed size, 15-20 g/100 seeds soybean grown for food or oil extraction is also traditionally used as vegetable soybean, called ‘tau rae’. Green pods attached to the stem are commonly boiled and sold in the countrywide local markets (Srisombun *et al.*, 2004).

Japan, China, Korea, and Taiwan were the major producers and consumers of vegetable soybean in the past. Among them, Japan produces large amount and consumes high quality vegetable soybean. For the time being, the production in Japan can not cope with demand. Therefore, fresh and frozen vegetable soybeans have to be imported from other countries. Until 1992, Taiwan had monopolized the export market to Japan. However, the production area in Taiwan has been reduced due to rising labor and costs of raw materials. Recently, 50% of vegetable soybean in Japanese market was imported from China, followed by 34% from Taiwan, 13% from Thailand and 3% from other countries including Indonesia and Vietnam (Shanmugasundaram and Yan, 2004).

The high quality vegetable soybean production and industry development for export has been promoted only in the past 20 years. The initial stage of vegetable soybean development for export was the establishment of a frozen vegetable soybean factory in the late 1980’s. The cooperation between Thailand and AVRDC in research, development, extension and market promotion at the early stage of industry development in 1991 stimulated vegetable soybean production for export and domestic use. The export of a small quantity commenced in 1990 and increased to almost 100,000 t in 2002. Vegetable soybean can be grown in three seasons in a year mainly in the northern region with the planted area of about 2,600 ha in 2003. The marketable pod yield averaged about 5.72 t/ha under farmer field conditions (Srisombun *et al*., 2004).

Quality of vegetable soybean

Quality requirements of vegetable soybean are grouped based on five major categories including appearance, taste, flavor, texture and nutritional value. The pod color is important, and bright-green is the most desirable. There are many taste-related substances in soybean seed, such as sugars, amino acids, organic acids, inorganic salts, flavonoids and saponins. Sensory evaluation of the stored vegetable soybean showed that the taste of the seeds is mainly determined by sucrose, glutamic acid and alanine (Masuda, 1991). Sucrose is the predominant sugar in vegetable soybean seed followed by glucose and fructose (Tsou and Hong, 1991). Vegetable soybean is generally harvested at about 33-38 days after flowering depending on its pod color and thickened. The level of sucrose content in the soybean seed increased during early developing stages, but the level tended to decline upon 35 days after flowering (Masuda, 1991).

The standard criteria for vegetable soybean exported to Japan are large pods and seed with two or more seeds per pod. Pod width and length should be more than 1.4 cm and 5 cm, respectively. Pods are bright green color with gray pubescence and spotless surface. Pod should be more than one hundred and fifty pods per five hundred gram. Eating quality for export are sweet taste (>11% TSS), volatile flavor, smooth texture and good taste (Shanmugasundaram *et al.*, 1991).

Relationship of grain quality and DTF with other agronomic traits

Seed weight (SW) is one of the yield components of grain soybean and it is positively correlated with seed yield (Burris *et al*., 1973; Smith and Camper, 1975). Similarly, Mian *et al*. (1996) reported that SW had a positive correlation with seed yield and a negative association with maturity in an F_4 population derived lines of ‘Young’ x PI416937. However, there were positive correlation with plant height, lodging and seed yield in F_2 population derived lines of PI97100 x ‘Coker 137’. Orf *et al.* (1999) also reported that seed yield in RIL population in soybean was only moderately correlated with height, maturity, and reproductive period, and even less with seed weight and flowering date.

Protein and oil contents in the soybean seed have been reported to be strongly negatively correlated (Mansur *et al.*,1996; Brummer *et al*., 1997; Chandler and Fehr, 2000; Tajuddin *et al.*, 2003). Moreover, the improvement for high protein content tends to decrease the yield due to the negative correlation between the two characters (Wasee, 1997).

Chandler and Fehr (2000) reported that total sugar was positively correlated with fiber for 23 soybean cultivars, similar correlation between the two traits for 16 small-seeded genotypes was also observed by Geater *et al.* (2000). However, sucrose content in soybean seed is closely associated with the quality and taste of many soyfood, as well as vegetable soybean (Masuda, 1991; Chandler and Fehr, 2000). Hymowitz *et al.* (1972) reported that total sugar content and oil content in soybean seed are positively associated, each was negatively correlated with protein content. Moreover, sucrose and raffinose content were positively correlated with oil content, while stachyose content was positively associated with protein.

Positive correlations of days to flower in vegetable soybean with plant height at maturity, number of nodes per plant and number of one-seeded pods per plant were reported by Wasee (1997). Flowering time (days to R_1) and maturity (days to R_8) in soybean have been reported to be highly correlated (Mansur *et al.*, 1996; Orf *et al*., 1999).

Wasee (1997) studied the correlation among agronomic characters from four crosses of vegetable soybean. It can be seen that the trend of associations was quite similar for all the crosses. Number of total pods per plant was positively correlated with number of two-seeded pods per plant and seed weight per plant. However, hundred seed weight was negatively correlated with plant height at maturity, number of nodes per plant, number of unfilled and damaged pods and number of total pods.

Inheritance of grain quality in soybean

Soybean SW is a highly heritable trait with heritability ranging from 44 to 94% (Brim, 1973). Hoeck *et al.* (2003) found that the broad-sense heritabilities for the three populations ranged from 0.45 to 0.85 on the plot basis and from 0.76 to 0.93 on the entry-mean basis, which were consistent with previous heritability estimates for small-seeded x normal-size crosses (Bravo *et al.*, 1980; Leroy *et al.*, 1991; Johnson *et al.*, 2001). However, standard unit heritability for seed weight based on the parent-offspring correlation and adjusted for inbreeding was 54% (Maughan *et al.*, 1996).

The contents of protein and lipid in grain soybean seed of RIL population estimates over two environments were highly heritable, with broad-sense heritability ranged from 0.74 to 0.79 and 0.73 to 0.78 respectively (Tajuddin *et al.*, 2003). Similarly, Hyten *et al.* (2004) also found broad-sense heritability of protein and oil content was 0.91 and 0.84 respectively. As well as, narrow-sense heritabilities of seed protein and oil contents estimates over three environments on a line mean basis in eight intraspecific soybean populations were highly heritable. Although the narrow-sense heritability of both traits is relative high, environmental variation can affect the ability to select these traits (Brummer *et al.*,1997).

Maughan *et al.* (2000) reported that broad-sense heritability of sucrose content calculated from variance components was 0.82, similar to the previous estimates of heritability for sugar content in soybean (Openshaw and Hadley, 1981).

Vegetable soybean variety improvement

In grain soybean breeding, in additional to increasing seed yield, improvement of the quality and quantity of soybean seed constituents is important (Tajuddin *et al*, 2003). Recently, vegetable soybean cultivars have been bred from varieties with emphasis on the green color of immature pods and acceptable taste of seeds, as well as the overall objectives of vegetable soybean breeding were to increase green pod yield and to improve the quality of green pod and green seed constituents (Masuda, 1991), In addition higher quality pods with superior quality are required. Thus the characteristics to be improved by a breeder are early maturity, large pod size with bright green color and spotless, smooth texture, white or grey pubescences, high ratio of 2-3 seeded-pods, sweet green seed, volatile flavors, good taste, disease and insect resistance (Takahashi, 1991).

The initial research in collaboration with AVRDC was instrumental for vegetable soybean development in Thailand. A number of varieties/lines were introduced from AVRDC and Japan to Thailand since 1986. At present, ‘AGS292’ and two new varieties, No.75 (‘Ryokkoh75’) and No.2808 are grown for export to Japan. ‘AGS292’ produced a graded pod yield of about 5.46 t/ha averaged from 13 environments during 2001-2002 in the research centers and it gave about 35 percent higher pod yield than that of No.75 (Srisombun *et al.*, 2004).

The improvement of vegetable soybean for large pods in early generations of selection was done by Wasee (1997). The results showed that ‘Kahori’, ‘AGS292’ and ‘AGS187’ appeared to be potential parents for large pods. ‘Kahori’ and ‘AGS189’ were good source for protein and oil while ‘AGS292’ was a good source for sugar. ‘AGS189’ was a good source for high yield. Moreover, sucrose content is considered a relatively more important character of good quality vegetable soybean in breeding programs.

Genetic markers

There are three types of genetic markers: 1) morphological markers which themselves are phenotypic traits or characters; 2) biochemical markers, which include allelic variants of enzymes called isozymes; and 3) DNA (or molecular) markers, which reveal site of variation in DNA (Collard *et al*., 2005). Morphological markers are usually visually characterized phenotypic characters such as flower color, seed shape, growth habits, or pigmentation. Isozyme markers are limited in number and are oftenly influenced by environmental factors or developmental stages of the plant. Despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Weeden *et al*., 1994).

DNA are the most widely used types of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in numbers and are not effected by environmental factors and/or developmental stage of the plant, highly heritable and are extremely useful for tagging traits that have low heritability or are difficult and/or time consuming and expensive to measure. Apart from the use of DNA markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm and cultivar identification (Baird *et al.,* 1997)

DNA markers may be broadly divided into three classes based on the method of their detection: 1) hybridization-based, 2) polymerase chain reaction (PCR)-based and 3) DNA sequence-based (Karp and Edwards, 1997). DNA markers are particularly useful if they reveal difference between individuals of the same or different species. Polymorphic markers may also be described as codominant or dominant. Codominant markers indicate difference in size whereas dominant markers are either present or absent. Codominant markers may have many different alleles whereas a dominant marker only has two alleles (Collard *et al*., 2005). DNA markers allowed the construction of genetic linkage maps of soybean from restriction fragment length polymorphisms (RFLP) (Lark *et al*., 1993; Shoemarker and Specht, 1995), random amplified polymorphic DNA (RAPD) (Chang *et al.,* 1997), amplified fragment length polymorphism (AFLP) (Keim *et al*., 1996) and microsatellite or simple sequence repeat (SSR) markers (Cregan *et al*.,1999 a).

Simple sequence repeats (SSR) or microsatellites are DNA sequences that consist of two to five nucleotide core units such as (AT), (CTT), and (ATGT) which are tandemly repeated. These small repetitive DNA sequences which are spread throughout the genomes of eukaryotes provide the basis of a polymerase chain reaction (PCR) based, multi-allelic, codominant genetic marker system. (Cregan and Quigley, 1997). Length polymorphisms arising from variations in the number of repeat units of these motifs can be used to distinguish between different alleles of these loci. However, DNA sequences flanking these hypervariable regions are generally conserved among genotypes of the same species (Cregan *et al.*, 1999 b), and so SSR primers can be easily developed from these genomic sequences.

SSR markers are abundance in plant nuclear DNA (Wang *et al.*, 1994) with high level of polymorphism. In soybean, up to 26 alleles of an SSR have been observed in a group of about 100 genotypes (Rongwen *et al.*, 1995). SSRs have been adopted for mapping studies in many important crops.

The development of SSR markers is rather tedious. It is necessary to screen a genomic library with an SSR probe, to sequence the positive clones, to synthesize the individuals (de Vienne *et al.*, 2003). For some plant species, numerous SSRs that are distributed throughout the genome have already been tested are publicly available. Sequences of almost 600 primers pairs of soybean are also available to day on the SOYBASE website.

Construction of linkage maps

The most important use of linkage maps is to identify chromosomal locations containing genes and QTL associated with traits of interest. Genetic maps are constructed based on the principle that genes and markers segregate via chromosome recombination during meiosis, thus allowing their analysis in the progeny. Gene and markers that are close together or tightly-linked are transmitted together from parent to progeny more frequently than genes or markers that are located further apart. In a segregating population, there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate recombinant fraction, which infer the genetic distance between markers, the relative order and distance between markers. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome. Markers that have a recombination frequency of 50% or more are described as ‘unlinked’ and assumed to be located far apart on the same chromosome or on different chromosomes. Linkage maps are constructed from the analysis of many segregating markers. The three main steps of linkage map construction are; 1) production of a mapping population ; 2) identification of marker polymorphism, and 3) linkage analysis of markers (Collard *et al*., 2005).

Construction of a linkage map requires a segregating plant population. The parents selected for the mapping population must differ for one or more traits of interest. The population size used in preliminary genetic mapping studies generally range from 50 to 250 individuals. However, larger populations are required for high-resolution mapping (Mohan *et al.,* 1997).

Several different populations may be utilized for mapping within a given plant species. Selection of a population for genomic mapping involves choosing parents and determining a mating scheme. Decision on selection of parents and mating design, as well as the type of markers, depend upon the objectives of the experiment (Liu, 1998). F_2 and backcross (BC) populations are the simplest types of mapping populations. Collard *et al*. (2005) suggested that the major advantages of these populations are easy to construct and require only a short time to produce. Inbreeding from individual F_2 plants allows the construction of recombinant inbred lines (RILs). The length of time (six to eight generations) needed for producing a RIL population is the major disadvantage. Double haploid (DH) populations may be produced by regenerating plants by the induction of chromosome doubling from pollen grains, however, the production of DH populations is only possible in species that amenable to tissue culture such as rice, wheat, sugarcane and barley. The major advantages of RIL and DH populations are that they produce homozygous lines that can be multiplied and reproduce without genetic change. This allows the plant breeder to conduct replicated trials across different location and years (Collard *et al*., 2005).

QTL analysis

QTL analysis is based on the principle of the detecting an association between phenotype and genotype of the markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant difference exist between groups with respect to the trait being measured (Tanksley, 1993). A significant difference between phenotypic means of the groups, depending on the marker and the type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait.

The investigation of QTL conducted in a single environment may lead to underestimate the number of QTL influencing a trait. It is also possible to have environmentally sensitive QTL, meaning that the expression of these QTL will only occur under certain environments. Paterson *et al*. (1991) suggested that, in such a case, the experiment conducted to identify QTL should be done in the location where these environmental conditions are satisfied. Lee *et al.* (1996 b) suggested that the phenotypic data for quantitative trait should be collected over a range of locations from within the base population of environments to identify putative QTL.

Lander and Botstein (1989) proposed selective genotyping or distribution extreme analysis for tagging QTL with significantly reduced time and expense of assaying molecular markers. Marker analysis is performed only on individuals in both extreme tails of the trait distribution (i.e. those with the lowest and highest values for the trait). If the allele frequency at any molecular marker locus differs significantly between the two extreme subpopulations, it is inferred that a QTL controlling the trait of interest is located near the marker. The disadvantage of this method for tagging QTL is that it is less efficient for determining individual effects than analyzing the whole population. A large number of positive or negative alleles of individuals in extreme subpopulations can confound the individual effects of any specific QTL. Additionally, it is not practical to use this method to investigate more than one trait, since the individuals with extreme phenotypes for one trait are unlikely to represent the extreme for other traits (Tanksley, 1993). This method was applied with the concept of bulked DNA (Michelmore *et al.,* 1991) and was reported to be efficiently used in the screening molecular marker association for several traits in soybean (Mansur *et al.,* 1993).

Three widely-used methods for detecting QTL are single-marker analysis, simple interval mapping and composite interval mapping (Liu, 1998; Tanksley, 1993). Single-marker analysis (also ‘single-point analysis or single-factor analysis) is the simplest methods used for detecting QTL associated with single markers. The statistical methods used for single-marker analysis include *t*-test, analysis of variance (ANOVA) and linear regression. Linear regression is the most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. The disadvantage of this method is the misclassification and underestimation due to crossover events between the marker locus and a QTL. A dense genetic linkage map and large population size would reduce these problems (Tanksley, 1993).

The simple interval mapping (SIM) method makes use of linkage maps and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously, instead of the analyzing single markers (Lander and Botstein, 1989). The use of linked markers for analysis compensates for recombination between the markers and the QTL, and is considered statistically more powerful compared to single-point analysis (Lander and Botstein, 1989; Liu, 1998).

More recently, composite interval mapping (CIM) has become popular for mapping QTL. This method combines interval mapping with linear regression and includes additional genetic markers in the statistical model in addition to an adjacent pair of linked markers for interval mapping (Zeng, 1994).

Marker-assisted selection (MAS)

The marker-assisted approaches are based on genetic linkage between a polymorphic marker locus and any locus controlling a trait of interest to the breeder (Cicek, 1997). Plant breeders often handle a large number of lines to select and phenotypic selection in always affected by environmental factors. Marker-assisted selection may greatly increase the efficient and effectiveness in plant breeding compared to conventional breeding methods (Henry, 1997). Once markers that are tightly linked to genes or QTL of interest have been identified, the breeder can facilitate specific molecular marker alleles as a diagnostic tool to identify plants carrying the genes or QTL, prior to field evaluation of large numbers of plants (Sanitchon, 2004). Currently, molecular markers have been used and become tools in marker-assisted selection. The molecular markers can be used for the development of particular genotypes as well as for improvement of a population by recurrent selection. The other uses of molecular markers in breeding programs are to optimize genetic resource conservation, marker choice of parents, identify favorable alleles, and develope genotypes accumulating such alleles. Some of these applications have already become routine. For example, marker-assisted backcrossing are used routinely in a large number of private and government programs. Other applications are for selection of quantitative traits which the main principles have been defined, but only a few experimental results are so far available (de Vienne *et al*., 2003).

MATERIALS AND METHODS

Plant materials

A population of recombinant inbred lines (RILs) was derived from a cross between two contrasting soybean lines ‘AGS292’ and ‘K3’. They are quite different in term of sugar and protein contents in the seed. ‘AGS292’ is a popular vegetable soybean cultivar with large seed and high sugar content. The experimental line ‘K3’ is a small-seeded grain soybean with high-protein content. It is a pure line derived by pedigree selection from the cross between G8891 and G7945. Both parents were obtained from the germplasm collection of the Asian Regional Center - Asian Vegetable Research and Development Center (ARC-AVRDC). The resulting progenies were advanced by a single seed descent method from F_2 plants until $F_{2:7}$ seeds were obtained. This population is considered as a RIL population to be grown and evaluated in the field. A total of 190 RILs were used in this study.

Field observation

The trait data of the parents and RIL population were observed in 2 trials in the field during late rainy season (August - November 2004) and dry season (November 2004 - February 2005) conducted in the field of the Tropical Vegetable Research Center of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand. The treatments were sown in single row plot each of 5.0 m long, with 0.60 m spacing between rows and 0.30 m between plants. Three seeds were sown in each hill and the seedlings were thinned down to 1 plant/hill at 10-14 days after germination. Each entry was replicated twice in a randomized complete block design. Days to flowering (DTF) was observed as the number of days from planting until a plant in the plot had an open first flower (R_1 stage as described by Fehr *et al.*, 1971). Seed weight (SW) was determined by weighing a 100 seed sample from each plot.

Evaluation of total soluble solid (TSS) content from brix reading

Brix reading to determine the total soluble solid content (TSS) of vegetable soybean seed at the $R_{6.5}$ growth stage was measured using a digital pocket refractometer PAL-1 (Atago Co., Japan). Juice samples were prepared following the method suggested by Wasee (1997). Briefly, 30 green pods were picked at the $R_{6.5}$ stage from 5 plants in each plot. The pods were kept in a styrofoam box filled with ice to minimize biochemical activity in the seed during evaluation. Twenty grams of fresh seed sample was taken from the green pods and ground to become fine soluble solid, then added with 20 ml of distilled deionized water and blended with blender (Moulinex, France) until it was homogeneous. The sample was filtrated with a muslin sheet and transferred to a 1.5 ml tube. The solution was centrifuged at 15000 rpm for 15 minutes, then approximately 0.3 ml of the clear supernatant was measured by a pocket refractometer.

Parental survey for marker polymorphism

SSR allele size profiling analysis of the parents was carried out at the Laboratory of Plant Genetics and Evolution, Hokkaido University using a DNA sequencer with 92 fluorescent-label primers following the method used by Abe *et al.* (2002). In addition, 69 none-label SSR primers were screened for polymorphism between the parents in this study. A total of 161 SSR loci were selected to survey the parents from an integrated soybean linkage map (Cregan *et al.*, 1999 a) in order to roughly cover 20 MLGs. The marker loci were considered for SSR analysis base on their allele size profile. The allele sizes with at least 8 base pair difference between the parents were chosen for SSR analysis in high resolution agarose (Cregan and Quigley, 1997). DNA was extracted from young leaves sampled from 20 parental plants following the method described by Doyle and Doyle (1990). The PCR reaction mixture contained 30 ng of total genomic DNA, 0.25 µM of 5’ and 3’ end primers, 200 µM of each dNTP, 0.5 units of *Taq* polymerase (TaKaRa, Japan), and 1x PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$) for a total volume of 20 µl. The PCR reactions were performed with a GeneAmp PCR System 9700

(Perkin Elmer/Applied Biosystems, Foster City, CA, USA) using the following program: 32 cycles at 94°C for 30 sec, 48°C for 30 sec, and 68°C for 30 sec. Following the amplifications, 1.5 µl of 6-FAM-labeled, 4.0 µl of HEX-labeled, and 2.0 µl of NED-labeled PCR products were combined and brought to a total volume of 20 µl by adding distilled water. An aliquot (1.5 µl) of the mixed PCR products combined with a loading buffer (1.5 µl) containing a ROX-labeled internal size standard (GeneScan-500) was denatured at 95°C for 5 min and then loaded and separated using an ABI 377 sequencer (Perkin Elmer/Applied Biosystems, Foster City, CA, USA). GeneScan software (version 3.1) was used to visualize the SSR variants and to estimate their sizes.

SSR analysis in RIL subpopulation

Out of 190 individual RILs the SSR analysis was assayed in 2 subpopulations by selective genotyping of 92 individual RILs (46 in each tail) with high and low TSS contents. DNA was extracted from young leaves from individual plant of the selective population following the modified method described by Rogers and Bendich (1994). The PCR reactions were performed as described in the parental survey, except for that none-label primers were used. PCR cycling was done in a MJ PTC-100 Programmable Thermal Controller. All PCR products were electrophoresed in 3-4% agarose gel . The gel was run in 0.5 x TBE buffer at 100 V for 45 minutes, stained with ethidium bromide, visualized under UV source, and photographed using Vilber Lourma TCX-20-M Gel Doc 2000 (Vilber Lourma, Cedex 1, France).

The RILs were scored base on marker genotypes of the parents. The RILs that possessed homozygous alleles derived from the vegetable soybean cultivar ‘AGS292’ were scored as A, the ones that possessed homozygous alleles from grain soybean experimental line ‘K3’ were scored as B, and the lines that possessed heterozygous alleles derived from both parents were scored as H.

Data analysis

Trait mean, normality index, correlation between three traits, and analysis of variance of each traits were determined using Statistical Analysis Systems version 6.12 (SAS Institute, 1990). Narrow-sense heritability was calculated from the variance component estimates based on plot basis according to Fehr (1987). Since the RILs are homozygous genetically, the dominant genetic variation (σ^2_d) can be neglected and the total genetic variance was considered comprising only additive genetic variation (σ^2_a). Thus, the heritability estimated from the RIL population is a narrow-sense one and can be determined from the following formula

$$h^2 = \sigma^2_g / [\sigma^2_g + (\sigma^2_{ge}/e) + (\sigma^2_e/re)]$$

Where h^2 represents heritability, σ^2_g is the genotypic variance component, σ^2_{ge} is the genotype x environment variation, σ^2_e is the experimental error variance, r is the number of replications and e is the number of environments. Standard errors associated with the heritability were estimated using approximation method as explained by Becker (1984).

Observed frequencies at marker loci were used to calculate χ^2 values to test for goodness-of-fit against the expected Mendelian ratio. Single-factor analysis of variance (General Linear Model - GLM) was used to associate polymorphic markers with SW, TSS and DTF (SAS Institute, 1990). Significant marker loci associated with each trait were identified when a marker at an individual environment was significant at $P \leq 0.05$ across two growing seasons. Significant marker loci were combined in a multiple-locus regression model (REG) to determine their combined effect. Interval mapping was not used because individual linkage groups were not fully saturated and many markers were unlinked (Lander and Botstein, 1989). Two-way analysis of variance was used to test for digenic interactions between markers significantly associated with each trait.

RESULTS

Variation of SW, TSS and DTF in the RILs

Mean, standard deviation, range, and parental values for 100 dry seed weight (SW), brix reading of total soluble solid content (TSS) of green seed, and days to flowering (DTF) after planting from the experiments conducted in late rainy and dry seasons of 2004/5 are presented in Table 1. For SW, ‘AGS292’ was higher than ‘K3’ in both environments, with the average values of 26.21 vs 8.42 g and 35.06 vs 12.63 g in late rainy and dry seasons, respectively. While in the RIL population, SW ranged from 12.20 to 28.67 g in late rainy season, and from 12.82 to 37.35 g in dry season. The experimental averages of SW in late rainy and dry seasons were 17.52 and 21.25 g, respectively. The averages of SW for ‘AGS292’ and ‘K3’ over two seasons were significantly different at 30.64 and 10.52 g. The range of SW in the RIL population was from 12.60 to 28.80 g with the mean falling between the two parents. SW of the parents were not significantly different from their RILs in late rainy season (Table 2). The effect of environment on SW was not significant in combined analysis (Table 4). Trangressive segregation in the population was also not significant

Brix reading of TSS content was higher in the vegetable soybean cultivar ‘AGS292’ than that of ‘K3’ in both environments, with the average values of 12.55 vs 8.35 and 9.95 vs 7.50% in late rainy and dry seasons, respectively. The readings in the RIL population ranged from 8.35 to 15.95% in late rainy season, and from 7.10 to 13.45% in dry season. The experimental average of brix readings were 11.58 in late rainy and 9.65% in dry season. TSS content of green seed averaged over two environments were 11.25% for ‘AGS292’ and 7.92% for ‘K3’, while the RIL population ranged from 8.30 to 13.70%, with the mean fell between the two parents (Table 1). The differences of TSS content between the parents and RIL population were not significant in both seasons (Table 2 and 3). However, trangressive segregation in the population was significant.

DTF of the vegetable soybean cultivar ‘AGS292’ was earlier than that of ‘K3’ in both environments, with the average of 25 vs 42 and 27 vs 40 days in late rainy and dry seasons, respectively. While in the RIL population, DTF ranged from 26 to 38 days in late rainy and from 26 to 40 days in dry season. The experimental average of DTF in both seasons were 31 and 33 days, respectively, while that in the RIL population ranged from 26 to 39 days, with the mean falling between the two parents. The average of DTF over two environments were 26 days for ‘AGS292’ and 41 days for ‘K3’ (Table 1).

The frequency distributions of SW, TSS content, and DTF among the RILs population, averaged over two seasons were continuous (Figure 1, 2 and 3), indicating that these traits are quantitatively inherited. Normality test of trait frequency distribution based on W-test method (Shapiro and Wilk, 1965) showed that the observations were normally distributed *($P > 0.10$).*

Table 1 Range and Mean±SD of 100 seed weight (g), brix reading (%), and days to flowering of 190 vegetable soybean RILs and their parents grown in late rainy and dry seasons of 2004, and combined over seasons. The narrow-sense heritabilities±SD were presented in the right column.

Season	Traits [a]	RIL		Parents		H^2 [b]
		Range	Mean	AGS292	K3	
Late Rainy	SW	12.20-28.67	17.52±2.40	26.21	8.42	0.754±0.093
	TSS	8.35-15.95	11.58±1.39	12.55	8.35	0.652±0.092
	DTF	26-38	31±2.58	25	42	0.942±0.100
Dry	SW	12.82-37.35	21.25±3.32	35.06	12.63	0.905±0.098
	TSS	7.10-13.45	9.65±1.04	9.95	7.50	0.582±0.092
	DTF	26-40	33±3.04	27	40	0.916±0.098
Combined	SW	12.60-28.80	19.38±2.57	30.64	10.52	0.620±0.065
	TSS	8.30-13.70	10.64±1.01	11.25	7.92	0.455±0.052
	DTF	26-39	32±2.61	26	41	0.291±0.032

[a] SW = 100 seed weight in grams, TSS = brix reading (percentage of total soluble solid content), DTF = Days to flowering

[b] Heritability in the narrow-sense

Table 2 ANOVA of 100 seed weight, brix reading, and days to flowering of parents and 190 vegetable soybean RILs grown in late rainy season of 2004.

Sources	Df	Mean square [a]		
		SW	TSS	DTF
Blocks	1	452.29	0.06	1.63
Genotypes	191	13.03**	3.91**	14.84**
Parents vs RILs	1	0.17	5.14	20.70**
Between Parents	1	316.31**	17.64**	289.00**
Between RILs	189	11.49**	3.83**	13.40**
Error	191	3.20	1.60	0.79

[a] SW = 100 seed weight, TSS = Brix reading (percentage of total soluble solid content), DTF = Days to flowering

** = Significantly different at the 0.01 probability level.

Table 3 ANOVA of 100 seed weight, brix reading, and days to flowering of parents and 190 vegetable soybean RILs grown in dry season of 2004.

Sources	Df	Mean square [a]		
		SW	TSS	DTF
Blocks	1	0.50	14.22	0.00
Genotypes	191	4700.74**	2.18**	19.09**
Parents vs RILs	1	26.67**	3.42	4.68**
Between Parents	1	503.17**	6.00*	156.25**
Between RILs	189	22.07**	2.15**	18.44**
Error	191	2.24	1.12	1.60

[a] SW = 100 seed weight, TSS = Brix reading (percentage of total soluble solid content), DTF = Days to flowering

*, ** = Significantly different at the 0.05 and 0.01 probability levels, respectively.

Table 4 Combined ANOVA of 100 seed weight, brix reading, and days to flowering of parents and 190 vegetable soybean RILs grown in late rainy and dry seasons of 2004.

Sources	df	Mean square [a]		
		SW	TSS	DTF
Environments (E)	1	2706.07	716.69**	396.75**
Blocks (Environment)	2	226.40**	7.14**	0.81**
Genotypes (G)	191	30.38 **	4.20**	29.31**
Parents vs RILs	1	11.32*	8.48*	8.82**
Between Parents	1	808.90**	22.11**	242.00**
Between RILs	189	26.36**	4.08**	11.76**
Genotype x Environment	191	7.26**	1.89**	4.62**
RIL x E	189	7.20**	1.90**	11.00**
Residual G x E	2	226.31**	7.23**	0.93
Pooled error	382	2.72	1.36	1.20

[a] SW = 100 seed weight, TSS = Brix reading (percentage of total soluble solid content), DTF = Days to flowering

*, ** = Significantly different at the 0.05 and 0.01 probability levels, respectively.

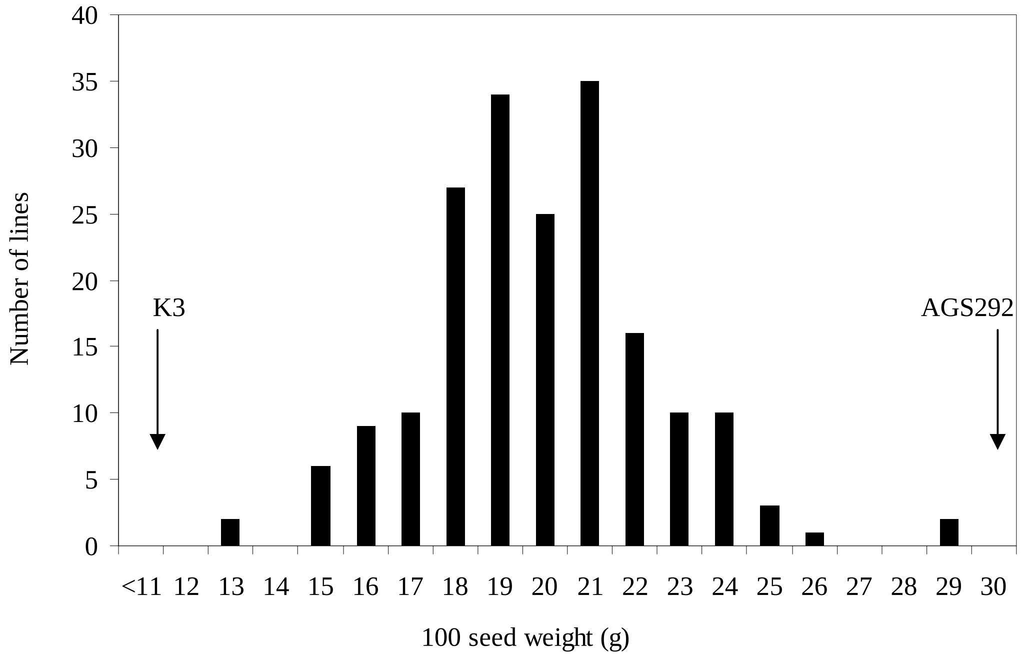

Figure 1 Frequency distribution of 100 seed weight (g) in 190 vegetable soybean RILs averaged across two seasons. Mean parental values of ‘AGS292’ and ‘K3’ are indicated by arrows.

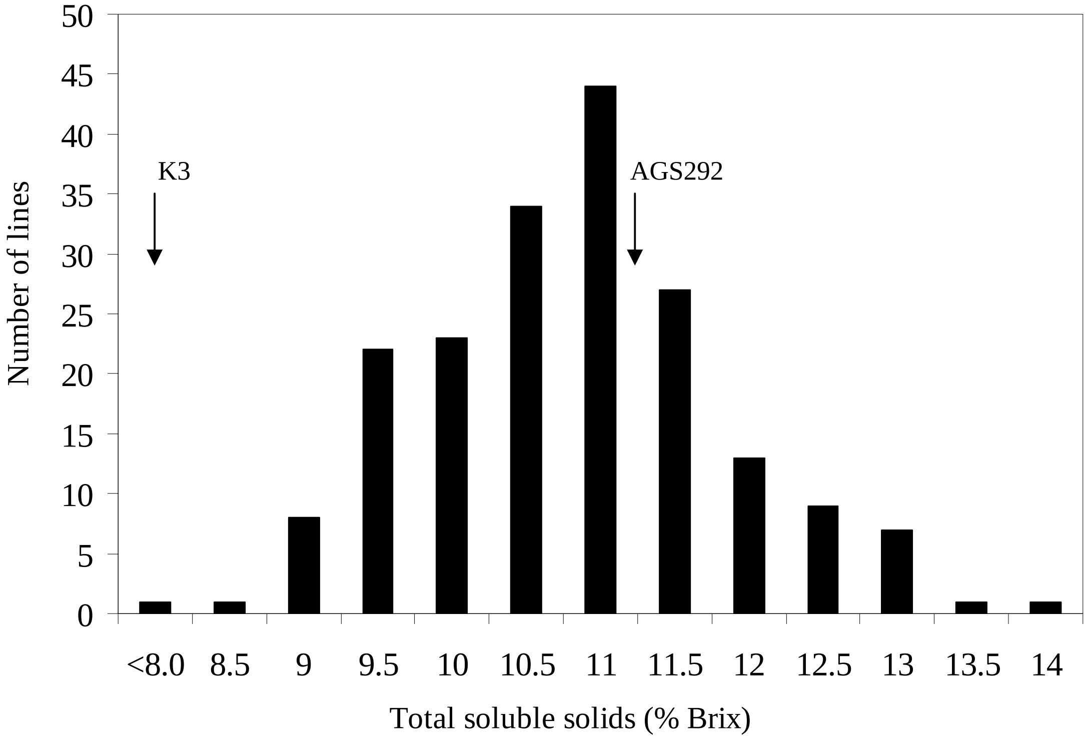

Figure 2 Frequency distribution of brix reading (total soluble solid content) in 190 vegetable soybean RILs averaged across two seasons. Mean parental values of 'AGS292' and 'K3' are indicated by arrows.

Figure 3 Frequency distribution of days to flowering in 190 vegetable soybean RILs averaged across two seasons. Mean parental values of 'AGS292' and 'K3' are indicated by arrows.

Narrow-sense heritability estimates

Narrow-sense heritability was estimated using variance components from the analysis of the RIL population in each season as well as combined analysis and presented in Table 1. The heritability for SW was less consistent in both seasons, giving the estimates of 0.754 and 0.905 from late rainy and dry seasons, respectively, with the combined heritability of 0.620. The heritability of TSS content for late rainy, dry, and combined seasons were 0.652, 0.582 and 0.455, respectively. The highest heritable values for DTF in late rainy and dry seasons were 0.942 and, 0.916 respectively. While the combined estimate over both seasons was low (0.291). This is the case because of significant interaction detected between genotypes and the growing seasons (Table 4).

Correlation between SW, TSS and DTF

The correlations between SW, TSS content and DTF in the same and different seasons are given in Table 5. The correlation between DTF of the same RIL grown in two seasons was the highest (r = 0.733), while the correlation between TSS was the lowest (r = 0.389). DTF was negatively correlated with SW and TSS in both seasons, as well as in combined analysis (Figure 4 and 5).

Table 5 Correlation between 100 seed weight, brix reading, and days to flowering of 190 vegetable soybean RILs grown in late rainy and dry seasons of 2004.

Trait [a]	SW late rainy	SW dry season	TSS late rainy	TSS dry season	DTF late rainy
SW dry season	0.647**				
TSS late rainy	0.133	0.004			
TSS dry season	0.040	0.002	0.389**		
DTF late rainy	-0.371**	-0.405**	-0.464**	-0.259**	
DTF dry season	-0.217**	-0.390**	-0.229**	-0.299**	0.733**

[a] SW = 100 seed weight, TSS = Brix reading (percentage of total soluble solid content), DTF = Days to flowering

** = Significant at the 0.01 probability level.

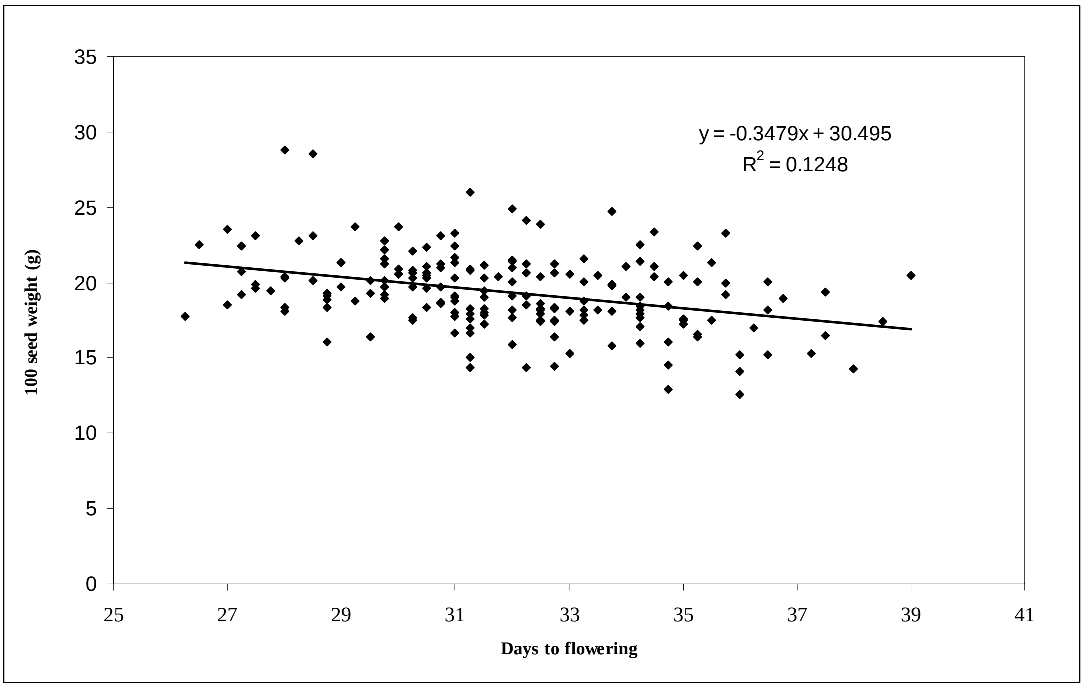

Figure 4 Correlation between days to flowering and 100 seed weight in 190 vegetable soybean RILs combined across two seasons.

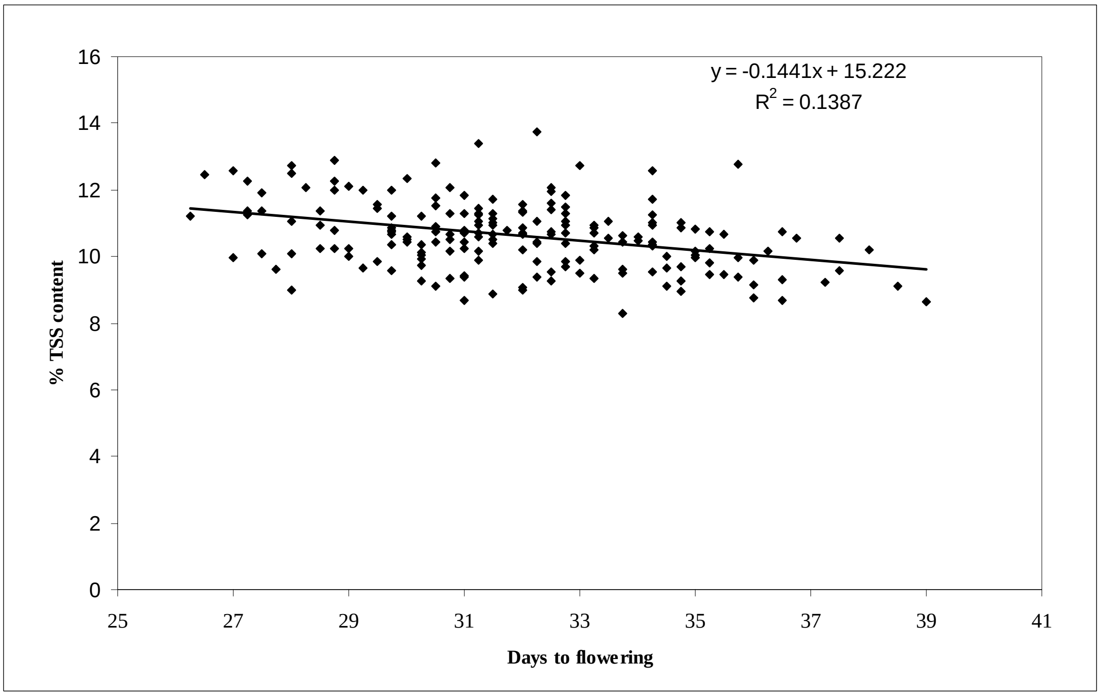

Figure 5 Correlation between days to flowering and TSS content in 190 vegetable soybean RILs combined across two seasons

SSR analysis in RIL subpopulation

The SSR allele size profiles of the parents were analyzed at 161 marker loci. Sixty-three polymorphic markers between the parents were used in this study. Allele size profiles of the parents at each marker locus were presented in Table 6. The size ranged from 110 to 294 base pairs and 76 to 302 base pairs at Satt055 and Satt394 for vegetable soybean cultivar ‘AGS292’ and grain soybean experimental line ‘K3’, respectively. The SSR analysis was assayed for linkage relationships with SW, TSS and DTF in selective genotyping of 92 RILs. The individuals having extremely high and low values of TSS were selected to form two subpopulations. A total of 24% (46 out of 190 individuals) in each tail of the TSS content distribution were genotyped.

The chi-squared values for goodness-of-fit test for each marker loci were presented in Table 7. The expected ratios of marker phenotypes were 1:1 in the RIL populations. Most of the marker loci mapped in the 92 RILs corresponded with Medelian segregation of 1:1. However, there were 11 marker loci in MLG A2 (Satt341 and Satt589), MLG C2 (Satt277, Satt307 and Satt316), MLG D1b+W (Sat_069), MLG F (Satt425), MLG G (Satt394), MLG J (Satt132), MLG L (Satt166), and MLG O (Satt477) that showed significant deviation from their expected ratios.

Table 6 The SSR allele size profiling (base pairs) of vegetable soybean cultivar 'AGS292' and grain soybean experimental line 'K3'.

Locus	MLG [a]	'AGS292'	'K3'
Satt236	A1	223	238
Satt197	B1	134	182
Satt063	B2	127	106
Satt565	C1	163	190
Satt277	C2	226	180
Satt307	C2	179	168
Satt316	C2	163	204
Satt184	D1a+Q	169	188
Satt005	D1b+W	151	163
Satt546	D1b+W	218	248
Satt350	D1b+W	242	257
Satt041	D1b+W	181	225
Satt141	D1b+W	162	141
Satt157	D1b+W	223	211
Satt045	E	124	137
Satt212	E	139	152
Satt335	F	151	166
Satt516	F	221	255
Satt288	G	246	199
Satt394	G	294	302
Satt568	H	237	246
Satt253	H	145	133
Satt367	I	220	196
Satt354	I	182	235
Satt431	J	224	205
Satt183	J	242	250
Satt055	K	110	76
Satt373	L	223	268
Satt463	M	126	114
Satt009	N	218	181
Satt387	N	201	210
Satt262	O	255	240
Satt477	O	150	137

[a] MLG = Molecular linkage group

Table 7 Segregation of SSR markers in soybean RIL population derived from the cross between 'AGS292' and 'K3' as evaluated by contingency chi-squared test for goodness-of-fit.

Locus	MLG [a]	Observed frequency [b]		χ^2	*P*
		A	B	(1A:1B)	
Satt236	A1	43	46	0.10	0.75
Satt511	A1	43	51	0.68	0.41
GMENOD2B	A2	44	48	0.17	0.68
Satt187	A2	39	55	2.72	0.10
Satt341	A2	37	57	4.26	0.04
Satt589	A2	36	58	5.15	0.02
Satt197	B1	48	43	0.27	0.60
Satt063	B2	43	47	0.18	0.67
Satt565	C1	43	47	0.18	0.67
Satt277	C2	33	56	5.94	0.01
Satt307	C2	30	53	6.37	0.01
Satt316	C2	31	52	5.31	0.02
Satt147	D1a+Q	43	51	0.68	0.41
Satt179	D1a+Q	44	39	0.30	0.58
Satt184	D1a+Q	52	43	0.87	0.35
Sat_069	D1b+W	58	36	5.15	0.02
Sat_135	D1b+W	47	46	0.01	0.92
Satt141	D1b+W	41	44	0.11	0.74
Satt157	D1b+W	44	46	0.04	0.83
Satt189	D1b+W	42	48	0.40	0.53
Satt282	D1b+W	40	52	1.57	0.21
Satt350	D1b+W	44	42	0.05	0.83
Satt412	D1b+W	44	46	0.04	0.83
Satt506	D1b+W	40	52	1.57	0.21
Satt546	D1b+W	51	40	1.33	0.25
Satt604	D1b+W	47	47	0.00	1.00
Satt372	D2	51	43	0.68	0.41
Satt458	D2	53	39	2.13	0.14
Satt486	D2	50	43	0.53	0.47
Satt045	E	43	43	0.00	1.00
Satt212	E	44	43	0.01	0.91
Satt230	E	44	49	0.27	0.60
Satt231	E	44	45	0.01	0.92

[a] MLG = Molecular linkage group

[b] A = homozygous alleles from 'AGS292' genome, B = homozygous alleles from 'K3' genome

Table 7 (cont'd)

Locus	MLG [a]	Observed frequency [b]		χ^2	*P*
		A	B	(1A:1B)	
Satt553	E	48	46	0.04	0.84
Satt335	F	46	45	0.01	0.92
Satt425	F	56	38	3.4	0.06
Satt510	F	50	44	0.38	0.54
Satt516	F	46	42	0.18	0.67
Satt288	G	37	44	0.60	0.44
Satt394	G	56	36	4.35	0.04
Satt253	H	45	49	0.17	0.68
Satt568	H	43	50	0.53	0.47
Sat_105	I	49	41	0.71	0.40
Satt049	I	48	41	0.55	0.46
Satt239	I	51	43	0.68	0.41
Satt354	I	44	42	0.05	0.83
Satt367	I	42	46	0.18	0.67
Satt132	J	36	58	5.15	0.02
Satt183	J	50	44	0.38	0.54
Satt431	J	44	47	0.10	0.75
Satt055	K	47	43	0.18	0.67
Satt166	L	32	54	5.63	0.02
Satt229	L	41	50	0.89	0.35
Satt284	L	51	43	0.68	0.41
Satt373	L	43	50	0.53	0.47
Satt523	L	51	40	0.89	0.34
Satt463	M	42	47	0.28	0.60
Satt540	M	42	52	1.06	0.30
Satt567	M	51	43	0.68	0.41
Satt009	N	47	43	0.18	0.67
Satt387	N	49	46	0.10	0.76
Satt262	O	47	43	0.18	0.67
Satt477	O	57	27	10.71	0.00

[a] MLG = Molecular linkage group

[b] A = homozygous alleles from 'AGS292' genome, B = homozygous alleles from 'K3' genome

Seed-weight QTL

Fifteen marker loci were identified by single-factor ANOVA as being associated with SW in the combined analysis (Table 8). They were located on ten independent MLGs (B1, C1, D2, E, F, I, J, L, N and O). Three markers were assigned on MLG D2, two markers each on MLG F, I and L, while the rest seven markers on MLG B1, C1, E, J, N and O were not linked with the other markers. However, one marker locus on MLG I and four marker loci on MLG D1b+W were not identified in the combined analysis. Six marker loci were identified in both seasons. Fourteen marker loci Sat_069, Satt189, Satt412, Satt506, Satt372, Satt458, Satt486, Satt335, Satt510, Sat_105, Satt239, Satt354, Satt229 and Satt262 were each detected in only one season. The combined data for SW revealed 15 linked loci on 10 MLG. Each locus explained 4.5 to 23.5% of the phenotypic variation. Multiple-locus regression on Table 9 identified seven markers that were significantly associated with SW in rainy season. In the combined analysis, seven of the eight marker loci marginally contributed 3.1 to 26.3 % of the variation after accounting for the other marker loci in the model and altogether explained 65.4% of the total variation. The small-seeded parent grain soybean experimental line ‘K3’ contributed alleles for small seed at 14 marker loci (Satt197, Satt565, Satt372, Satt458, Satt486, Satt045, Satt335, Satt510, Sat_105, Satt354, Satt166, Satt229, Satt009 and Satt262) and large size at one locus (Satt431) (Table 8). The large-seeded parent vegetable soybean cultivar ‘AGS292’ contributed alleles for small size at one locus (Satt431) and alleles for large size at 14 marker loci (Satt197, Satt565, Satt372, Satt458, Satt486, Satt045, Satt335, Satt510, Sat_105, Satt354, Satt166, Satt229, Satt009 and Satt262) and small size at one locus (Satt431). Two-way analysis of variance revealed no significant epistasis interactions between QTL for SW in this population across the two environments.

QTL associated with TSS content

A total of fourteen putative markers were found associating with TSS content in the combined analysis (Table 10). They were located on five independent MLGs (A1, D1a+Q, D1b+W, D2, and I). Eight markers were assigned on MLG D1b+W, two markers each on MLG A1, and D2, while the rest two markers on MLG D1a+Q and I were not linked with the other markers. However, one marker locus on MLG D1b+W was not identified in the combined analysis. Ten marker loci were identified to be significant in both seasons. Five marker loci Sat_069, Satt189, Satt546, Satt458 and Satt486, were each detected in only one season. In the combined analysis, the marker loci individually explained 5.1 to 21.7% of the phenotypic variation for TSS content. Three markers identified using multiple-locus regression were significantly associated with TSS content at one environment (Table 11). These marker loci contributed 6.8 to 21.7% of the variation after accounting for the other marker loci in the model and together explained 36.6% of the total variation for TSS content combined across environments. The grain soybean parent ‘K3’ contributed alleles for low TSS content in fresh seed at 12 marker loci (Satt147, Sat_135, Satt141, Satt189, Satt350, Satt412, Satt506, Satt546, Satt604, Satt458, Satt486, and Satt049) and high TSS content at two loci (Satt236 and Satt511) (Table 10). The high TSS content parent vegetable soybean cultivar ‘AGS292’ contributed alleles for low TSS content at two loci (Satt236 and Satt511) and alleles for high TSS content at 12 marker loci (Satt147, Sat_135, Satt141, Satt189, Satt350, Satt412, Satt506, Satt546, Satt604, Satt458, Satt486, and Satt049). No significant epistatic interaction was detected among the marker loci in the two-way analysis of variance.

QTL associated with days to flowering

Base on SF-ANOVA, 10 marker loci were identified as being associated with DTF in the combined analysis (Table 12). They were located on five independent MLGs (A2, D1b+W, J, L, and O). Five markers were assigned on MLG D1b+W, two markers on MLG J, while the rest three markers on MLG A2, L and O were not linked with other markers. However, 11 marker loci on MLG D1a+Q, D1b+W, D2, E, G, H and I were not identified in combined analysis. Seven of 21 of the marker loci were significant in two environments (Sat_135, Satt189, Satt350, Satt132, Satt431, Satt229, and Satt262). Fourteen marker loci Satt187 Satt184, Satt141, Satt412, Satt506, Satt604, Satt458, Satt486, Satt230, Satt288, Satt568, Sat_105, Satt049 and Satt354 were each detected in only one environment. The marker loci individually explained 4.5 to 26.6% of the phenotypic variation for DTF combined across seasons. Multiple-locus regression identified six markers that were significantly associated with DTF at one environment (Table 13). Four of the six marker loci contributed 4.4 to 26.0 % of the variation after accounting for the other marker loci in the model and altogether explained 51.6% of the total variation for DTF combined across environments. The longer flowering date parent ‘K3’ contributed alleles for longer flowering date at 10 marker loci (Satt187, Sat_135, Satt141, Satt189, Satt350, Satt412, Satt132, Satt431, Satt229, and Satt262) (Table 12). The earliness flowering date parent ‘AGS292’ contributed alleles for shorter flowering date at the same marker loci.

Table 8 Marker loci significantly associated with 100 seed weight (g) of 92 soybean RILs from the cross between ‘AGS292’ and ‘K3’ grown in late rainy and dry season 2004, and combined over two seasons.

		Environment				Combined			
Locus	MLG [a]	Rainy 04		Dry 04/05		*P*	R^2 (%)	Allelic mean (SW) [b]	
		P	R^2 (%)	*P*	R^2 (%)			AGS292	K3
Satt197	B1	0.0082	7.4	0.0359	4.7	0.0123	6.7	19.9	18.5
Satt565	C1	0.0182	6.0	0.0379	4.7	0.0184	6.0	20.0	18.7
Sat_069	D1b+W	0.0317	4.9	NS		NS [c]			
Satt189	D1b+W	0.0278	5.4	NS		NS			
Satt412	D1b+W	0.0384	4.8	NS		NS			
Satt506	D1b+W	0.0409	4.6	NS		NS			
Satt372	D2	0.0001	14.7	NS		0.0145	6.3	19.9	18.5
Satt458	D2	0.0012	11.0	NS		0.0402	4.6	19.8	18.5
Satt486	D2	0.0003	13.6	NS		0.0072	7.5	20.0	18.4
Satt045	E	0.0383	4.9	0.0177	6.4	0.0153	6.6	19.9	18.4
Satt335	F	0.0170	6.1	NS		0.0404	4.5	19.8	18.6
Satt510	F	0.0097	7.0	NS		0.0171	6.0	19.9	18.5
Sat_105	I	NS		0.0283	5.4	0.0334	5.0	19.8	18.6
Satt239	I	NS		0.0328	4.9	NS			
Satt354	I	NS		0.0069	8.2	0.0217	6.0	19.9	18.5
Satt431	J	0.0092	7.2	<.0001	17.1	0.0002	14.6	18.2	20.3
Satt166	L	<.0001	19.2	<.0001	20.7	<.0001	23.5	21.1	18.4
Satt229	L	NS		0.0095	7.3	0.0166	6.3	20.0	18.6
Satt009	N	0.0051	8.7	0.0055	8.4	0.0027	9.8	20.1	18.4
Satt262	O	0.0258	5.5	NS		0.0300	5.2	19.9	18.6

[a] MLG = Molecular linkage group

[b] SW = 100 seed weight

[c] NS = not significant

Table 9 Marker loci significantly associated with 100 seed weight (g) of 92 soybean RILs from the cross between 'AGS292' and 'K3' grown in late rainy and dry season 2004, and combined over seasons using multiple-locus regression.

Locus	MLG [a]	Environment					
		Rainy 04		Dry 04/05		Combined	
		P	Partial R^2 (%)	*P*	Partial R^2 (%)	*P*	Partial R^2 (%)
Satt197	B1	0.0387	3.2	NS [b]		0.0464	3.1
Satt189	D1b+W	0.0035	8.6	NS		NS	
Satt372	D2	0.0016	15.4	NS		0.0002	15.6
Satt045	E	0.0246	3.6	0.0034	8.6	0.0288	3.3
Satt510	F	0.0067	6.1	NS		0.0117	5.4
Satt431	J	0.0266	4.5	0.0025	10.4	0.0034	8.2
Satt166	L	<.0001	22.4	<.0001	22.8	<.0001	26.3
Satt229	L	NS		NS		0.0304	3.5
Total variation explained			63.8		41.8		65.4

[a] MLG = Molecular linkage group

[b] NS = not significant

Table 10 Marker loci significantly associated with brix reading of total soluble solid content (%TSS) of 92 soybean RILs from the cross between 'AGS292' and 'K3' grown in late rainy and dry season 2004, and combined over seasons.

Locus	MLG[a]	Environment				Combined			
		Rainy 04		Dry 04/05		*P*	R^2 (%)	Allelic mean (% TSS)[b]	
		P	R^2 (%)	*P*	R^2 (%)			AGS292	K3
Satt236	A1	<.0001	17.5	<.0001	17.4	<.0001	21.7	9.9	11.2
Satt511	A1	0.0031	9.1	0.0024	9.6	0.0008	11.6	10.1	11.1
Satt147	D1a+Q	0.0016	10.3	0.0006	12.2	0.0002	13.7	11.2	10.1
Sat_069	D1b+W	NS[c]		0.0244	5.4	NS			
Sat_135	D1b+W	0.0268	5.3	0.0057	8.1	0.0064	7.9	11.0	10.2
Satt141	D1b+W	0.0096	7.6	0.0148	6.8	0.0045	9.1	11.1	10.2
Satt189	D1b+W	NS		0.0406	4.7	0.0253	5.6	10.9	10.2
Satt350	D1b+W	0.0276	5.6	0.0128	7.1	0.0092	7.8	11.0	10.2
Satt412	D1b+W	0.0155	6.5	0.0248	5.6	0.0086	7.6	11.0	10.2
Satt506	D1b+W	0.0240	5.5	0.0418	4.5	0.0150	6.4	11.0	10.3
Satt546	D1b+W	NS		0.0217	5.6	0.0251	5.4	10.9	10.2
Satt604	D1b+W	0.0023	9.7	0.0284	5.1	0.0026	9.4	11.1	10.2
Satt458	D2	0.0193	5.9	NS		0.0309	5.1	10.9	10.3
Satt486	D2	0.0020	10.0	NS		0.0053	8.2	11.0	10.2
Satt049	I	0.0404	4.7	0.0470	4.5	0.0238	5.7	10.9	10.3

[a] MLG = Molecular linkage group

[b] TSS = Brix reading (percentage of total soluble solid content)

[c] NS = not significant

Table 11 Marker loci significantly associated with TSS content of 92 soybean RILs from the cross between 'AGS292' and 'K3' grown in late rainy and dry season 2004, and combined over seasons using multiple-locus regression.

Locus	MLG [a]	Environment				Combined	
		Rainy 04		Dry 04/05			
		P	Partial R^2 (%)	*P*	Partial R^2 (%)	*P*	Partial R^2 (%)
Satt236	A1	0.0007	15.7	0.0011	15.1	<.0001	21.7
Satt147	D1a+Q	0.0269	5.6	0.0092	8.5	0.0124	6.8
Satt049	I	0.0142	7.3	NS [b]		0.0088	8.1
Total variation explained			28.6		23.6		36.6

[a] MLG = Molecular linkage group

[b] NS = not significant

Table 12 Marker loci significantly associated with days to flowering (DTF) of 92 soybean RILs from the cross between ‘AGS292’ and ‘K3’ grown in late rainy and dry season 2004, and combined over seasons.

Locus	MLG [a]	Environment				Combined			
		Rainy 04		Dry 04/05		*P*	R^2	Allelic mean (DTF) [b]	
		P	R^2 (%)	*P*	R^2 (%)		(%)	AGS 292	K3
Satt187	A2	NS[c]		0.0463	4.2	0.0401	4.5	31.1	32.4
Satt184	D1a+Q	0.0279	5.2	NS		NS			
Sat_135	D1b+W	0.0135	6.5	0.0414	4.5	0.0175	6.1	31.2	32.7
Satt141	D1b+W	0.0238	5.9	NS		0.0291	5.5	31.0	32.5
Satt189	D1b+W	0.0267	5.5	0.0413	4.6	0.0242	5.6	31.2	32.0
Satt350	D1b+W	0.0200	6.5	0.0404	4.8	0.0200	6.3	31.1	32.6
Satt412	D1b+W	0.0084	7.6	NS		0.0173	6.3	31.0	32.5
Satt506	D1b+W	0.0290	5.2	NS		NS			
Satt604	D1b+W	0.0476	4.2	NS		NS			
Satt458	D2	0.0361	4.8	NS		NS			
Satt486	D2	0.0115	6.8	NS		NS			
Satt230	E	0.0122	6.7	NS		NS			
Satt288	G	0.0320	5.5	NS		NS			
Satt568	H	0.0215	5.7	NS		NS			
Sat_105	I	0.0317	5.1	NS		NS			
Satt049	I	0.0193	6.1	NS		NS			
Satt354	I	0.0241	5.8	NS		NS			
Satt132	J	0.0004	12.8	0.0001	13.9	0.0001	15.1	30.4	32.8
Satt431	J	<.0001	18.8	<.0001	27.4	<.0001	26.6	30.4	33.4
Satt229	L	0.0033	9.31	0.0041	8.9	0.0021	10.2	30.8	32.7
Satt262	O	0.0167	6.3	0.0317	5.1	0.0163	6.4	31.2	32.7

[a] MLG = Molecular linkage group

[b] DTF = Days to flowering

[c] NS = not significant

Table 13 Marker loci significantly associated with days to flowering of 92 soybean RILs from the cross between ‘AGS292’ and ‘K3’ grown in late rainy and dry season 2004, and combined over seasons using multiple-locus regression.

Locus	MLG [a]	Environment					
		Rainy 04		Dry 04/05		Combined	
		P	Partial R^2 (%)	*P*	Partial R^2 (%)	*P*	Partial R^2 (%)
Satt350	D1b+W	0.0170	4.7	NS		NS	
Satt412	D1b+W	NS [b]		NS		0.0079	6.2
Satt568	H	0.0022	10.0	NS		NS	
Sat_105	I	0.0024	11.6	NS		NS	
Satt132	J	0.0155	5.4	0.0240	4.6	0.0205	4.4
Satt431	J	0.0011	16.2	<.0001	26.5	<.0001	26.0
Satt229	L	0.0019	17.7	0.0040	8.1	0.0001	15.0
Total variation explained			65.6		39.2		51.6

[a] MLG = Molecular linkage group

[b] NS = not significant

DISCUSSION

In this study, total soluble solid content in green seed of individual RILs were determined by brix reading. Brix reading in fresh seed samples reflects the total sugar content. Chandler and Fehr (2000) reported that total sugar is not routinely measured in the soybean breeding program because the current methods of analysis are too time consuming for assessment large number of genotypes. The brix reading method using pocket refractometer would be more practical and yet sufficiently sensitive to predict the difference among genotypes for total sugar content in vegetable soybean.

The RILs demonstrated significant positive transgressive segregation for TSS content ($P<0.05$). This observation supports the previous study that the vegetable soybean cultivar ‘AGS292’ contained high TSS content (Wasee, 1997). However, the alleles with high TSS content in the RIL population came from grain soybean line ‘K3’ due to those alleles carrying high TSS content at locus Satt236 (Figure 2). Moreover, brix values of the parents were not significantly different from that of the recombinant inbred lines in both seasons (Table 2 and 3) and yet showed normal distribution.

The differences between parents and between recombinant inbred lines were significant in all three traits in both seasons (Table 4). This indicated that the traits were diverse among the parents and inherited the variation well to their progenies. The effect of environment on SW was not significant in this study, meaning that this trait is rather stable against the tested environments. However, the interaction between genotype and environment was highly significant. Both environments and interaction between genotypes and environments of DTF were significant.

In grain soybean, Maughan *et al*. (2000) reported that a broad-sense heritability of sucrose content was rather high at 0.82. A high estimate was also detected in sugar content of grain soybean (Openshaw and Hadley, 1981). In our experiment SW, TSS content and DTF in a single season were highly heritable. The heritabilities reduced slightly when the combined data were used. This is the case in the tropical countries where vegetable soybean can be grown two seasons per year. The environmental difference between two seasons in the tropic is normally higher than that in the temperate countries where the crop is grown in a relatively fixed date of planting and environmental regimes each year. From the results of this study, it is recommended that vegetable soybean grown in the tropical be selected particularly for the intended growing season. A superior selection method is a single seed descent across wet and dry seasons. Selection for disease resistance and some qualitative characters can be done from earlier generations to reduce the number of RILs for more intensive selection in later generations. Then, vegetable soybean lines with high grain quality and desirable flowering date in each season can be finally identified.

Correlation between the same trait grown in different seasons was shown in Table 5. Although the correlation coefficients were significant in all traits, the magnitude in TSS content was the lowest. This implied that sweetness in vegetable soybeans is rather environmental dependent. On the other hand, SW and DTF were highly correlated in different seasons implying that these two traits were rather consistent across the environments. Moreover, DTF was negatively correlated with SW and TSS in both seasons. Similar negative correlation between DTF and SW was reported by Mansur *et al*. (1996). SW was also reported to correlate negatively with maturity (Mian *et al*., 1996). The poor green seed quality seemed to correlate with longer flowering date which is the trait of ‘K3’. This field soybean line contained high protein content which expressed strong negative correlation with oil content and sugar content (Openshaw and Hadley, 1984; Hymowitz *et al*., 1972; Tajuddin *et al*., 2003).

The results of relationship among SW, TSS content and DTF in the RIL population obtained from this study will be useful for further study on grain quality in vegetable soybean, which there is a little or no information available at the moment.

Several putative QTL associated with SW were reported by Main *et al*. (1996), Hoeck *et al*. (2003) and Zhang *et al*. (2004). Hoeck *et al*. (2003) identified SSR markers associated with QTL for seed weight in soybean across populations and environments and found that SSR marker loci at Satt045, Satt565, Satt431 and

Satt009 were associated with seed size. These markers were detected in the present investigation as well. They also compared the cost of phenotypic selection and marker-assisted selection for seed size and suggested that phenotypic selection was more effective and less expensive. A major QTL for seed size on MLG L at SSR marker locus Satt166 was reported by Hyten *et al.* (2004). The same SSR marker locus as major seed weight QTL was also detected in this study,

QTL for seed-weight on MLG B1, C1, E, J, L and O were detected in two environments. The consistency level of the QTL across environments for SW was high (Table 8) owing to high heritability of this trait. Similar high consistency of QTL for SW across environments were reported by Mian *et al.* (1996) and Hoeck *et al.* (2003). Individually, the 15 marker loci explained between 4.5 and 23.5% of the variation in SW. Multiple-locus regression identified seven markers that were significantly associated with SW combined over seasons (Table 9). The narrow-sense heritability of SW in the population, using the combined data was only 62.0 % (Table 1). Thus, most of the variation could be explained by the seven marker loci. The 'AGS292' alleles contributed to larger SW at 14 marker loci, while the 'K3' alleles did so at Satt431 (Table 8). Thus, both parents potentially can contribute to larger or smaller SW in the progeny.

TSS content was the only grain quality trait to demonstrate transgressive segregation. Transgressive segregation for TSS content was expected in the RIL population because of the diversity of the two parents for this trait. There was a trend for the parent with the greater trait value to contribute more QTL alleles with positive effects (Table 10). A QTL for TSS content on MLG A1 was a major QTL at which experimental line 'K3' allele increased TSS content while SSR marker loci on MLG D1a+Q, D1b+W,D2 and I were QTL at which the vegetable soybean cultivar 'AGS292' increased TSS content. The 12 QTL alleles that increased TSS content came from vegetable soybean cultivar 'AGS292' which had a mean TSS content of 9.95%, which is greater than the mean for experimental line 'K3' (7.92%) in combined analysis over two seasons (Table 1). In only two alleles of the 'K3' that increased TSS content.

Individually, 14 markers explained between 5.4 and 21.7% of the variation in TSS content combined across seasons (Table 10). Multiple-locus regression identified three markers that were significantly associated with TSS content at one environment (Table 11). The heritability of TSS content in the population, using the combined data was 45.5 % (Table 1). Thus, the variation in TSS could be explained by the these marker loci. The 'K3' alleles contributed to higher TSS content at two of the 14 loci, while the 'AGS292' alleles did so at the remaining 12 loci (Table 10). Therefore, the parent 'K3' can potentially contribute to a higher TSS content of the progeny, and this information is derived from the positive transgressive segregation observed in this population. Tests for digenic epistatic interactions among significant marker loci for TSS content were all non-significant.

Ten of 15 markers were identified in two environments (Table 10). Thus, the level of consistency of the QTL for TSS content across environments was considered rather high. Some QTL were easily influenced by environment. The same phenomenon of QTL for seed protein and oil contents were reported by Lee *et al*. (1996 b) and Brummer *et al*. (1997). With respect to the environmental effect, Brummer *et al*.(1997) differentiated two types of QTL identified as (1) environmentally stable QTL, which were detected in both environments, and (2) environmentally sensitive QTL, which were found in only one environment. The environmentally stable QTL may be suitable for marker-assisted strategies in a soybean breeding program.

Two marker loci Satt236 and Satt511 were placed on MLG A1, while Satt458 and Satt486 were placed on MLG D2. Nine marker loci at Sat_069, Sat_135, Satt141, Satt189, Satt350, Satt412, Satt506, Satt546 and Satt604 were placed on the same MLG D1b+W and linked to each other in this study (Table 10). These markers may also linked to the same QTL.

Molecular marker analysis in this study revealed that at least 15 genetic factors were involved in the variation in brix reading of total soluble solid, three are major QTL in MLG A1 and D1a+Q, and the other thirteen were the minor ones located in

MLG D1b+W, D2 and I. The QTL in MLG A1, D1a+Q, D1b+W and I showed consistent effects in both environments, whereas the effects of the QTL tagged by Sat_069, Satt189, Satt546, Satt458 and Satt486 differ between environments. Maughan *et al*. (2000) identified a QTL controlling sucrose content in MLG A1 and suggested that the marker alleles that were associated with the increase in sucrose content were always associated with an increase in oil content. A strong positive correlation between oil and sugar content in soybean was found, indicating that QTL conditioning these two traits may located on the same chromosomal regions. Several oil QTL in MLG A1 and D1a+Q were reported by Brummer *et al*. (1997) and Hyten *et al*. (2004). Additionally, one QTL for oil content in MLG D1b+W was also found by Zhang *et al.* (2004). Similar genomic positions for TSS content tagged by selective genotyping were effectively found as for the oil and sucrose QTL detected in RIL and segregating F_2 populations (Maughan *et al*. 2000; Hyten *et al*. 2004). The selective genotyping population showed 48% of total TSS content distribution. However, several other QTL may still be detected. These results suggested that molecular marker analysis used for tagging QTL associated with TSS content in a selective genotyping population may assisted plant breeder to improve the high grain quality of vegetable soybean in the future. Simultaneous investigation of two or more traits by selective genotyping would require additional genotyping effort, but could still be worthwhile.

Days to flowering was an important trait that effect directly many other agronomic traits and grain quality of vegetable soybean. There was a trend for the parent with the greater trait value to contribute more QTL alleles with positive effects. The mean DTF of vegetable soybean cultivar ‘AGS292’ was shorter than the mean of experimental line ‘K3’ in both seasons (Table 1). A QTL for days to flowering on MLG J was a major QTL at which the parent ‘AGS292’ alleles contributed to shorter flowering date.

Individually, ten markers explained between 4.5 and 26.6% of the variation in DTF combined across environments (Table 12). Multiple-locus regression identified six markers that were significantly associated with DTF at one environment (Table

13). However, the narrow-sense heritability of DTF was less consistent across the environment. Although, the narrow-sense heritability of DTF estimates at one environment was considered rather high, with 94.2% for late rainy season and 91.6% for dry season, while the narrow-sense heritability estimates using combined data was only 29.1% (Table 1). Thus, the phenotypic variation of DTF could be explained by the six marker loci. The 'AGS292' alleles contributed to shorter DTF at all marker loci (Table 12). Therefore, the parent 'AGS292' can potentially contribute to a shorter DTF in their progenies. Tests for digenic epistatic interactions among significant marker loci for TSS content were all non-significant.

Major QTL for days to flowering showed consistent effects in both seasons. Minor QTL on MLG D1b+W, J, L and O also showed consistent effects across environments. However, the effects of the QTL on MLGs A2, D1a+Q, D2, E, G, H and I were different between environments. Some QTL were easily influenced by environment. The major QTL on MLG J detected in this study was not detected by Tasma *et al.*(2001); Zhang *et al*. (2004) and Yamanaka *et al*. (2001). They found major QTL on MLG C2 and L.

Two marker loci Satt458 and Satt486 were placed on MLG D2 and Satt132 and Satt431 were placed on MLG J. Seven marker loci Sat_135, Satt141, Satt189, Satt350, Satt412, Satt506, and Satt604 were placed on MLG D1b+W and linked to each other in this study. Three markers Sat_105, Satt049 and Satt354 were placed on MLG I. These markers may also linked to the same QTL for days to flowering (Table 12).

Molecular marker analysis in this study revealed that at least 21 genetic factors were involved in the variation in days to flowering, two are major QTL in MLG J, and the other 19 were the minor ones located in MLG A2, D1a+Q, D1b+W, D2, E, G, H, I, L and O. Several QTL associated with DTF have been previously reported in difference populations. They were located on different MLGs (Keim *et al*., 1990; Shoemaker and Specht, 1995; Mansur *et al*., 1996; Lee *et al*.,1996 a; Cregan *et al*.,1999 a; Tasma *et al*., 2001; Zhang *et al*., 2004). Similarly, QTL for days to flowering were also located on different MLGs in this study. These results suggested that the putative QTL for days to flowering may be population-specific as indicated by different genomic region that control the same trait in different mapping populations.

CONCLUSION

A RIL population was developed from the cross between vegetable soybean cultivars ‘AGS292’ and grain soybean experimental line ‘K3’. The traits data of the parents and RIL population were observed in two trials to investigate the inheritance of seed weight (SW), total soluble solid (TSS) content, and days to flowering (DTF). TSS content in green seed of individual RILs were determined by brix reading using a pocket refractometer. Brix reading in fresh seed samples reflects the total sugar content of vegetable soybean seed. Total sugar is not routinely measured in the soybean breeding program because the current analytical method is too time consuming for assessment large number of genotypes.

The differences between parents and between recombinant inbred lines were significant in all three traits in both rainy and dry seasons. This indicated that the traits were diverse among the parents and inherited the variation well to their progenies.

TSS content was the only seed quality trait to demonstrate transgressive segregation. There was a trend for the parent with the greater trait value to contribute more QTL alleles with positive effects.

Narrow-sense heritability for SW was not consistent across seasons, giving the estimates of 0.754 and 0.905 from late rainy and dry seasons, respectively, with the combined heritability of 0.620. The heritability of TSS content for late rainy, dry, and combined seasons were 0.652, 0.582 and 0.455, respectively. The highest heritable values for DTF in late rainy and dry seasons were 0.942 and 0.916, respectively. While the combined estimate over both seasons was low (0.291). From the results of this study, it is recommended that vegetable soybean grown in Thailand be selected particularly for the intended growing season. A superior selection method is a single seed descent across wet and dry seasons.

A total of 161 SSR loci were chosen to survey the parents from an integrated soybean linkage map in order to cover 20 MLGs. The parental survey was carried out with 92 fluorescent-label primers and 69 none-labeled SSR primers to screen polymorphism. Sixty-three polymorphic primers were used for SSR analysis in selective genotyping population with the highest and lowest percentage of TSS content. The same method was used for tagging QTL for TSS content, SW and DTF. The QTL analysis was performed in selective genotyping of 92 RILs.

Single-point analysis of 63 SSR marker loci revealed that twenty genetic factors were involved in the variation of SW in the combined analysis, there were two major QTL in MLGs J and L, and the other 18 were the minor ones located in MLGs B1, C1, D1b+W, D2 E, F, I, N and O.

Fifteen genetic factors were involved in the variation in brix reading of total soluble solid content, three are major QTL in MLG A1 and D1a+Q, and the other 13 were the minor ones located in MLGs D1b+W, D2 and I. The QTL in MLG A1, D1a+Q, D1b+W and I showed consistent effects in both growing seasons.

Twenty-one genetic factors were involved in the variation in days to flowering, two are major QTL in MLG J, and the other 19 were the minor ones located in MLGs A2, D1a+Q, D1b+W, D2, E, G, H, I, L and O.

Multiple-locus regression analysis of significant markers of combined data across two seasons were found that at least seven QTL were controlling SW, three QTL controlling TSS, and four QTL controlling DTF.

The selective genotyping population showed only 48% of TSS content distribution. However, several other QTL may still be detected. As well as the investigation of grain quality and days to flowering using this subpopulation would require additional genotyping effort. Therefore, mapping of the whole population using significant markers for the traits of interest should be done in the future work.

LITERATURE CITED

Abe, J., M.D.H. Xu, Y. Suzuki, A. Kanazawa and Y. Shimamoto. 2002. Germplasm pools of soybean in Asia revealed by nuclear SSRs. **Theor. Appl. Genet**. 106: 445-453.

Baird, V., A. Abbott, R. Ballard, B. Sonsinski and S. Rajapakse. 1997. DNA diagnostics in horticulture, p. 111-130 *In* P. Gresshoff, ed. **Current Topics in Plant Molecular Biology: Technology Transfer of Plant Biotechnology**. CRC Press, Boca Raton.

Becker, W.A. 1984. **Manual of Quantitative Genetics** 4th ed. Academic Enterprises, Pullman, Washington, D.C.

Bravo, J.A., W.R. Fehr and S.R. de Cianzio. 1980. Use of pod width for indirect selection of seed weight in soybean. **Crop Sci**. 20: 507-510.

Brim, C.A. 1973. Quantitative genetics and breeding. p. 155-186. *In* R.J. Wicox ed. **Soybeans: Improvement, Production, and Uses.** Madison, Wisconsin.

Brummer, E.C., G.L. Graef, J. Orf, J.R. Wilcox and R.C. Shoemaker. 1997. Mapping QTL for seed protein and oil content in eight soybean populations. **Crop Sci.** 37: 370-378.

Burris, J.S., O.T. Edje and A.H. Wahab. 1973. Effect of seed size on seedling performance in soybean. II. Seedling growth and photosynthesis and field performance. **Crop Sci.** 13: 207-210.

Chandler, W.G. and W.R. Fehr. 2000. Association of total sugar content with other seed traits of diverse soybean cultivars. **Crop Sci.** 40: 552-1555.

Chang, S.J.C., T.W. Doubler, V. Kilo, R.J. Suttner, M.E. Schmidt and P.T. Gibson. 1997. Association of field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN). **Crop Sci.** 37: 965-971.

Cicek, M. 1997. **Genetic Analysis of Quantitative Trait Loci Associated with Seed Sucrose Content Using Molecular Markers in an Interspecific *Glycine* Cross.** M.S. thesis, Virginia Polytechnic Institute and State University.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer and E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. **Euphytica** 142: 169-196.

Cregan, P.B. and C.V. Quigley. 1997. Simple sequence repeat DNA marker analysis. pp 173-185. *In* G. Caetano-Anolles and P.M. Gresshoff, eds. **DNA Markers: Protocols, Applications, and Overviews**. Wiley-Liss, Inc., New York.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. Van Toai, D.G. Lohnes, J. Chung and J.E. Specht. 1999 a. An integrated genetic linkage map of the soybean genome. **Crop Sci.** 39: 1464-1490.

Cregan, P.B., J. Mudge, E.W. Fickus, L.F. Marek, D. Danesh, R. Denny, R.C. Shoemaker, B.F. Matthews, T. Jarvik and N.D. Young. 1999 b. Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. **Theor. Appl. Genet.** 98: 919-928.

de Vienne, D., S. Santoni and M. Falque. 2003. Principal sources of molecular markers. pp. 1-46. *In* D. de Vienne. ed. **Molecular Marker in Plant Genetics and Biotechnology**. Science Publishers, Inc. Enfield, New Hampshire.

Doyle, L.J. and J.J. Doyle. 1990. Isolation of plant DNA from fresh tissue. **Focus** 12: 13-14.

Fehr, W.R., C.E. Caviness, D.T. Burmood and J.S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. **Crop Sci.** 11: 929-931.

Fehr, W.R. 1987. **Principles of Cultivar Development**. Vol 1: Theory and technique. Macmillan Publishing Company, New York.

Geater, C.W., W.R. Fehr and L.A. Wilson. 2000. Soybean seed traits influence natto quality. **Crop Sci.** 40: 1529-1534.

Henry, R. 1997. Molecular markers in plant improvement, pp. 99-132. *In* **Practical Applications of Plant Molecular Biology**. Chapman and Hall, London.

Hoeck, J.A., W.R. Fehr, R.C. Shoemaker, G.A. Welke, S.L. Johnson and S.R. Cianzio. 2003. Molecular marker analysis of seed size in soybean. **Crop Sci.** 43: 68-74.

Hymowitz, T., F.I. Collins, J. Panczner and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. **Agron. J.** 64: 613-616.

Hymowitz, T. and F.I. Collins. 1974. Variability of sugar content in seed of *Glycine max* (L.) Merill and *G. soya* Sieb and Zucc. **Agron. J.** 66: 239-240.

Hyten, D.L., V.R. Pantalone, C.E. Sams, A.M. Saxton, D. Landau-Ellis, T.R. Stefaniak and M.E. Schmidt. 2004. Seed quality QTL in a prominent soybean population. **Theor. Appl. Genet.** 109: 552-561.

Johnson, S.L., W.R. Fehr, G.A. Welke and S.R. Cianzio. 2001. Genetic variability for seed size of two- and three-parent soybean populations. **Crop Sci**. 41: 1029-1033.

Karp, A. and K.J. Edwards. 1997. DNA markers: a global overview. pp 1-13. *In* G. Caetano-Anolles and P.M. Gresshoff, eds. **DNA Markers: Protocols, Applications, and Overviews**. Wiley-Liss, Inc., New York.

Keim, P., B.W. Diers, T.C. Olson and R.C. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. **Genetics** 126: 735-742.

Keim, P., R.C. Shoemaker and R.G. Palmer. 1996. A high density soybean genetic map based on AFLP. **Crop Sci.** 36: 786-792.

Krober, O.A. and J.L. Cartter. 1962. Quantitative interrelationships of protein and nonprotein constituents of soybeans. **Crop Sci.** 2: 171-172.

Lander, E.S. and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. **Genetics** 121: 185-199.

Lark, K.G., J.M. Weisemann, B.F. Matthews, R. Palmer, K. Chase and T. Macalma. 1993. A genetic map of soybean (*Glycine max* L.) using an intraspecific cross of two cultivars: ‘Minsoy’ and ‘Noir 1’. **Theor. Appl. Genet.** 86: 901-906.

Leroy, A.R., S.R. de Cianzio and W.R. Fehr. 1991. Direct and indirect selection for small seed of soybean in temperate and tropical environments. **Crop Sci.** 31: 697-699.

Lee, S.H., M.A. Bailey, M.A.R. Main, T.E. Carter Jr, D.A. Ashley, R.S. Hussey, W.A. Parrott and H.R. Boerma. 1996 a. Molecular markers associated with soybean plant height, lodging, and maturity across locations. **Crop Sci.** 36: 728-735.

Lee, S.H., M.A. Bailey, M.A.R. Main, T.E. Carter Jr, E.R. Shipe, D.A. Ashley, W.A. Parrott, R.S. Hussey and H.R. Boerma. 1996 b. RFLP loci associated with soybean seed protein and oil content across populations and locations**. Theor. Appl. Genet.** 93: 649-657.

Liu, B.H. 1998. **Statistical Genomics: Linkage, Mapping, and QTL Analysis**. CRC Press, Boca Raton, New York.

Lumpkin, T.A. and J. Konovsky. 1991. A critical analysis of vegetable soybean production, demand and research in Japan, pp.199-212. *In* S. Shanmugasundaram. ed. **Proceeding of Workshop on Vegetable Soybean: Research Needs for Production and Quality Improvement.** Kenting, Taiwan: Asian Vegetable Research and Development Center, Publication No. 91-346.

Mansur, L.M., J.H. Orf. and K.G. Lark. 1993. Determining the linkage of quantitative trait loci to RFLP marker using extreme phenotypes of recombinant inbreds of soybean (*Glycine max* (L.) Merr.). **Theor. Appl. Genet**. 86: 914-918.

Mansur, L.M., J.H. Orf, K. Chase, T. Jarvik, P.B. Cregan. and K.G. Lark. 1996. Genetic mapping of agronomic traits using recombinant inbred lines of soybean. **Crop Sci**. 36: 1327-1336.

Masuda, R. 1991. Quality requirement and improvement of vegetable soybean, pp.92-101. *In* S. Shanmugasundaram. ed. **Proceeding of Workshop on Vegetable Soybean: Research Needs for Production and Quality Improvement.** Kenting, Taiwan: Asian Vegetable Research and Development Center, Publication No. 91-346.

Maughan, P.J., M.A. Saghai Maroof and G.R. Buss. 1996. Molecular-marker analysis of seed-weight: genomic location, gene action, and evidence for orthologous evolution among three legume species. **Theor. Appl. Genet.** 93: 574-579.

Maughan, P.J., M.A. Saghai Maroof and G.R. Buss. 2000. Identification of quantitative trait loci controlling sucrose content in soybean (*Glycine max*). **Mol. Breed.** 6: 105-111.

Mian, M.A.R., M.A. Bailey, J.P. Tamulonis, E.R. Shipe, T.E. Carter Jr, W.A. Parrott, D.A. Ashley, R.S. Hussey and H.R. Boerma. 1996. Molecular markers associated with seed weight in two soybean populations. **Theor. Appl. Genet.** 93: 1011-1016.

Michelmore, R.W., I. Paran and V. Kesseli. 1991. Identification of markers linked to disease-resistance gene by bulked segregant: A rapid method to detect markers in specific genomic regions by using segregating populations. **Proc. Natl. Acad. Sci.** 88: 9828-9832.

Mohan, M., S. Nair, A. Bhagwat, T.G. Krishna, M. Yano, C.R. Bhatia and T. Sasaki. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. **Mol. Breed.** 3: 87-103.

Openshaw, S.J. and H.H. Hadley. 1981. Selection to modify sugar content of soybean seeds. **Crop Sci.** 21: 805-808.

Openshaw, S.J. and H.H. Hadley. 1984. Selection indexes to modify protein concentration of soybean seeds. **Crop Sci.** 24: 1-4.

Orf, J.H., K. Chase, F.R. Adler, L.M. Mansur and K.G. Lark. 1999. Genetic of soybean agronomic traits: II. Interactions between yield quantitative trait loci in soybean. **Crop Sci.** 39: 1652-1657.

Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowich, S.E. Lincoln, E.S. Lander and S.D. Tanksley. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. **Genetics** 127: 181-197.

Paterson, A.H. 1996. Making genetic maps. pp. 23-39. *In* A.H. Paterson, ed. **Genome Mapping in Plants**. R.G. Landes Company, San Diego, California; Academic Press, Austin, Texas.

Rogers, S.O. and A.J. Bendich. 1994. Extraction of total cellular DNA from plants, algae and fungi, pp. D1: 1-8. *In* S.B. Gelvin, and R.A. Schilperoot, eds. **Plant Molecular Biology Manual.** 2nd ed., Kluwer Academic Publishers, Dordrecht, Netherlands.

Rongwen, J., M.S. Akkaya, A.A. Bhagwat, U. Lavi and P.B. Cregan. 1995. The use of microsatellite DNA markers for soybean genotypes identification. **Theor. Appl. Genet.** 90: 43-48.

SAS Institute, 1990. **SAS language: Reference, Version 6.** SAS institute. Cary, North Carolina.

Sanitchon. J. 2004. **DNA Markers Linked to Sudden Death Syndrome Resistance in Soybean**. Ph.D. thesis, Kasetsart University.

Shanmugasundaram S., S.T. Cheng, M.T. Huang and M.R. Yan. 1991. Vegetable soybean varietal improvement in Taiwan, pp.30-42. *In* S. Shanmugasundaram. ed. **Proceeding of Workshop on Vegetable Soybean: Research Needs for Production and Quality Improvement.** Kenting, Taiwan: Asian Vegetable Research and Development Center, Publication No. 91-346.

Shanmugasundaram S. and M.R. Yan. 2004. Global expansion of high value vegetable soybean, pp. 915-926. *In* F. Moscarsi, C.B. Hoffmann-Campo, O.F. Saraiva, P.R. Galerani, F.C. Krzyzanowski and M.C. Carrao-Panizzi, eds. **Proceeding of the VII World Soybean Research Conference-VI International Soybean Processing and Utilization Conference- III**

Congresso Brasileiro De Soja. Brasillian Agricultural Research Corporation, National Soybean Research Center, Foz do lguassu, PR, Brazil.

Shapiro, S.S. and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). **Biometrika**. 52: 591-611.

Shoemaker, R.C. and J.E. Specht. 1995. Integration of the soybean molecular and classic genetic linkage groups. **Crop Sci**. 35: 436-446.

Smith, T.J. and H.M. Camper, Jr. 1975. Effect of seed size on soybean performance. **Agron J**. 67: 681-684.

Srisombun, S., S. Ratanarat, S. Kaewmeechai and S. Shanmugasundaram. 2004. Vegetable soybean research and industry development in Thailand, pp.929-935. *In* F. Moscarsi, C.B. Hoffmann - Campo, O.F. Saraiva, P.R. Galerani, F.C. Krzyzanowski and M.C. Carrao-Panizzi. eds. **Proceeding of the VII World Soybean Research Conference-VI International Soybean Processing and Utilization Conference- III Congresso Brasileiro De Soja.** Brasillian Agricultural Research Corporation, National Soybean Research Center, Foz do lguassu, PR, Brazil.

Tajuddin. T., S. Watanabe, N. Yamanaka and K. Harada. 2003. Analysis of quantitative trait loci for protein and lipid contents in soybean seeds using recombinant inbred lines. **Breeding Sci**. 53: 133-140.

Takahashi, N. 1991. Vegetable soybean varietal improvement in Japan - Past, Present and Future, pp.26-29. *In* S. Shanmugasundaram. ed. **Proceeding of Workshop on Vegetable Soybean: Research Needs for Production and Quality Improvement.** Kenting, Taiwan: Asian Vegetable Research and Development Center, Publication No. 91-346.

Tanksley, S.D. 1993. Mapping polygenes. **Annu. Rev. Genet.** 27: 205-233.

Tasma, I.M., L.L. Lorenzen, D.E. Green and R.C. Shoemaker. 2001. Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. **Mol. Breed**. 8: 25-35.

Tsou, S.C.S. and T.L. Hong. 1991. Research on vegetable soybean quality in Taiwan, pp.103-107. *In* S. Shanmugasundaram. ed. **Proceeding of Workshop on Vegetable Soybean: Research Needs for Production and Quality Improvement.** Kenting, Taiwan: Asian Vegetable Research and Development Center, Publication No. 91-346.

Wang, Z., J.L. Weber, G. Zhong and S.D. Tanksley. 1994. Survey of plant short tandem DNA repeats. **Theor. Appl. Genet.** 88: 1-6.

Wasee, S. 1997. **Genetic Studies in Early Generations of Some Agronomic and Quality Characters of Vegetable Soybean (*Glycine max* (L.) Merrill).** Ph.D. thesis, Universiti Putra Malaysia.

Weeden, N., G. Timmerman and J. Liu. 1994. Identifying and mapping genes of economic significance. **Euphytica** 73: 191-198.

Yamanaka, N., S. Ninomiya, M. Hoshi, Y. Tsubokura, M.Yano, Y. Nagamura, T. Sasaki and K. Harada. 2001. An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. **DNA Res**. 8: 61-72.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. **Genetics** 136: 1457-1468.

Zhang, W.K., Y.J. Wang, G.Z. Luo, J.S. Zhang, C.Y. He, X.L. Wu, J.Y. Gai and S.Y. Chen. 2004. QTL mapping of ten agronomic traits on the soybean (*Glycine* *max* L.Merr.) genetic map and their association with EST markers. **Theor. Appl. Genet.** 108: 1131-1139.

APPENDIX

Appendix Table 1 Brix reading of total soluble solid content (%TSS) of vegetable soybean cultivar 'AGS292', grain soybean experimental line 'K3' and their 190 RILs.

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
1	11.8	7.5	9.7
2	11.5	11.4	11.4
3	11.4	7.7	9.5
4	11.5	10.5	11.0
5	13.6	11.6	12.6
6	10.3	7.7	9.0
7	14.6	10.3	12.5
8	10.1	10.3	10.2
9	13.4	9.1	11.3
10	10.3	8.4	9.4
11	10.1	11.4	10.8
12	12.1	9.4	10.7
13	12.4	10.4	11.4
14	10.3	8.8	9.5
15	11.8	9.4	10.6
16	12.3	8.7	10.5
17	11.9	9.9	10.9
18	11.7	8.7	10.2
19	9.6	9.7	9.7
20	13.1	12.4	12.7
21	11.0	9.8	10.4
22	13.1	10.8	11.9
23	13.6	10.4	12.0
24	11.6	9.1	10.4
25	12.3	9.3	10.8
26	11.5	9.4	10.4
27	9.9	8.8	9.4
28	11.5	10.1	10.8
29	14.2	9.8	12.0
30	12.4	10.1	11.3
31	11.0	7.3	9.1
32	10.3	10.0	10.2
33	15.7	11.1	13.4
34	11.2	9.0	10.1

Appendix Table 1 (cont'd)

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
35	11.2	10.4	10.8
36	12.0	10.8	11.4
37	10.6	9.2	9.9
38	12.3	9.7	11.0
39	12.5	10.0	11.2
40	9.4	9.2	9.3
41	11.3	9.1	10.2
42	9.8	8.5	9.1
43	11.1	8.8	10.0
44	12.0	13.5	12.7
45	11.7	8.9	10.3
46	10.8	9.2	10.0
47	10.0	10.3	10.1
48	11.2	9.5	10.4
49	11.4	9.2	10.3
50	9.5	10.0	9.7
51	11.4	10.8	11.1
52	9.3	9.2	9.2
53	10.6	8.4	9.5
54	12.0	9.4	10.7
55	9.8	11.5	10.6
56	10.6	9.4	10.0
57	10.8	10.6	10.7
58	10.3	9.6	9.9
59	13.2	9.3	11.2
60	13.4	8.8	11.1
61	13.3	10.9	12.1
62	13.5	10.7	12.1
63	12.0	9.9	10.9
64	12.7	9.0	10.9
65	9.5	10.1	9.8
66	10.5	10.4	10.4
67	9.3	8.5	8.9
68	8.4	8.9	8.6
69	11.2	10.3	10.7
70	11.4	9.7	10.6
71	11.7	10.4	11.0

Appendix Table 1 (cont'd)

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
72	12.7	8.2	10.4
73	12.3	9.1	10.7
74	12.9	11.1	12.0
75	13.8	8.9	11.3
76	13.1	10.1	11.6
77	10.1	8.2	9.2
78	13.6	11.1	12.3
79	11.8	9.9	10.8
80	9.5	8.4	9.0
81	11.2	10.2	10.7
82	12.6	9.0	10.8
83	9.7	10.1	9.9
84	8.9	7.7	8.3
85	10.4	8.7	9.5
86	12.0	8.8	10.4
87	13.5	12.1	12.8
88	10.6	9.1	9.9
89	12.3	10.3	11.3
90	9.8	8.8	9.3
91	16.0	11.5	13.7
92	13.8	12.0	12.9
93	12.3	9.8	11.0
94	12.5	10.1	11.3
95	10.3	8.7	9.5
96	13.3	9.3	11.3
97	12.7	11.9	12.3
98	12.6	10.7	11.6
99	10.4	9.2	9.8
100	12.0	11.1	11.6
101	12.4	8.8	10.6
102	11.5	8.7	10.1
103	10.3	7.1	8.7
104	11.7	9.9	10.8
105	12.8	9.2	11.0
106	10.8	8.5	9.6
107	12.4	9.9	11.1
108	13.5	11.7	12.6

Appendix Table 1 (cont'd)

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
109	11.7	8.7	10.2
110	11.7	9.3	10.5
111	11.4	8.9	10.1
112	10.6	8.3	9.4
113	13.5	9.0	11.2
114	13.5	10.6	12.1
115	11.0	9.4	10.2
116	10.7	9.8	10.3
117	11.7	9.9	10.8
118	11.4	8.4	9.9
119	11.2	9.5	10.4
120	11.0	10.2	10.6
121	11.2	9.9	10.6
122	12.4	10.5	11.5
123	11.3	10.3	10.8
124	10.7	11.0	10.9
125	13.2	9.6	11.4
126	12.9	8.9	10.9
127	11.5	10.4	10.9
128	12.7	11.5	12.1
129	10.2	9.8	10.0
130	9.2	8.8	9.0
131	12.1	9.8	10.9
132	14.0	9.5	11.7
133	12.7	10.1	11.4
134	11.4	9.9	10.7
135	11.4	10.0	10.7
136	11.4	9.5	10.5
137	14.7	11.0	12.8
138	11.4	10.8	11.1
139	9.8	7.8	8.8
140	12.3	10.4	11.3
141	11.8	8.7	10.2
142	10.6	10.2	10.4
143	9.5	10.3	9.9
144	11.5	10.0	10.7
145	12.4	10.3	11.3

Appendix Table 1 (cont'd)

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
146	10.1	11.4	10.7
147	11.5	9.6	10.5
148	9.9	8.3	9.1
149	11.1	8.2	9.6
150	11.8	11.3	11.5
151	9.8	8.8	9.3
152	13.5	9.6	11.5
153	13.1	10.6	11.8
154	10.7	9.4	10.0
155	11.6	9.9	10.7
156	12.2	9.7	10.9
157	9.6	9.8	9.7
158	10.6	8.6	9.6
159	10.9	9.7	10.3
160	11.6	9.4	10.5
161	9.6	8.7	9.1
162	12.9	9.7	11.3
163	13.7	10.3	12.0
164	14.4	10.6	12.5
165	12.9	8.7	10.8
166	11.7	10.1	10.9
167	14.8	8.7	11.8
168	12.3	9.3	10.8
169	12.1	8.4	10.3
170	11.8	11.2	11.5
171	9.7	10.4	10.0
172	12.7	9.5	11.1
173	11.2	9.5	10.3
174	11.2	9.8	10.5
175	11.0	8.7	9.9
176	10.8	8.4	9.6
177	10.6	8.3	9.4
178	11.8	9.9	10.8
179	12.6	11.1	11.9
180	8.5	8.9	8.7
181	11.0	10.1	10.5
182	9.6	9.2	9.4

Appendix Table 1 (cont'd)

RIL No.	Brix reading (%TSS)		Mean
	Rainy season	Dry season	
183	12.6	9.6	11.1
184	13.2	10.2	11.7
185	14.1	10.5	12.3
186	9.8	9.1	9.4
187	10.9	7.7	9.3
188	10.9	10.3	10.6
189	10.8	8.3	9.5
190	10.6	10.0	10.3
AGS292	12.6	10.0	11.3
K3	8.4	7.5	7.9

Appendix Table 2 One hundred seed weight (g) of vegetable soybean cultivar 'AGS292', grain soybean experimental line 'K3' and their 190 RILs.

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
1	18.6	22.1	20.3
2	16.6	18.3	17.5
3	16.7	20.2	18.5
4	19.1	27.0	23.1
5	16.4	20.6	18.5
6	16.9	19.9	18.4
7	19.8	25.1	22.5
8	14.2	21.0	17.6
9	18.4	23.2	20.8
10	18.2	19.0	18.6
11	18.6	23.9	21.2
12	19.2	20.8	20.0
13	18.0	22.2	20.1
14	18.8	22.3	20.6
15	17.8	20.3	19.1
16	19.1	22.0	20.6
17	14.8	20.8	17.8
18	19.0	23.0	21.0
19	18.9	28.6	23.7
20	17.8	22.9	20.4
21	15.2	17.6	16.4
22	21.8	24.5	23.1
23	20.1	23.1	21.6
24	15.2	20.6	17.9
25	23.3	24.5	23.9
26	18.4	23.4	20.9
27	15.9	20.4	18.2
28	18.4	20.9	19.6
29	16.4	19.9	18.2
30	15.2	18.1	16.6
31	22.4	24.3	23.4
32	15.3	21.1	18.2
33	15.8	25.9	20.9
34	28.7	29.0	28.8

Appendix Table 2 (cont'd)

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
35	16.6	21.6	19.1
36	17.6	21.6	19.6
37	17.0	19.2	18.1
38	12.2	13.7	12.9
39	19.5	24.8	22.2
40	16.7	24.1	20.4
41	17.6	20.6	19.1
42	17.3	23.6	20.4
43	17.2	29.8	23.5
44	14.5	16.1	15.3
45	15.0	19.3	17.1
46	22.3	24.2	23.3
47	16.3	24.3	20.3
48	16.2	19.1	17.7
49	20.7	19.4	20.1
50	13.7	18.5	16.1
51	15.5	19.3	17.4
52	15.1	15.5	15.3
53	18.4	26.6	22.5
54	17.4	20.0	18.7
55	13.8	17.9	15.8
56	19.3	20.2	19.7
57	19.0	20.3	19.7
58	18.5	22.8	20.6
59	16.9	27.3	22.1
60	21.1	19.9	20.5
61	18.4	24.1	21.2
62	18.6	24.1	21.3
63	17.8	19.7	18.8
64	19.8	20.3	20.1
65	16.5	25.1	20.8
66	19.4	20.3	19.9
67	17.1	20.9	19.0
68	18.2	22.8	20.5
69	13.2	15.6	14.4
70	19.3	18.6	18.9
71	18.3	19.8	19.0

Appendix Table 2 (cont'd)

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
72	15.1	16.8	16.0
73	17.8	18.6	18.2
74	19.0	19.6	19.3
75	18.8	20.1	19.5
76	17.1	21.5	19.3
77	13.0	15.1	14.1
78	17.9	23.2	20.5
79	15.1	19.9	17.5
80	13.8	15.3	14.6
81	18.2	19.0	18.6
82	14.2	16.2	15.2
83	12.3	12.8	12.6
84	16.9	22.7	19.8
85	16.0	20.6	18.3
86	19.3	21.3	20.3
87	19.4	19.1	19.2
88	20.3	27.9	24.1
89	17.9	17.7	17.8
90	17.4	22.6	20.0
91	13.8	14.8	14.3
92	13.9	18.2	16.0
93	19.3	23.0	21.2
94	17.3	17.8	17.5
95	19.9	22.7	21.3
96	15.3	24.2	19.7
97	20.5	24.3	22.4
98	17.2	17.6	17.4
99	14.8	18.0	16.4
100	14.6	17.2	15.9
101	13.1	15.6	14.3
102	14.5	20.5	17.5
103	16.5	19.9	18.2
104	16.7	20.9	18.8
105	15.1	18.9	17.0
106	16.0	22.9	19.4
107	14.2	20.3	17.2
108	21.7	23.4	22.5

Appendix Table 2 (cont’d)

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
109	16.0	18.0	17.0
110	19.9	22.3	21.1
111	19.7	20.1	19.9
112	14.2	19.2	16.7
113	15.7	19.9	17.8
114	21.2	24.3	22.8
115	20.4	22.9	21.6
116	17.1	25.5	21.3
117	21.5	25.1	23.3
118	18.6	17.9	18.2
119	21.2	24.2	22.7
120	21.0	26.4	23.7
121	15.5	17.5	16.5
122	16.5	19.4	18.0
123	18.5	22.4	20.4
124	19.2	23.6	21.4
125	14.0	21.3	17.7
126	17.3	20.5	18.9
127	19.6	21.7	20.7
128	17.4	18.4	17.9
129	17.9	23.0	20.5
130	19.5	22.5	21.0
131	16.4	20.0	18.2
132	16.9	18.5	17.7
133	18.0	20.4	19.2
134	15.9	19.0	17.5
135	18.1	17.5	17.8
136	18.5	22.1	20.3
137	18.4	18.3	18.3
138	22.6	29.4	26.0
139	14.1	16.3	15.2
140	20.1	20.1	20.1
141	13.2	15.3	14.3
142	18.0	19.0	18.5
143	13.9	16.2	15.0
144	19.2	22.5	20.8
145	15.3	19.7	17.5

Appendix Table 2 (cont'd)

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
146	17.5	18.6	18.0
147	19.0	27.2	23.1
148	19.7	23.3	21.5
149	14.7	21.4	18.1
150	19.7	25.0	22.4
151	16.6	20.2	18.4
152	21.0	21.4	21.2
153	16.8	19.9	18.4
154	17.6	24.5	21.0
155	23.7	26.1	24.9
156	13.4	23.1	18.2
157	16.2	20.5	18.4
158	16.9	23.4	20.1
159	19.0	18.6	18.8
160	17.2	23.5	20.3
161	16.0	18.8	17.4
162	19.8	23.0	21.4
163	17.1	20.5	18.8
164	17.8	22.8	20.3
165	15.9	17.2	16.5
166	17.0	25.0	21.0
167	18.3	23.1	20.7
168	18.0	20.4	19.2
169	20.2	24.7	22.5
170	14.0	18.8	16.4
171	15.3	19.3	17.3
172	16.9	19.4	18.1
173	14.6	20.5	17.5
174	18.5	19.7	19.1
175	17.5	22.8	20.1
176	18.0	20.8	19.4
177	16.4	21.6	19.0
178	17.9	20.3	19.1
179	19.3	24.1	21.7
180	16.9	25.7	21.3
181	15.1	19.3	17.2
182	17.7	22.3	20.0

Appendix Table 2 (cont'd)

RIL No.	100 seed weight (g)		Mean
	Rainy season	Dry season	
183	18.7	23.8	21.3
184	17.1	18.9	18.0
185	16.3	20.3	18.3
186	19.8	21.5	20.6
187	18.7	20.8	19.7
188	15.9	20.4	18.2
189	20.0	29.6	24.8
190	19.8	37.3	28.6
AGS292	26.2	35.1	30.6
K3	8.4	12.6	10.5

Appendix Table 3 Days to flowering of vegetable soybean cultivar 'AGS292', grain soybean experimental line 'K3' and their 190 RILs.

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
1	32	37	35
2	33	33	33
3	33	36	34
4	27	30	29
5	27	28	27
6	28	29	28
7	27	26	27
8	35	36	35
9	27	28	27
10	31	31	31
11	30	30	30
12	33	34	33
13	28	29	29
14	32	34	33
15	34	35	34
16	29	31	30
17	32	35	33
18	30	32	31
19	30	29	29
20	29	28	28
21	32	34	33
22	27	28	28
23	28	32	30
24	33	36	34
25	31	34	33
26	28	32	30
27	34	33	33
28	31	31	31
29	32	33	33
30	30	33	31
31	33	37	35
32	31	32	31
33	30	33	31
34	28	29	28
35	32	30	31
36	28	27	28

Appendix Table 3 (cont’d)

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
37	33	33	33
38	35	35	35
39	28	32	30
40	34	32	33
41	31	34	32
42	31	30	31
43	27	28	27
44	34	32	33
45	31	38	34
46	35	37	36
47	32	29	30
48	30	31	30
49	34	37	35
50	36	34	35
51	35	31	33
52	38	37	37
53	35	36	35
54	27	35	31
55	34	34	34
56	29	30	29
57	30	30	30
58	31	30	30
59	28	33	30
60	32	36	34
61	30	32	31
62	28	31	29
63	34	33	33
64	33	37	35
65	32	29	30
66	34	34	34
67	31	32	32
68	38	40	39
69	32	34	33
70	37	37	37
71	32	37	34
72	34	35	34
73	31	33	32

<u>Appendix Table 3</u> (cont'd)

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
74	29	29	29
75	31	33	32
76	29	31	30
77	35	37	36
78	28	32	30
79	35	36	35
80	35	35	35
81	30	36	33
82	35	39	37
83	35	37	36
84	35	33	34
85	32	33	33
86	32	31	32
87	33	39	36
88	32	33	32
89	30	32	31
90	34	39	37
91	31	34	32
92	29	29	29
93	30	33	32
94	30	33	31
95	33	39	36
96	29	33	31
97	27	28	27
98	32	33	33
99	34	37	35
100	33	32	32
101	31	32	31
102	30	31	30
103	34	39	37
104	31	31	31
105	31	32	31
106	28	28	28
107	31	32	32
108	34	35	34
109	36	37	36
110	32	36	34

Appendix Table 3 (cont'd)

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
111	27	28	28
112	31	31	31
113	26	27	26
114	29	28	28
115	34	33	33
116	30	28	29
117	30	32	31
118	33	33	33
119	29	31	30
120	30	30	30
121	36	39	38
122	30	33	31
123	30	34	32
124	31	34	32
125	31	33	32
126	29	31	30
127	33	33	33
128	30	36	33
129	35	35	35
130	32	32	32
131	34	35	34
132	33	36	34
133	27	28	27
134	35	36	36
135	31	33	32
136	31	32	31
137	30	32	31
138	31	32	31
139	33	39	36
140	31	34	32
141	38	38	38
142	30	35	32
143	32	31	31
144	31	32	31
145	32	34	33
146	31	31	31
147	31	31	31

Appendix Table 3 (cont'd)

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
148	31	33	32
149	35	33	34
150	31	31	31
151	33	37	35
152	31	35	33
153	33	33	33
154	32	37	35
155	31	34	32
156	30	33	32
157	33	33	33
158	31	29	30
159	27	31	29
160	30	32	31
161	37	40	39
162	31	38	34
163	30	29	29
164	28	28	28
165	34	37	35
166	30	32	31
167	29	33	31
168	28	32	30
169	29	33	31
170	30	30	30
171	36	35	35
172	28	28	28
173	32	35	33
174	32	33	32
175	29	30	30
176	38	38	38
177	32	31	31
178	28	30	29
179	30	32	31
180	32	31	31
181	33	31	32
182	37	35	36
183	33	32	32
184	30	33	32

Appendix Table 3 (cont'd)

RIL No.	Days to flowering		Mean
	Rainy season	Dry season	
185	30	28	29
186	32	33	32
187	29	32	30
188	32	35	34
189	34	34	34
190	30	27	29
AGS292	25	28	26
K3	42	40	41

CURRICULUM VITAE

NAME : Pornpan Pooprompan

BIRTH DATE : January 5, 1959

BIRTH PLACE : Nakhon Pathom

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
1982	Maejo University	B.Ag. Tech.
1991	Kasetsart University	M.S. (Agriculture)

POSITION/TITLE : Lecturer

WORK PLACE : Faculty of Agricultural Production, Maejo University

SCHOLARSHIP/AWARD : Center for Agricultural Biotechnology, Thailand Research Fund.