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Molecular Marker Analysis of Days to Flowering in Vegetable Soybean (*Glycine max* (L.) Merrill)

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ABSTRACT

Days to flowering (DTF) in vegetable soybean is an important reproductive character of agronomic interest. This trait is useful for developing vegetable soybean cultivars with desirable flowering date, and eventually harvesting date. The objective of this study was to identify simple sequence repeat (SSR) markers associating with quantitative trait loci (QTL) for DTF in recombinant inbred lines (RILs) derived from the cross between the vegetable soybean cultivar 'AGS292' and the grain soybean line (G8891xG7945-31-3-5-5) or 'K3' grown in two environments. The SSR allele size profiling of the parents were analyzed with 162 markers to identify their polymorphism. The analysis was assayed for linkage relationships of DTF in a sub-population of 92 RILs. Molecular marker analysis of 63 polymorphic SSR markers revealed that at least two major and nineteen minor QTL were involved in controlling DTF. The QTL near SSR markers (Satt132 and Satt431) in molecular linkage group (MLG) J had the greatest effect on DTF. These results suggested that the putative QTL for DTF might be population-specific as indicated by different genomic region that control the same trait in different mapping populations. The QTL found in this study could facilitate vegetable soybean breeders in performing marker-assisted selection (MAS) as early as in the seedling stage to improve vegetable soybean cultivars with desirable flowering date.

Key words: vegetable soybean, *Glycine max*, days to flowering, quantitative trait loci, simple sequence repeat, marker-assisted selection

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). Days to flowering (DTF) is a major trait to be considered by the farmers growing vegetable soybean. The cultivars with too short flowering date do not

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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. 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). Tasma *et al.* (2001) reported that the numbers of days to flowering and maturity in field soybean were useful for developing soybean cultivars with wider geographical adaptation.

Several QTL associated with DTF have been previously mapped on different molecular linkage groups in soybean. The investigation of QTL conducted in a single environment may lead to underestimating the number of QTL influencing a trait. It is also possible to have environmentally sensitive QTL, meaning that expression of these QTL will occur only 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 were satisfied. Lee *et al.* (1996) 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.

Since most vegetable soybean breeding projects belong to private companies, no publication on an important reproductive character study is publicly available so far. The objective of this study was to identify SSR markers associating with QTL for DTF of a RIL population derived from the cross between 2 contrasting parents, viz. vegetable soybean cultivar 'AGS292' and the grain soybean experimental line 'K3'.

MATERIALS AND METHODS

Plant materials

A population of recombinant inbred lines (RILs) was obtained from a cross between two contrasting soybean lines 'AGS292' and 'K3'. They were markedly different in terms of nutritional content in the seed, as well as flowering date. 'AGS292' was a popular vegetable soybean cultivar with large seed and high sugar content. It was a pure line selected from the Japanese cultivar 'Taishoshiroge' by AVRDC - the World Vegetable Center, Taiwan. The experimental line (G8891/G7945-31-3-5-5) or 'K3' was a small-seeded grain soybean with high-protein content and had longer flowering date than vegetable soybean cultivar 'AGS292'. It was a pure line derived by pedigree selection from the cross between 'G8891' and 'G7945' (both are from collection of AVRDC) by the soybean breeding project of Kasetsart University, Thailand. The resulting progenies from the AGS292xK3 cross were advanced by a single seed descent method from F_2 plants until 190 $F_{2:7}$ seeds were obtained. This population was considered as a RIL population to be grown and evaluated for DTF in the field in two seasons.

Field observation

The trait data of the parents and their RIL population were observed in 2 trials in the field during the late rainy season (August - November 2004) and dry season (November 2004 - February 2005) at 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 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).

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 93 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 162 SSR loci were selected to survey the parents from an integrated soybean linkage map (Cregan *et al*., 1999) in order to roughly cover 20 MLGs. The marker loci were considered for SSR analysis based 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 sub-population

Out of 190 individual RILs, the SSR analysis was assayed in a sub-population by randomizing 92 individual RILs. DNA was extracted from young leaves from individual plant of the subpopulation 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 performed in a MJ PTC-100 Programmable Thermal Controller. All PCR products were electrophoresed in 3-4% agarose gel. The gel was run in 0.5x 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 based 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 ones that possessed heterozygous alleles derived from both parents were scored as H.

Data analysis

Trait mean, normality index, and analysis of variance of DTF was 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 were homozygous genetically, the dominant genetic variation (σ^2_d) could 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 was a narrow-sense one and could be determined from the 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. 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 (SF-ANOVA) was used to associate polymorphic markers with DTF (SAS Institute, 1990). Significant marker loci associated with DTF was identified when a marker at an individual environment was significant at $P \leq 0.05$ across two environments. 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 DTF.

RESULTS

Variation of DTF in the RILs

Mean, standard deviation, range, and parental values for DTF are presented in Table 1. DTF of the vegetable soybean cultivar 'AGS292' was earlier than that of 'K3' in both environments, with the averages of 25 vs 42 and 27 vs 40 days in the late rainy and dry seasons, respectively. While in the RIL population, DTF ranged from 26 to 38 days in the late rainy and from 26 to 40 days in dry seasons. 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 DTF among the 190 RILs, averaged over two seasons were continuous (Figure 1), indicating that the trait was 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)*.

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 heritable values for DTF in the late rainy and dry seasons were 94.2 and 91.6% respectively. While the combined estimate over both seasons was low (29.1%). This was the case because of significant interaction detected between genotypes and the growing seasons (data not shown).

SSR analysis in RIL sub-population

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

Table 1 Range and Mean±SD of days to flowering of 190 soybean RILs and their parents grown in the late rainy and dry seasons of 2004, and combined over seasons. The narrow-sense heritabilities were presented in the right column.

Season	RIL		Parents		H^2 1/
	Range	Mean	AGS292	K3	(%)
Late rainy	26-38	31±2.58	25	42	94.2
Dry season	26-40	33±3.04	27	40	91.6
Combined	26-39	32±2.61	26	41	29.1

1/ Heritability in the narrow-sense

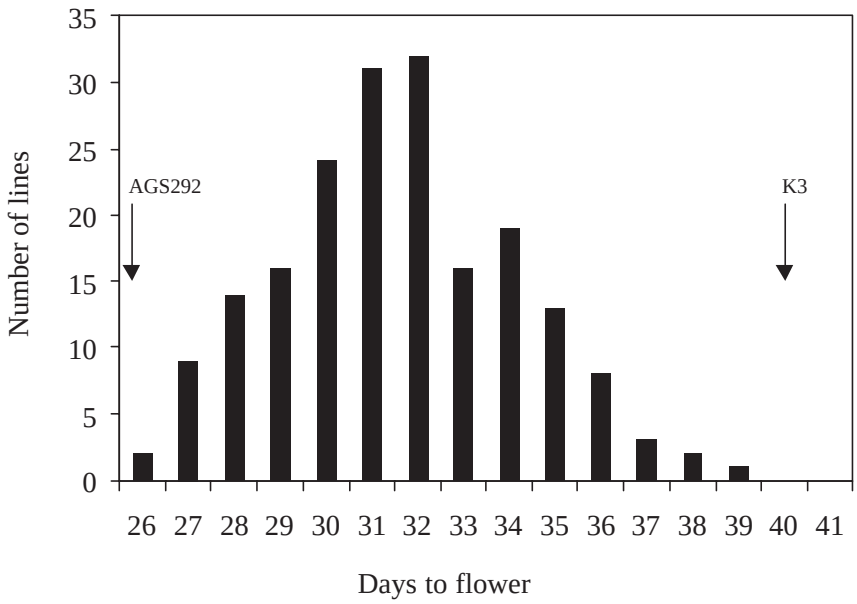

Figure 1 Frequency distribution of days to flowering in 190 RILs averaged across seasons. Mean parental values of 'AGS292' and 'K3' are indicated by arrows.

(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 (data not shown).

QTLs associated with days to flowering

Based on SF-ANOVA, ten marker loci were identified as being associated with DTF in the combined analysis (Table 2). 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 the other markers. However, eleven marker loci on MLG D1a+Q, D1b+W, D2, E, G, H and I were not identified in the combined analysis. Seven (Sat_135, Satt189, Satt350, Satt132, Satt431, Satt229, and Satt262) of twenty-one marker loci were significant in two environments. Fourteen marker loci, viz. 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 over seasons. Multiple-locus regression identified six markers that were significantly associated with DTF in the late rainy season 2004 (Table 3). Four of the seven marker loci contributed 4.4 to 26.0 % of the variation after accounting for the other marker loci in the model and together explained 51.6% of the total variation for DTF combined across seasons. The grain soybean experimental line 'K3' contributed alleles for longer flowering date at ten marker loci (Satt187, Sat_135, Satt141, Satt189, Satt350, Satt412, Satt132, Satt431, Satt229, and Satt262) (Table 2), while the vegetable soybean cultivar 'AGS292' contributed alleles for shorter flowering date at the same loci.

DISCUSSION

In this experiment, DTF in a single

season was highly heritable. The heritability reduced slightly when the combined data across 2 experimental seasons 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 tropic 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 the later generations. Then, vegetable soybean lines with desirable flowering date in each season can be identified later.

Days to flowering of vegetable soybean is an important trait that affects directly to the other agronomic traits, including grain quality. There

Table 2 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 seasons 2004/2005, and combined over seasons.

Locus	MLG [1/]	Environment				Combined			
		Rainy 04		Dry 04/05		*P*	R^2 (%)	Allelic mean (DTF) [2/]	
		P	R^2 (%)	*P*	R^2 (%)			AGS292	K3
Satt187	A2	NS[3/]		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

[1/] Molecular linkage group

[2/] Days to flowering

[3/] Non-significant

was a trend for the parent with the greater trait value to contribute more QTL alleles with positive effects. The average of DTF for vegetable soybean cultivar 'AGS292' was shorter than that of the experimental line 'K3' in both environments (Table 1). A QTL for days to flowering on MLG J was a major QTL at which the parental 'AGS292' alleles contributed to shorter flowering date.

The narrow-sense heritability of DTF was less consistent across the seasons. The estimate in each season was considered rather high (94.2% for late rainy and 91.6% for dry seasons), while the estimate from combined data was only 29.1% (Table 1). The phenotypic variation of DTF could be explained by six marker loci from which 'AGS292' alleles contributed to shorter DTF at all loci (Table 2). Thus 'AGS292' could potentially contribute a shorter DTF to its progenies. Tests for digenic epistatic interaction among significant marker loci for DTF content were all non-significant.

Major QTL for DTF showed consistent effects in both environments. 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 differed between environments. Some QTLs were easily influenced by environment. The MLG J location of the major QTL 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. Several other QTL were also detected in difference populations and located on different MLGs (Keim *et al*., 1990; Shoemaker and Specht, 1995; Mansur *et al*., 1996; Cregan *et al.*,1999; Lee *et al.*,1996).

Two marker loci Satt458 and Satt486 were placed on MLG D2, while 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 DTF (Table 2).

CONCLUSION

Molecular marker analysis in this study revealed that at least 21 genetic factors were

Table 3 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 2004/2005, and combined over seasons using multiple-locus regression.

Locus	MLG [1/]	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 [2/]	
Satt412	D1b+W	NS		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

[1/] Molecular linkage group

[2/] Non-significant

involved in the variation in DTF, two were major QTL in MLG J, while the other nineteen were the minor ones located in MLG A2, D1a+Q, D1b+W, D2, E, G, H, I, L and O. Some QTL associated with DTF have been previously reported in different populations to locate on different MLGs. Similar results were detected in this study, suggesting that the putative QTL for days to flowering might be population-specific as indicated by different genomic regions that controled the same trait in different mapping populations.

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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.

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**. 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. An integrated genetic linkage map of the soybean genome. **Crop Sci**. 39: 1464-1490.

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, 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.

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

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. Molecular markers associated with soybean plant height, lodging, and maturity across locations. **Crop Sci**. 36: 728-735.

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.

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.

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

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

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.). **Proc. VII World Soybean Research Conf. - VI Int. Soybean Processing and Utilization Conf. - III Congresso Brasileiro De Soja**. Foz do lguassu.

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.

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.

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.

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.