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THESIS

THE GENETICS OF DOMESTICATION-RELATED TRAITS OF YARDLONG BEAN [*VIGNA UNGUICULATA* (L.) WALP. SSP. *UNGUICULATA* CV.-GR. *SESQUIPEDALIS*]

ALISA KONGJAIMUN

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Alisa Kongjaimun 2012: The Genetics of Domestication-related Traits of Yardlong Bean [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*]. Doctor of Philosophy (Plant Breeding), Major Field: Plant Breeding, Faculty of Agriculture at Kamphaeng Saen. Thesis Advisor: Professor Peerasak Srinives, Ph.D. 115 pages.

The genetics of domestication-related traits of yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*] is of particular interest because the genome of this legume has experienced divergent domestication. Initially cowpea was domesticated from wild cowpea in Africa, subsequently, in Asia a vegetable form of cowpea, yardlong bean, evolved from cowpea. Information on the genetics of domestication-related traits would be useful for yardlong bean and cowpea breeding programs, as well as comparative genome study among members of the genus *Vigna*. The objectives of this study were (1) to develop a genetic linkage map of yardlong bean using SSR markers from related *Vigna* species (2) to locate QTLs for traits related to the phenotypic differences between yardlong bean and wild cowpea and (3) to compare them with the previously reported QTLs in closely related *Vigna*.

Two linkage maps were developed from BC_1F_1 and F_2 populations from the cross between yardlong bean (*V. unguiculata* ssp. *unguiculata* cv.-gr. *sesquipedalis*) accession JP81610 and wild cowpea (*V. unguiculata* ssp. *unguiculata* var. *spontanea*) accession TVnu457. Using these linkage maps, QTLs for 24 domestication-related traits were analyzed and mapped. QTLs were detected for traits related to seed, pod, stem and leaf.

Most traits were controlled by between one and eleven QTLs. QTLs for domestication-related traits showed co-location on several narrow genomic regions on almost all linkage groups, especially on linkage groups 3, 7, 8, and 11. Major QTLs for sizes of seed, pod, stem and leaf were principally located on linkage group 7. Pleiotropy or close linkage of genes for the traits is suggested in these chromosome regions.

This is the first report of QTLs for domestication-related traits in yardlong bean. The results provide a foundation for marker-assisted selection of domestication-related QTLs in yardlong bean and enhance understanding of domestication in the genus *Vigna*.

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THE GENETICS OF DOMESTICATION-RELATED TRAITS OF YARDLONG BEAN [*VIGNA UNGUICULATA* (L.) WALP. SSP. *UNGUICULATA* CV.-GR. *SESQUIPEDALIS*]

INTRODUCTION

The Leguminosae genus *Vigna* is a pantropical genus comprising about 100 species mainly found in Africa and Asia (Maréchal *et al.,* 1978). Nine *Vigna* species have been domesticated, of which two were domesticated in Africa and seven were in Asia. The African *Vigna* consists of cowpea [*V. unguiculata* (L.) Walp.] and bambara groundnut [*V. subterranea* (L.) Verdc.] (Smartt, 1990). The Asian *Vigna* comprises mungbean [*V. radiata* (L.) Wilczek], blackgram [*V. mungo* (L.) Hepper], moth bean [*V. aconitifolia* (Jacq.) Maréchal], azuki bean [*V. angularis* (Willd.) Ohwi & Ohashi], rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi], jungli bean [*V. trilobata* (L.) Verdc.] and creole bean [*V. reflexo-pilosa* Hayata] (Tomooka *et al.,* 2002). All domesticated *Vigna* except creole bean have the chromosome number of 2n=2x=22. These crops are adapted to various agroclimatic conditions and fit well into many cropping systems. Dry seeds, young pods and sprouts from these crops are consumed. Plant parts of the crops are used as fodder or hay for farm animals. Among these *Vigna* crops, cowpea is the most important in term of planting area.

Yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*] is characterized by its very long (30-90 cm in length) pods with seeds usually 8-12 mm long. It is also known as asparagus bean, string bean, snake bean and sitao. Unlike other *Vigna* crops which are grown primarily for seeds, yardlong bean is cultivated mainly for crisp and tender pods that are consumed both fresh and cooked. Yardlong bean is believed to have been domesticated from cultivated cowpea in Asia. It is widely grown as vegetable in China, South and Southeast Asia.

Yardlong bean and cowpea differ phenotypically as a result of divergent selection during the evolution of the crop. The domestication syndrome is a suite of morphological and physiological traits that distinguish domesticated crops from their wild ancestors (Hammer, 1984). Domestication-related traits include changes in plant architecture (e.g. no branching in maize, determinate growth habit in beans), gigantism in the consumed plant organs (e.g. seed size in bean), reduced seed dispersal (i.e. non-shattering or non-dehiscence in bean and rice) and loss of seed dormancy. Crop domestication is an accelerated evolutionary process that is the result of the synergistic effect of human (both intentional and unintentional selections) and natural selection. Plant scientists are interested in studying the genetic basis of crop domestication with an ultimate goal of identifying useful allele(s), gene(s) or genome region(s) that have not been exploited in the wild relatives of the cultivated crops which could have a positive impact on crop improvement. The search and localization for genes involved in crop domestication are done by means of QTL analyses in many crops. Some genes for domestication have been cloned (Purugganan and Fuller, 2009, Izawa *et al.,* 2009). In addition, the divergence within the primary genepool of cowpea to a crop for seeds and pods outside the natural range of the wild relatives of cowpea can provide insights into crop evolution and agriculture. Unfortunately, there is a lack of archaeobotanical information on cowpea and particularly yardlong bean in Asia (Fuller and Harvey, 2006).

The genetics of domestication syndrome traits have been reported in a limited number of legume crops including azuki bean, common bean, pea, soybean and rice bean. Among the nine cultivated *Vigna* species, comprehensive QTL mappings for domestication syndrome traits were reported for azuki bean (Kaga *et al.,* 2008) and rice bean (Isemura *et al.,* 2010); both of which are Asian *Vigna* crops. QTL analyses for domestication traits in cowpea and yardlong bean have recently been published (Andargie *et al.,* 2011, Xu *et al.,* 2011). However, these papers examined only 2 traits of domestication-related characters.

Genetics of domestication-related traits of yardlong bean is of particular interest because genome of the bean has experienced divergent domestication; from cowpea that is grown primarily for its seeds. Cowpea was domestciated from wild cowpea in Africa. Subsequently, in Asia, cultivated cowpea or its weedy relative was selected for the vegetable crop, yardlong bean. Information on the genetics of domestication related traits would be useful for yardlong bean and cowpea breeding programs, and comparative genome studies among members of the genus *Vigna*.

OBJECTIVES

The objectives of this study are:

1. To develop a genetic linkage map of yardlong bean using SSR markers from related *Vigna* species

2. To locate QTLs for traits related to the phenotypic differences between yardlong bean and wild cowpea

3. To compare QTL of domestication-related traits of yardlong bean with previously reported QTLs in closely related *Vigna*

LITERATURE REVIEW

The genus *Vigna* are distributing across a wide region of Asia. Among them, two African *Vigna*; yardlong bean and cowpea, and four Asian *Vigna*; mung bean (*Vigna radiata*), rice bean (*V. umbellata*), black gram (*V. mungo*) and azuki bean (*V. angularis*) are economically important in Asia. Numerous variation in morphological and physiological traits associated with domestication are observed between the cultivated and wild forms. These differences, collectively called the domestication syndrome, result from selection over several thousand years of adaptation to cultivated environments, human nutritional requirements and preferences (Hawkes, 1983). The cultivated *Vigna* species listed above are ideal materials for studying comparative genomics of the gene evolution related to domestication within and among *Vign*a species and for characterizing quantitative trait loci (QTLs) of useful traits for breeding of these crop. The genomic structures and genomic regions associated with domestication among these species were studied in the two Asian *Vigna* species, azuki bean (Kaga *et al.,* 2008) and rice bean (Isemura *et al.,* 2010). QTL analyses for domestication traits in cowpea and yardlong bean have recently been published (Andargie *et al.,* 2011, Xu *et al.,* 2011). However, these papers examined only 2 traits of domestication-related characters, viz. seed weight and pod shattering in cowpea, and flower and seed coat color in yardlong bean.

Domestication of Yardlong bean

Yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*] (2n =2x = 22) is one of the most important vegetable legumes of Southeast and East Asia. It is also known as asparagus bean, string bean, snake bean, snake pea, snap pea, bodi, bora, and sitao. It is believed to have been domesticated in Southeast Asia from vegetable (pod) cowpea selected from the Unguiculata Group (subsp. *unguiculata* in the original paper) in India (Steele and Mehra, 1980). The bean is grown principally for its immature long pods which are consumed fresh or as cooked pods. Yardlong bean can be grown year-round and the green tender pods can be harvested two to four times starting at about 50 days after planting. The bean provides important sources of dietary proteins, amino acids, vitamins, and minerals for Asian people. Yardlong bean is characterized by its very long and succulent pods (30-90 cm in length), whereas cowpea (*Vigna unguiculata* (L.) Walp. ssp. *unguiculata* Unguiculata Group and Biflora Group (or Cylindrica)) has shorter pods (20-30 cm in length) (Verdcourt, 1970). Yardlong bean belongs to one of the four cultivar Groups within the subspecies *unguiculata* including Unguiculata, Biflora, Sesquipedalis, and Textilis (Maréchal *et al.,* 1978). Cowpea is a member of the African *Vigna* (subgenus *Vigna*) and is genetically related to Asian *Vigna* (subgenus Ceratotrotopis) including azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi) and mungbean (*Vigna radiata* (L.) Wilczek) (Tomooka *et al.,* 2002).

Domestication-related Traits of Yardlong bean

Traits related to domestication of yardlong bean are similar to those in other legumes. They include pod length, increased seed size, increased number of seeds per pods, increased leaf size, increased plant size, seed color, non-dormant seeds, non-shattering pods, early flowering, and early maturity. Pod length of yardlong bean is about ten times longer than other species in the genus *Vigna* in which yardlong bean belongs to. This trait is very much worth to deeping investigate.

Molecular Studies on Domestication of Yardlong bean

The genetic of domestication of yardlong bean has been studied using different molecular marker techniques. Genetics markers that reveal polymorphisms at the DNA level are known as molecular or DNA markers. In cowpea and yardlong bean, DNA markers such as restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs) or microsatellites have been used to study the genetics relationships (Fatokun *et al.,* 1993; Li *et al.,* 2001), develop genetic linkage map, and locate quantitative trait locus (QTL) for seed weight (Fatokun *et al.,* 1992), and aphid resistance (Myers *et al.,* 1996). The *Vigna* are reported that using SSR marker and QTL analysis such as cowpea, azuki bean, mung bean and common bean.

Simple sequence repeat (SSR)

Simple sequence repeat (SSR) or microsatellites are tandom repeats of DNA sequence of 1 to 6 base pairs (Akkaya *et al.,* 1992; Richard and Christopher, 1999). The repeat regions are generally composed of di- tri- tetra- and sometimes greater perfectly repeated nucleotide sequence (Tautz and Renz, 1984). SSR markers are generally very highly polymorphic, mainly based on the number of repeats at the same locus and can be easily assayed by PCR (Morgante and Olivieri, 1993). The high variability of repeated numbers among individuals led to the use of SSR marker for development of genome-specific DNA fingerprints (Powell *et al.,* 1996). SSR markers are codominant in nature (i.e. heterozygotes can be distinguished from dominant parental alleles). SSR has become the molecular markers of choice for a wide range of applications such as genetic mapping and genome analysis (Chen *et al.,* 1997; Li *et al.,* 2000).

Quantitative trait loci (QTL)

Quantitative trait loci (QTL) are genome region(s) that control traits showing continuous phenotypic variation. QTL analysis is done to determine association between phenotypic variation and genetic marker(s). Four methods are popularly used to identify QTL, although several methods are available.

1. Single marker analysis. This method uses t-test, ANOVA, likelihood ratio test, or regression to compare the phenotypic means of genotypes at each marker. Statistical difference indicates association between a marker and the QTL. Single marker analysis can be done without marker order and linkage map. However, the method cannot locate the QTL and QTL effect estimated from this method is usually biased.

2. Simple interval mapping (SIM). SIM can be analyzed using likelihood (Lander and Botstein, 1989), regression (Haley and Knott, 1992) and the combination of both likelihood and regression (Kearsey and Hyne, 1994). The method determines

the likelihood of the presence of a QTL flanked by a pair of markers. The likelihood statistics are used to test the presence of QTL, and the maximum likelihood or minimum variance residue is used to indicate the location of the QTL. QTL location can be identified by SIM with more precise QTL effect, compare to single marker analysis.

3. Composite interval mapping (CIM). CIM is proposed by Zeng (1994) to replace SIM for estimating less biased location and effect of QTL when more than one QTLs locate on the same chromosome. CIM analysis uses interval mapping and multiple regressions. Markers other than the flanking markers are also included in the analysis as cofactor to “absorb” the effect of the other QTL and residual variance (Zeng, 2005). Location and effect of QTL estimated by CIM is more precise than SIM.

4. Muliple interval mapping (MIM). MIM uses multiple marker intervals simultaneously to construct multiple putative QTLs in the model for QTL mapping which tends to be more powerful and precise in detecting QTL than SIM and CIM. MIM can readily search for and analyze epistatic QTL and estimate the individual genotypic value and the heritabilities of quantitative traits. On the basis of the MIM result, genetic variance components contributed by individual QTL were also estimated, and marker-assisted selection can be performed (Kao *et al.,* 1999).

MATERIALS AND METHODS

Mapping population

Mapping populations comprised BC_1F_1 and F_2 populations. They were derived from a cross between yardlong bean accession “JP81610” and wild cowpea (*V. unguiculata* ssp. *unguiculata* var. *spontanea;* Andersson and de Vicente, 2010) accession “TVnu457”. JP81610 is a landrace from Sri Lanka, whereas TVnu457 (or JP89083) originated from Africa. Both accessions were obtained from the Gene bank, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. TVnu457was pollinated onto JP81610 to produce F_1 hybrid plants. An F_1 plant was self-pollinated to produce the F_2 population and, at the same time another F_1 plant was crossed as female parent with JP81610 to develop the BC_1F_1 population. One hundred and eighty-eight F_2 plants were grown under natural condition at an experimental field of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand from October 2008 to February 2009. While each of 190 plants of the BC_1F_1 population were grown in a 20 cm diameter pot in a vinyl greenhouse of the Gene bank, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan from May to August 2009. The BC_1F_1 population was treated with a short-day treatment of 11 hour day length after sowing for a month. The treatment was maintained until the last plant began to flower. Plants were maintained under ambient temperature and humidity.

DNA extraction

Total genomic DNA of parents, BC_1F_1 and F_2 populations were extracted from fresh leaf tissue using the CTAB method (Lodhi *et al.*, 1994). DNA concentration was estimated and adjusted to 5 ng/µl for SSR analyses by comparing with a known concentration of standard λ-DNA on 1.5% agarose gel.

Trait measurement

A total of 24 traits related to fitness and domestication were evaluated following Kaga *et al.* (2008) (Table 1). Of these, 21 were treated as quantitative traits and three, pod dehiscence, epicotyl color and seed coat color, were treated as qualitative traits. The F_2 population of 188 plants, together with 10 plants of each parent, were grown in a net house at the experimental field of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand (13°48'N, 99°5'E) from October 2008 to February 2009. The BC_1F_1 population of 190 plants, together with 10 plants of each parent, were grown in 20 cm diameter pots in a vinyl greenhouse of the Gene bank, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan (36°2'N, 140°8'E) from May to August 2009.

In both F_2 and BC_1F_1 populations, the seedling traits i.e. primary leaf length (LFPL), primary leaf width (LFPW), epicotyl color (ECC), and epicotyl length (ECL) were recorded when the first trifoliate leaf opened. While stem length (STL), stem thickness (STT) and branch number (BRN) were recorded when the tenth trifoliate leaf was fully developed. Whole stem length (STLW) was an additional trait evaluated only in the BC_1F_1 population (Table 1).

The seed-related traits were investigated using seeds and pods from both F_2 and BC_1F_1 plants. Seed coat permeability (SDP) as an index of seed dormancy was determined using ten unscarified seeds from each BC_1F_1 plants. The seeds were placed on filter paper, incubated at 25°C for 7 days, and the number of seeds that imbibed water was counted daily. Seed dimensions: seed length (SDL), seed width (SDW) and seed thickness (SDT), were the averaged of 10 seeds. The 100-seed weight (SD100WT) was evaluated using intact seeds of each plant.

The pod traits i.e. pod length (PDL), pod width (PDW), pod dehiscence (PDD), and number of twists along the length of dehisced pods (PDT) when kept at room temperature, were evaluated from 10 pods of each plant. The PDT was used as an index of pod structure. Pod dehiscence was scored as dehiscent or indehiscent on the basis of whether seeds shattered from pods or not.

The number of days from planting to first flowering (FLD) was recorded. The days to first mature pod (PDDM) was defined as number of days from first flowering to harvesting of the first mature pod. After harvesting all pods, total pod number (PDTN), and total seed weight (SDTWT) were measured in each plant. The number of seeds per pod (SDNPPD) was recorded using 10 pods. The spacing between seeds (PDSBS) was evaluated with the formula: [(PDL*10) – (SDL*SDNPPD)]/SDNPPD using the data from 10 pods. Seed color from each plant was scored as black or brown.

Table 1 Domestication-related traits examined in BC_1F_1 and F_2 populations of the cross between yardlong bean and wild cowpea (based on Kaga *et al*. 2008. Genetics. Table1)

General attribute	Organ	Trait	Trait abbreviation	QTL/ gene	Evaluation method	Evaluated population
Seed dormany	Seed	Seed – coat permeability (%)	SDP	*Sdp*	Percent of imbibed seeds at 25°C in incubator (use 20 seeds)	$BC_1F_{1:2}/F_{2:3}$
Pod dehiscence	Pod	Number of twists (count)	PDT	*pdt*	Number of twists along the length of the shattered pod	BC_1F_1
		Pod – dehiscence	PDD	*Pdd*	Dehiscence or indehiscence	F_2
Gigantism	Seed	100 seed weight (g)	SD100WT	*Sd100wt*	Weight of 100 seeds (use 100 seeds)	$BC_1F_{1:2}/F_{2:3}$
		Length (mm)	SDL	*Sdl*	Maximum distance from top to bottom of the seed (use 10 seeds)	$BC_1F_{1:2}/F_{2:3}$
		Width (mm)	SDW	*Sdw*	Maximum distance from hilum to its opposite side (use 10 seeds)	$BC_1F_{1:2}/F_{2:3}$

Table 1 (Continued)

General attribute	Organ	Trait	Trait abbreviation	QTL/ gene	Evaluation method	Evaluated population
		Thickness (mm)	SDT	*Sdt*	Maximun distance between both sides of the hilum (use 10 seeds)	$BC_1F_{1:2}/F_{2:3}$
	Pod	Length (cm)	PDL	*Pdl*	Length of straight pod (use 10 pods)	BC_1F_1/F_2
		Width (cm)	PDW	*Pdw*	Maximum width (use 10 pods) measure after soaking in water to flatten the pod to measure	BC_1F_1/F_2
		Spacing between seeds (mm)	PDSBS***	*Pdsbs*	Spacing between seeds is calculated by formula: [(PDL*10) – (SDL*SDNPPD)]/SDNPPD	BC_1F_1/F_2
	Stem	Thickness (mm)	STT	*Stt*	Stem diameter under the primary leaf (measured at flowering stage)	BC_1F_1/F_2
	Leaf	Primary leaf length (cm)	LFPL	*Lfpl*	Distance from pulvinus to leaf tip	BC_1F_1/F_2

Table 1 (Continued)

General attribute	Organ	Trait	Trait abbreviation	QTL/ gene	Evaluation method	Evaluated population
		Primary leaf width (cm)	LFPW	*Lfpw*	Maximum width	BC_1F_1/F_2
Plant type	Epicotyl	Length (cm)	ECL	*Ecl*	Length from cotyledon to primary leaf	BC_1F_1/F_2
	Stem	Length (up to 10th node) (cm)	STL10	*Stl10*	Length from node on primary leaf to node 10 of trifoliate leaf	BC_1F_1/F_2
		Length (whole)	STLW ***	*Stlw*	Length from node on primary leaf to terminal shoot	BC_1F_1
	Branch	Number (count)	BRN	*Brn*	Number of branches on main stem from node 1 to node 10 of trifoliate leaf (measured at post maturity stage just before discarding)	BC_1F_1/F_2
Earliness	Flower	Days to first flower (day)	FLD	*Fld*	Number of days from planting to 1st flowering	BC_1F_1/F_2

Table 1 (Continued)

General attribute	Organ	Trait	Trait abbreviation	QTL/ gene	Evaluation method	Evaluated population
	Pod	Days to maturity of 1st pod (day)	PDDM	*Pddm*	Number of days from 1st flowering to harvesting of 1st pod	BC_1F_1/F_2
Yield potential	Seed	Total weight (g)	SDTWT	*Sdtwt*	Total weight of harvested seeds	BC_1F_1/F_2
		Number of seeds per pod (seeds/pod)	SDNPPD	*Sdnppd*	Number of seeds per pod	BC_1F_1/F_2
	Pod	Total number (pod)	PDTN	*Pdtn*	Total number of harvested pods	BC_1F_1/F_2
Pigmentation	Epicotyl	Epicotyl color	ECC	*Ecc*	Red or green	BC_1F_1/F_2
	Seed	Seed coat color	SDC	*Sdc*	Black or brown	BC_1F_1/F_2

*** not included in Kaga *et al.*, 2008

Data analysis

Mean, standard deviation, and broad-sense heritability were calculated, and the frequency distribution of phenotypes in the BC_1F_1 and F_2 populations were examined for each trait. The correlation coefficient (r) between traits was also calculated. The total number of seeds per plant was used as an index of seed productivity and treated as the dependent (Y) variable. Since stem length, branch number, leaf size (the product between maximum length and width), 100-seed weight, pod length, total number of pods per plant, and number of seeds per pod are possibly correlated with total number of seeds, these traits were treated as the independent (X) variables.

Development of cowpea SSR markers

Cowpea genomic sequences, 260,642 in total, were downloaded from the CGKB database (Chen *et al.,* 2007; http://cowpeagenomics.med.virginia.edu/CGKB/) and were searched for SSR motifs using the Read2Marker program with default parameter (Fukuoka *et al.,* 2005). Redundant genomic sequences in the database were automatically removed by the program and finally 6,580 unique SSR motifs were identified. From those sequences, 1,900 SSR primer pairs were successfully designed using Primer3 with default parameter (Rozen and Skaletsky, 2000). Among these, SSR primers for sequences carrying more than 26 repeats were screened for polymorphisms between the two parents. It should be noted that the cowpea SSR markers reported by Gupta and Gopalakrishna (2010), Xu *et al.* (2010), Xu *et al.* (2011) and Andagie *et al.* (2011) also used sequences from CGKB database. Although our and those studies used different criteria and software for primer design, some of the primers may detect the same SSR.

SSR marker analysis

Four hundred and eighty cowpea SSRs were developed in this study (Appendix Table 1), 315 azuki bean SSRs (Wang *et al.,* 2004) and 231 mungbean SSRs (Somta *et al.,* 2009; Tangphatsornruang *et al.,* 2009) were screened for polymorphism between the parents. PCR mixture in a total volume of 5 µl, containing 1.0 µl of template DNA, 2.5 µl of 2xQIAGEN Multiplex PCR Master Mix, 1.0 µl of Q-solution, 0.2 µl of 20 ρmol primers mix, and 0.2 µl of Taq. The 5'-end of the reverse primer was fluorescent labeled with one of the three following fluorescent dyes, Fam, Hex and NED (Applied Biosystems, Foster City, CA, USA). PCR reactions were performed in a GeneAmp PCR System 9700 (Applied Biosystems). The PCR thermal cycling for mungbean and azuki bean SSRs was set as described by Somta *et al.* (2008) and Wang *et al.* (2004), respectively. In the case of cowpea SSRs, thermal cycling was programmed as follows: 95°C for 15 min followed by 40 cycles of 94°C for 30 s, 50°C for 60 s, 72°C for 60 s and a final cycle at 72°C for 10 min. After amplification, 1µl of PCR product was mixed with 10 µl of Hi-Di formamide and 0.125 µl of Rox size standard (Applied Biosystems), and run on an ABI Prism 3100 or 3130xl Genetic Analyzer (Applied Biosystems). Allele size for the highest stutter peak with the height ranging between 500 and 10,000 RFU were recorded and used to create bins for automatic assignment of genotypes. The genotyping was conducted by the GeneMapper 3.0 software (Applied Biosystems) with default settings.

After marker screening, two or four differentially labeled primers were mixed into single PCR reaction mixture and amplified. Fluorescent signal strengths of each amplified fragment were leveled by increasing non-fluorescent labeled primer pairs while reducing the labeled primers. Such multiplex sets were used to genotype the BC_1F_1 population with the same PCR amplification and detection described above.

Linkage map construction

BC_1F_1 population was used for linkage map construction and QTL analysis; whereas the F_2 population was used for confirmation of the QTL results. The linkage map of the BC_1F_1 and an F_2 populations was constructed with JoinMap 4.0 (Van Ooijen, 2006). For each marker, chi-square analysis was calculated for goodness-of-fit to a 1:1 for BC_1F_1 population and 1:2:1 for F_2 population, segregation ratio of genotypic classes at P = 0.05, 0.01 and 0.001. Markers were assigned to a linkage group based on recombination frequencies and LOD values. A minimum LOD score of 3 and recombination frequency of 0.25 were used. The recombination frequencies were converted into map distances (cM) using the mapping function of Kosambi (1944). Double crossovers between adjacent loci were confirmed manually. Linkage groups were named following azuki bean linkage groups (Han *et al.,* 2005).

QTL analysis

QTL analysis was conducted using the software package MultiQTL ver. 2.6 according to the procedures described by Kaga *et al.* (2008). Initially, the entire genome was scanned for QTLs using general interval mapping with the following approach. First, a single QTL model was fitted for each trait-chromosome (linkage group) combination. Chromosome-wise statistical significance thresholds (*P*= 0.001) for declaring putative QTL were determined by 10,000 runs of a permutation test (Churchill and Doerge, 1994), and parameters (position, additive effect, and the percentage of explained variance) for significant QTL were obtained.

Multiple interval mapping (MIM) (Kao *et al.,* 1999) was then conducted to reduce the background variation by taking into account QTL effects from other chromosomes. On the basis of parameters defined for each putative QTL above, the chromosome was included or removed iteratively into/from the MIM model at a more stringent level of significance (*P* = 0.01) than default. The stepwise selection of chromosomes based on significance using a permutation test was repeated until the process converged when no QTL on the remaining chromosomes was found. The QTL effects were reevaluated by fitting all positive QTL in the order of their power and by a global permutation test (10,000 runs) to obtain more precise estimates of significance. QTLs were named following Somta *et al.* (2006). Randomness of the genomic distribution of the QTLs was determined using Chi-square tests (Isemura *et al.,* 2007). Randomness of the QTLs along a linkage group was tested using Poisson distribution test (Isemura *et al.,* 2007).

RESULTS

SSR markers transferability and segregation distortion

In total, 1,026 SSR markers from three legume crops were screened to reveal polymorphism between the yardlong bean and wild cowpea parents. Single locus amplification of markers from cowpea, mungbean and azuki bean was successful at the rate of 67.9%, 55.9% and 57.7%, respectively (Table 2). Of these, 465 markers revealed polymorphism between the parents with the highest percentage of markers from cowpea, followed by azuki bean and mungbean. Among these polymorphic markers, 226 were successfully used in multiplex PCR analysis for genotyping the BC_1F_1 population.

Chi-square analysis for the goodness-of-fit of marker segregation demonstrated that 204 (90.3%) markers segregated into the expected 1:1 ratio while 22 (9.7%) showed significant segregation distortion at $P \leq 0.05$ (Appendix Table 2). Seventeen (77.3%) of the distorted markers showed an excess of homozygosity for yardlong bean alleles, while the rest showed an excess of heterozygous genotypes.

Construction of SSR linkage map

All the 226 polymorphic SSR marker loci (165 from cowpea, 46 from azuki bean and 15 from mungbean) could be assigned to 11 linkage groups (LGs) which correspond to the haploid number of chromosomes of yardlong bean (Figure 1). The linkage groups spanned 852.4 cM in total length, with an average distance between adjacent markers of 3.96 cM (Table 3). The length of the linkage groups ranged from 43.1 cM (LG11) to 147.3 cM (LG1), with an average of 77.5 cM per LG. The number of marker loci per LG varied from 10 (LG6) to 35 (LG1), with an average of 20.5 loci per LG. All LGs except LG11 had a length of 50 cM or longer (Table 3; Figure 1). Four gaps (distance between the adjacent markers greater than 15 cM) existed on the map, one each on LG1 (cp05566-cp04208), LG4 (cp03825-CEDG127), LG6 (cp00080-cp01038) and LG9 (cp05238-CEDG172) (Figure 1). The largest gap was 34.1 cM in LG6.

Seven linkage groups contained at least one distorted marker including LG1, LG3, LG5, LG6, LG8, LG10 and LG11 (Figure 1). Of the 22 distorted markers, one each (4.54%) was located on LG1, LG5, LG6, LG8 and LG10, 4 (18.18%) were located on LG3, and 13 (59.09%) were located on LG11 (Appendix Table 2). All the markers on LG11 showed distorted segregation.

The F_2 map was constructed using 113 SSR markers (78 from cowpea, 26 from azuki bean and 9 from mungbean) covering 977.1 cM with an average distance between the adjacent markers of 9.58 cM (Table 4; Figure 2). The length of the LGs range from 28.3 cM (LG11) to 140.4 cM (LG1) with a mean of 88.83 cM. The number of marker loci per LG varied from 6 (LG9) to 16 (LG1) with the mean of 10.27 loci. The length of the F_2 map was equivalent to 114.6% of the BC_1F_1 map. All of the LGs except LG11 had a length of 60 cM or longer. The largest gap of 41.3 cM was on LG9. Segregation distortion was observed at 48.7% (55 out of 113) of mapped markers ($P< 0.05$). The distorted markers were distributed on all LGs (Figure 2.). In most cases, on the same LG the distorted markers appeared to be clustered or close to one another. All the markers on LG11 and nine out of eleven (81.8%) markers on LG7 showed distortion (Appendix Table 3). Orders and linkages of all the markers were the same in both linkage maps.

Table 2 Amplification and polymorphism of SSR markers from azuki bean, cowpea and mungbean in cultivated yardlong bean accession JP81610 and wild cowpea accession TVnu457

SSR sources	No. of SSRs screened	No. of SSRs amplified single locus in both parents (%)	No. of polymorphic SSRs (%)[1]	No. of SSRs successfully used in multiplex PCR (%)[2]
Cowpea	480	326 (67.9)	297 (91.1)	165 (55.6)
Azuki bean	315	179 (55.9)	112 (62.6)	46 (41.1)
Mungbean	231	133 (57.7)	56 (42.1)	15 (26.8)
Total	1,026	638 (62.2)	465 (72.9)	226 (48.6)

[1] (No. of polymorphic SSRs/ No. of SSRs amplified single locus in both parents) x 100

[2] (No. of SSRs successfully used in multiplexed PCR/ No. of polymorphic SSRs) x 100

Table 3 Number of markers and average distance between markers in each linkage group in the yardlong bean map developed from the BC_1F_1 population derived from the cross between cultivated yardlong bean and wild cowpea

Linkage group	Length (cM)	Average interval (cM)	No. of SSR markers			
			Total	cowpea	azuki bean	mungbean
1	147.3	4.33	35	26	9	0
2	76.4	4.29	22	18	3	1
3	78.8	3.94	21	12	6	3
4	67.4	3.37	21	15	5	1
5	64.9	2.95	23	17	4	2
6	79.5	8.84	10	7	2	1
7	85.6	4.75	19	15	1	3
8	86.0	2.96	30	21	5	4
9	60.3	5.02	13	10	3	0
10	63.2	3.51	19	13	6	0
11	43.1	3.59	13	11	2	0
Total	852.4	3.96	226	165	46	15

Table 4 Number of markers and average distance between markers in each linkage group in the yardlong bean map estimated from F_2 population derived from the cross between cultivated yardlong bean and wild cowpea

Linkage group	Length (cM)	Average interval (cM)	No. of SSR markers			
			Total	cowpea	azuki bean	mungbean
1	140.4	9.36	16	12	4	0
2	93.4	8.49	12	9	3	0
3	105.7	8.13	14	10	3	1
4	87.2	10.90	9	5	4	0
5	73.7	9.21	9	6	1	2
6	107.7	13.46	9	6	2	1
7	107.1	10.71	11	8	0	3
8	103.4	11.49	10	7	1	2
9	70.5	14.09	6	5	1	0
10	59.8	6.64	10	5	5	0
11	28.3	4.72	7	5	2	0
Total	977.1	9.58	113	78	26	9

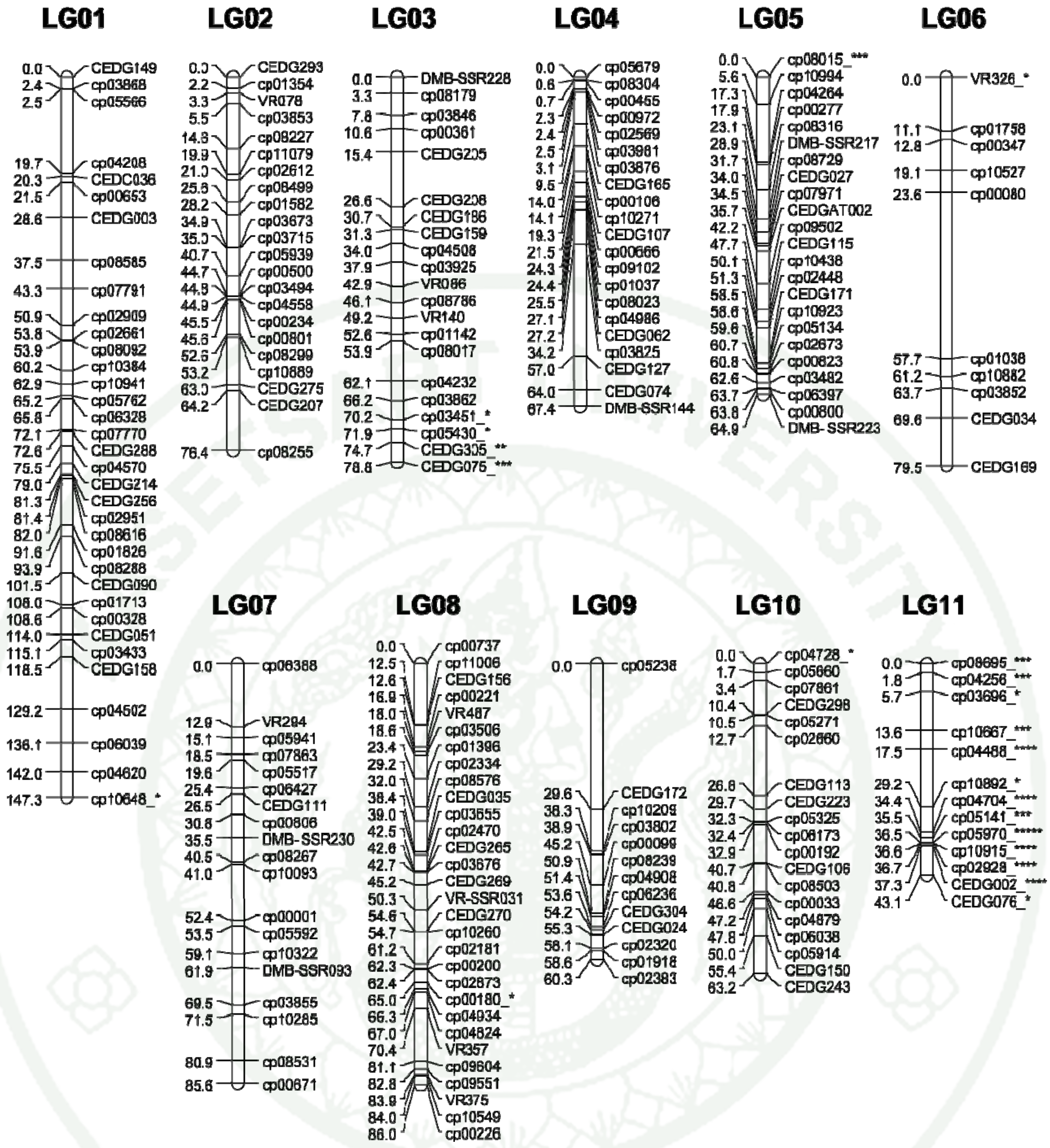

Figure 1 An SSR genetic linkage map of yardlong bean constructed from a BC_1F_1 population. This map was constructed from 190 BC_1F_1 individuals of (*V. unguiculata* ssp. *unguiculata* cv.-gr. *sesquipedalis* x wild *V. unguiculata* ssp. *unguiculata* var. *spontanea*) x *V. unguiculata* ssp. *unguiculata* cv.-gr. *sesquipedalis*. Map distances (cM) are shown on the left side and marker names are shown on the right side of the linkage groups. “cp-” and “CED-” represent the SSR marker loci from cowpea and azuki bean, respectively,“DMB-SSR-”, “VR-”, and “VR-SSR-” represent the SSR marker loci from mungbean. *,** and *** indicate significant segregation distortion at the 5%, 1% and 0.1% significance levels, respectively.

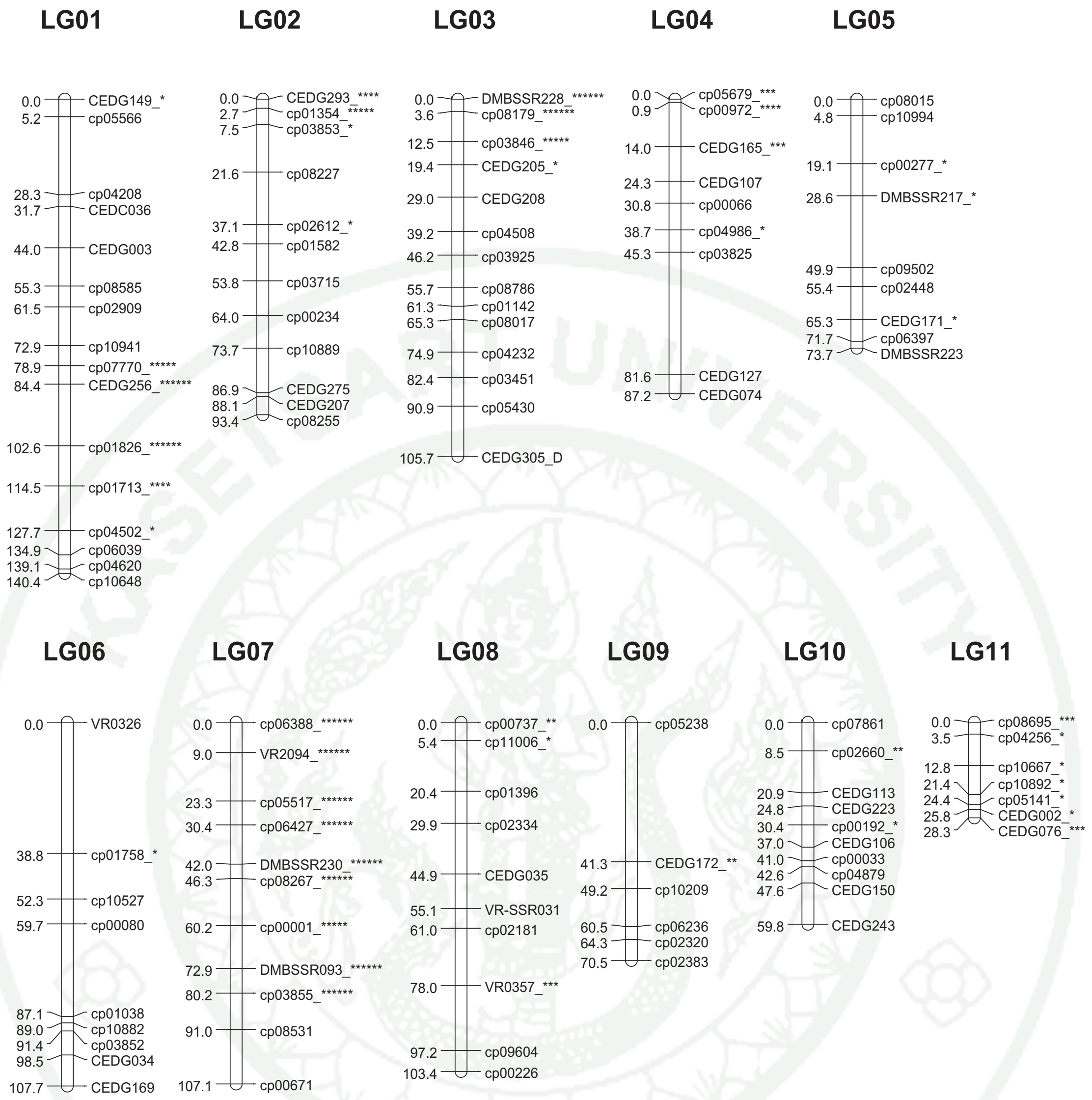

Figure 2 An SSR genetic linkage map of yardlong bean constructed from an F_2 population. This map was constructed from 188 F_2 individuals of (*V. unguiculata* ssp. *unguiculata* cv.-gr. *sesquipedalis* x *V. unguiculata* ssp. *unguiculata* var. *spontanea*). Map distances (cM) are shown on the left side and marker names on the right side of the linkage groups. “cp-” and “CED-” represent the SSR marker loci from cowpea and azuki bean, respectively, and “DMB-SSR-”, “VR-”, and “VR-SSR-” represent the SSR marker loci from mungbean. *,** and *** indicate significant segregation distortion at the 5%, 1% and 0.1% significance levels, respectively.

Variation of domestication-related traits

The mean, range, standard deviation of traits in the parents, $BC_1F_1$and F_2 populations as well as their broad-sense heritabilities are shown in Table 5 and Table 6. The mean of BC_1F_1 population was between their parents for all traits except days to first flowering and number of seeds per pod. Similarly, the mean of F_2 population generally fell between those of the parents for all traits except days to maturity of first pod and number of seeds per pod. The parents were clearly different in all traits observed. The cultivated parent was higher or larger than the wild parent in seed coat permeability, number of twists along the pod, stem length (1^{st} to 10^{th} nodes and the 1^{st} node to terminal shoot) and the size of organs such as leaf, stem, seed and pod. A pronounced difference was observed for SD100WT, PDL, PDW, PDSBS, STL10 and PDTN. In the case of qualitative traits, the cultivated parent had black seed coat and indehiscent pod whereas the wild parent had black mottle over tan seed coat and pods were dehiscent. Both parents had green epicotyls.

The measured traits showed nearly normal distribution in both populations (Appendix Figure 1 and 2). The F_2 mean for percentage of imbibed seeds (SDP) showed a biased distribution towards the cultivated parent. Clear transgressive segregation was observed in STT, PDSBS, ECL, STL10, STLW, BRN, FLD, PDDM, SDTWT, SDNPPD and PDTN in BC_1F_1 or F_2 or both populations.

Generally, the traits measured showed high broad-sense heritability (Table 5 and 6). SDP, SDTWT, SDL, SDW, PDL, PDSBS and STL10 showed high heritability (>70%) whereas STT, LFPL, LFPW, ECL, FLD and PDDM showed medium to low heritability (<70%). SDT and PDW showed high heritability in only the BC_1F_1 population, while SDTWT, SDNPPD and PDTN showed high heritability in only the F_2 population.

There were significant positive correlations ($P < 0.05$) between related traits, such as between pod length and pod width, and between seed size-related traits and pod size-related traits (Appendix Table 4 and 5). PDT and PDTN were negatively correlated with seed-related traits, STLW and earliness traits (FLD and PDDM).

Table 5 The mean, standard deviation, minimum, maximum and heritability values of parents, the BC_1F_1 or $BC_1F_{1:2}$ populations derived from the cross between cultivated yardlong bean and wild cowpea

Traits	Units	Cultivated yardlong bean, JP81610				Wild cowpea, TVnu457				BC_1F_1 or $BC_1F_{1:2}$				Heritability (%)
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	
SDP	(%)	97.7	2.7	93.0	100.0	0.0	0.0	0.0	0.0	46.9	31.4	0.0	100.0	99.6
PDT	count	0.0	0.0	0.0	0.0	6.2	0.3	5.8	6.6	3.4	4.6	0.0	13.0	99.9
SD100WT	g	17.5	1.4	14.4	19.6	2.4	0.2	1.8	2.6	10.5	1.9	6.4	18.7	71.4
SDL	mm	11.2	0.4	10.5	11.9	3.6	0.1	3.5	3.9	8.9	0.8	4.6	10.7	85.2
SDW	mm	6.3	0.2	5.9	6.7	2.5	0.1	2.3	2.6	5.0	0.4	3.9	5.9	78.1
SDT	mm	4.1	0.2	3.9	4.4	1.8	0.1	1.7	1.9	3.4	0.2	2.7	4.1	72.1
PDL	cm	64.4	5.7	56.8	73.9	8.5	0.3	8.0	9.0	31.6	7.8	15.1	57.1	73.1
PDW	cm	1.3	0.1	1.2	1.4	0.5	0.0	0.5	0.5	0.9	0.1	0.7	1.3	88.2
PDSBS	mm	30.0	3.5	25.1	38.1	3.1	0.7	2.1	4.0	17.4	6.7	4.4	40.2	86.1
STT	mm	7.4	1.1	5.7	9.1	5.4	1.0	4.2	6.8	6.0	1.1	3.9	8.5	-
LFPL	cm	8.0	0.7	7.2	9.3	3.4	0.3	2.9	4.0	5.9	0.5	4.3	7.5	6.3
LFPW	cm	5.5	0.3	5.0	5.9	1.8	0.3	1.2	2.2	3.7	0.3	2.8	4.5	13.9
ECL	cm	4.2	0.4	3.6	5.0	2.2	0.4	1.7	3.0	3.3	0.6	2.0	5.7	65.8
STL10	cm	177.8	14.1	157.0	195.5	63.6	6.3	53.0	73.0	142.4	32.9	61.0	219.5	89.0
STLW	cm	154.8	23.8	121.0	195.0	21.6	14.6	11.0	48.0	99.5	45.4	17.0	220.0	81.1
BRN	count	2.7	1.6	1.0	6.0	7.1	0.9	6.0	9.0	3.8	1.8	0.0	9.0	45.8
FLD	day	57.6	2.7	55.0	64.0	56.8	4.4	50.0	66.0	56.0	4.4	47.0	67.0	29.5

Table 5 (Continued)

Traits	Units	Cultivated yardlong bean, JP81610				Wild cowpea, TVnu457				BC$_1$F$_1$ or BC$_1$F$_{1:2}$				Heritability (%)
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	
PDDM	day	18.4	1.5	16.0	21.0	12.3	0.9	11.0	14.0	15.9	1.7	7.0	24.0	47.2
SDTWT	g	15.4	5.1	10.9	28.0	12.8	4.2	7.0	22.4	15.7	5.5	4.6	31.5	27.8
SDNPPD	count	15.7	2.2	11.6	19.8	12.9	1.1	11.3	14.3	12.5	3.2	5.1	19.5	69.7
PDTN	count	6.7	2.2	3.0	10.0	89.3	21.6	47.0	119.0	17.3	8.1	2.0	50.0	-
ECC	-	Green				Green				Green				
SDC	-	Black				Brown				Black				

Table 6 The mean, standard deviation, minimum, maximum and heritability values of parents, F_2 or $F_{2:3}$ populations derived from the cross between cultivated yardlong bean and wild cowpea

Traits	Units	Cultivated yardlong bean, JP81610				Wild cowpea, TVnu457				F_2 or $F_{2:3}$				Heritability (%)
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	
SDP	(%)	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	78.2	36.5	0.0	100.0	100.0
PDD	-	Indehiscence				Dehiscence				Dehiscence:Indehiscence = 111:77(ratio=9:7, X2 = 0.60, P=0.44)				
SD100WT	g	19.4	1.4	16.6	20.9	2.5	0.2	2.2	2.7	5.7	1.9	2.0	16.1	72.6
SDL	mm	11.9	0.4	11.3	12.5	3.9	0.2	3.6	4.1	7.2	1.1	4.3	9.9	92.5
SDW	mm	3.7	0.2	3.3	4.0	2.0	0.1	1.8	2.2	2.7	0.3	0.8	3.8	81.0
SDT	mm	6.2	0.2	5.9	6.5	2.7	0.3	2.4	3.9	4.0	0.5	2.6	5.8	65.7
PDL	cm	78.1	3.1	72.9	84.0	9.9	0.4	8.8	10.4	21.3	5.8	10.2	41.3	85.7
PDW	cm	11.5	0.8	10.0	13.0	0.5	0.0	0.4	0.5	0.7	0.1	0.5	1.0	49.9
PDSBS	mm	26.6	1.8	23.8	30.8	1.9	0.2	1.6	2.2	9.9	3.7	2.1	26.3	87.8
STT	mm	15.3	2.0	11.2	18.4	9.7	1.8	6.3	11.9	13.4	2.0	7.4	18.8	15.1
LFPL	cm	7.6	0.7	6.5	8.8	2.9	0.4	2.2	3.4	4.7	0.6	2.4	6.1	18.0
LFPW	cm	5.2	0.5	4.5	6.0	1.6	0.2	1.2	2.0	2.9	0.4	1.9	3.7	2.6
ECL	cm	6.6	0.9	5.0	8.0	4.2	0.5	3.2	5.0	5.2	1.1	2.5	8.5	62.2
STL10	cm	120.8	9.5	105.0	134.3	30.0	9.5	16.0	46.0	68.3	19.9	30.0	118.5	77.1
FLD	day	55.1	3.5	50.0	62.0	43.9	5.9	37.0	62.0	43.9	4.3	37.0	58.0	-
PDDM	day	18.6	4.1	15.0	28.0	14.6	3.3	9.0	19.0	20.6	2.2	15.0	29.0	-

Table 6 (Continued)

Traits	Units	Cultivated yardlong bean, JP81610				Wild cowpea, TVnu457				F_2 or $F_{2:3}$				Heritability (%)
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	
SDTWT	g	209.0	55.5	115.0	307.5	94.9	23.3	54.3	131.8	161.1	99.8	12.9	631.1	81.8
SDNPPD	count	20.3	0.7	19.1	21.6	17.3	0.6	15.9	17.9	12.8	3.0	7.0	20.3	95.7
PDTN	count	128.5	20.3	59.0	130.0	504.8	131.9	248.0	701.0	461.9	248.6	15.0	1609.0	85.6
ECC	-	Green				Green				Green				
SDC	-	Black				Brown				Brown:Black = 33:155 (ratio=3:13, X^2 = 0.18, P=0.67				

QTLs for domestication-related traits

The results of the QTL analysis for each trait in each population are shown in Table 7. Only QTLs with $P \leq 0.05$ were considered. In total, 153 QTLs were identified for the 21 traits. One to eleven QTLs were detected for each trait except for BRN and SDTWT in the BC_1F_1 population, from which no significant QTL was detected.

Seed coat permeability (SDP): One key trait in domestication is reduction or loss of seed dormancy that enables uniform germination. Like several cereal and legume crops, domestication of cowpea/yardlong bean has resulted in reduced seed dormancy. SDP in the yardlong bean and wild cowpea were 100% and 0%, respectively. Six QTLs were detected on LGs 1, 2, 4, 7, 8 and 11, where all alleles, except *Sdp7.1*- of the yardlong bean parent increase percentage of permeable seeds. The QTL located on LG1 had the largest effect on phenotypic variance explained (Table 7).

Pod dehiscence (PDT and PDD): Loss of pod dehiscence in legume crops is advantageous for harvesting seeds but reducing seed dispersal in wild species. Pod dehiscence can be characterized as qualitative or quantitative trait using visual score (PDD; dehiscent vs. indehiscent) or number of twists along the shattered pod (PDT).

The qualitative nature of dehiscence was examined only in the F_2 population (Table 6) and the progenies segregated for PDD in the ratio 111 dehiscent to 77 indehiscent plant. The segregation ratio fitted a 9:7 ($\chi^2 = 0.60$, P =0.44) indicating that duplicated recessive genes control pod indehiscence.

The quantitative nature of pod dehiscence was investigated only in the BC_1F_1 population (Table 5). The yardlong bean JP81610 had no twist on the pod while the wild cowpea TVnu457 had twists along the pod. Four QTLs were detected on LGs 1, 4, 7 and 9. Of these QTLs, the one on LG7 had the largest effect on phenotypic variance. Alleles of the cultivated parent reduce the number of twists on the pod at all QTLs.

Organ size (seed size, pod size, spacing between seeds and stem thickness): Domestication of yardlong bean has resulted in about an 8-fold increase in seed weight (Table 6). Eight to ten QTLs for traits related to seed size (SD100WT, SDL, SDW and SDT) were located on all LGs. At all QTLs, alleles from the cultivated parent increase the size of each trait, except alleles of the QTLs on LG11 which decrease SD100WT, SDL and SDT. The QTLs with the largest phenotypic contribution for SD100WT (24.6%), SDL (26.5%) and SDW (20.9%) were located on LG7, and for SDT (26.4%) was located on LG3 (Table 7).

The most remarkable domesticated trait of yardlong bean is its length of pods. Domestication of yardlong bean has resulted in an about 8-fold increase in pod length. Seven to nine QTLs were identified for pod-related traits on LGs 1, 2, 3, 4, 5, 7, 8, 9 and 11 (Table 7). The alleles from the yardlong bean increased pod size at almost all QTLs. Alleles from the wild cowpea reduced the value of pod size only on LG9 for PDL and on LG11 for PDSBS. The QTLs located on LG7 had largest effect for PDL and PDW. For PDSBS, the QTLs with large effect were located on LGs 3, 7 and 11. Generally, QTLs for pod size were clustered with or located close to QTLs for seed size-related traits, especially the QTLs on LGs 3, 4, 7, 8 and 11 (Figure 3). The QTLs on LG7 for PDL, PDW and PDSBS were also located near to QTL for pod dehiscence.

Four QTLs for leaf length were found on LGs 3, 6, 7 and 11 in the BC_1F_1 population. However, only the QTL on LG11 was confirmed in the F_2 population. For leaf width, nine QTLs were detected on LGs 1, 2, 3, 6, 7, 8, 9 and 11. In both traits, the QTLs on LG7 had highest effect and were at the same position (Table 7, Figure 3).

Nine QTLs for stem thickness were detected on LGs 1, 2, 3, 4, 6, 7, 9, 10 and 11. The QTL on LG7 had the highest effect. Except for the QTLs on LGs 1, 9, 10 and 11, the cultivated alleles at these QTLs increased STT.

Growth habit (epicotyl length: ECL, stem length within the first 10 internodes: STL10 and whole stem length: STLW): ECL, STL10 and STLW in the cultivated parents were higher than those in the wild parent. Six QTLs for ECL were found on LGs 2, 4, 6, 7, 9 and 10. The cultivated alleles at QTLs *Ecl2.1-*, *Ecl6.1-* and *Ecl9.*1- decreased epicotyl length. Eleven QTLs for STL10 were detected on all LGs. The cultivated alleles at QTLs *Stl102.1-*, *Stl105.1-*, *Stl106.1-*, *Stl108.1-* and *Stl109.1-* lowered internode length. Seven QTLs for STLW were identified on LGs 1, 2, 3, 5, 6, 8 and 10. At QTLs *Stlw2.1-*, *Stl3.1-*, *Stlw5.1-*, *Stlw6.1-* and *Stlw8.1*, the alleles from the cultivated parent decreased upper internode length. For all growth habit traits, the QTLs on LG10 had the largest effect with alleles increasing trait values. The QTLs for epicotyl and internode lengths were located on the same or similar position on LGs 5, 7 and 10. QTLs for lower and upper internode lengths were also mapped to the same or similar location on LGs 2, 6 and 10.

Flowering time (FLD): Days to first flowering of the cultivated and wild parents were almost the same (58 vs. 57 days) in Japan, but relatively different (55 vs. 44 days) in Thailand. However, high variation existed in both populations. Ten QTLs were detected on all LGs except LG3 for FLD. QTLs on LGs 2, 10 and 11 had large effect on phenotypic variance. Unexpectedly, alleles from the cultivated parent at all QTLs but LG5 and LG11 delayed flowering.

Maturity time (PDDM): The cultivated parent had much larger seed and pod than the wild parent. It is expected that yardlong bean would take longer time to pod maturity than the wild parent due to more translocation of dry matter to seeds and pods. Six QTLs associated with days to maturity were found on LGs 1, 2, 3, 4, 6 and 7. The alleles from the cultivated parent that delayed maturity are on LGs 2, 3, 4, 6 and 7, whereas a QTL on LG1 hastened maturity. The QTL on LG3 had the largest effect and was located near the QTLs for seed- and pod-related traits.

Yield-related traits (PDTN, SDNPPD and SDTWT): The cultivated parent produced a markedly lower number of pods than the wild parent. Ten QTLs were detected on all LGs, except LG5. Of these QTLs, only at LG6 that the allele from the yardlong bean increased PDTN. This QTL had the largest effect accounting for 57.1% of the phenotypic variation.

Although the yardlong bean has much longer pod than the wild cowpea, the average seed number per pod is only slightly different (3 seeds). Two QTLs were found on LGs 7 and 11 for SDNPPD. The QTL on LG11 had largest effect explaining 70% of the phenotypic variation in the BC_1F_1 population. This QTL was co-located with QTLs for flowering time, stem thickness, leaf size, and seed- and pod-related traits. As expected, the alleles from the yardlong bean parent increased number of seeds per pod at both QTLs (Table 7, Figure 3).

The wild parent has smaller seed size but higher total seed weight than the cultivated parent. SDTWT was investigated only in the F_2 population. Six QTLs were found on LGs 2, 4, 5, 6, 7 and 10. The QTL with the largest effect was located on LG7. This QTL was clustered with stem-, seed- and pod-related traits. The alleles from the cultivated parent at all QTLs increased the total seed weight.

Seed coat color (SDC): Seed coat color was characterized as a qualitative trait. The cultivated parent has black seed coat whereas the wild parent has brown seed coat. F_2 progeny segregated for seed coat color at the ratio 33 (brown) to 155 (black). Although the segregation ratio fit a 3:13 (χ^2 = 0.18, P = 0.67), it also fit a 1:3 at P = 0.01 (χ^2 = 5.56, P = 0.018). The BC_1F_1 progenies showed no segregation and all had black seed coat. These suggested that seed coat color may be governed by dominant and recessive epistasis or by a single gene and black is dominant over brown. However, when the trait was mapped as a morphological marker, seed coat color was located near to marker cp03855 which showed high segregation distortion.

Distribution of domestication- related trait QTLs

Although it is not known whether all the 153 QTLs detected for domestication-related traits had independent actions on each trait, the observed number of QTLs was compared with the expected number based on each LG length (Table 8.) The observed and expected numbers agreed well throughout all LGs, except for LG11 that the number of QTLs was statistically higher than expected. The departure in LG11 caused the significance in total $\chi 2$ values for both BC_1F_1 and F_2 at 17.5 and 17.8, respectively.

Table 7 QTLs detected in the $BC_1F_{1:2}$, F_2 and $F_{2:3}$ populations derived from the cross between cultivated yardlong bean and wild cowpea

		$BC_1F_{1:2}$						$F_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
SDP	*Sdp1.1+*	1	27.3	0.0009	90.2	41.1	8.1	1						
	Sdp2.1+	2	4.3	0.0009	2.2	4.3	2.6	2						
	Sdp4.1+	4	2.2	0.0112	46.9	2.9	2.1	4			NI[1/]			
	Sdp7.1-	7	1.9	0.0300	30.8	1.9	-1.7	7						
	Sdp8.1+	8	3.1	0.0019	82.8	3.0	2.2	8						
	Sdp11.1+	11	4.9	0.0009	17.5	5.2	2.9	11						
PDT	*Pdt1.1-*	1	11.7	0.0009	93.4	11.8	-3.3	1			NI			
	Pdt4.1-	4	3.0	0.0009	24.5	2.6	-1.5	4						
	Pdt7.1-	7	30.1	0.0009	9.3	47.6	-6.5	7						
	Pdt9.1-	9	2.2	0.0084	58.1	1.9	-1.3	9						
SD100WT	*Sd100wt1.1+*	1	17.2	0.0009	116.2	11.0	1.3	1	18.9	0.0009	89.0	16.8	1.1	-0.5
	Sd100wt2.1+	2	8.4	0.0009	2.9	4.6	0.8	2	4.7	0.0009	45.7	3.6	0.5	0.3
	Sd100wt3.1+	3	12.4	0.0009	0.6	7.1	1.0	3	16.4	0.0009	11.5	18.7	1.2	0.1
	Sd100wt4.1+	4	10.6	0.0009	6.2	6.1	1.0	4	12.8	0.0009	24.3	8.9	0.7	0.4
	Sd100wt5.1+	5	5.7	0.0009	64.9	2.9	0.7	5						
	Sd100wt6.1+	6	5.1	0.0009	38.7	5.2	0.9	6						
	Sd100wt7.1+	7	31.2	0.0009	22.9	24.6	1.9	7	11.9	0.0009	51.6	13.1	1.0	0.3
	Sd100wt8.1+	8	9.5	0.0009	21.6	5.5	0.9	8	14.1	0.0009	23.7	11.4	0.9	0.0

Table 7 (Continued)

		BC$_1$F$_{1:2}$						F$_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Sd100wt10.1+	10	8.0	0.0009	28.9	4.3	0.8	10	4.4	0.0009	5.8	2.6	0.4	0.2
	Sd100wt11.1-	11	11.8	0.0009	31.9	7.6	-1.1	11						
SDL	*Sdl1.1+*	1	12.6	0.0009	89.8	10.4	0.5	1	29.4	0.0009	94.9	20.8	0.7	-0.1
	Sdl2.1+	2	6.4	0.0009	56.5	4.8	0.4	2	8.0	0.0009	47.7	2.8	0.2	0.2
	Sdl3.1+	3	8.8	0.0009	18.5	7.2	0.4	3	25.0	0.0009	8.6	18.8	0.7	0.3
	Sdl4.1+	4	16.0	0.0009	35.0	13.5	0.6	4	21.9	0.0009	24.3	7.5	0.4	0.1
	Sdl5.1+	5	3.8	0.0009	15.9	2.7	0.3	5	5.9	0.0009	49.9	1.6	0.2	0.0
	Sdl6.1+	6	2.4	0.0075	34.5	2.1	0.2	6	4.9	0.0009	7.3	1.7	0.2	0.2
	Sdl7.1+	7	13.6	0.0009	23.4	13.5	0.6	7	41.8	0.0009	46.3	26.5	0.7	0.5
	Sdl8.1+	8	12.7	0.0009	56.2	9.8	0.5	8	22.6	0.0009	23.9	9.1	0.5	0.0
	Sdl11.1-	11	11.9	0.0009	34.4	9.3	-0.5	11						
SDW	*Sdw1.1+*	1	22.1	0.0009	93.2	18.4	0.3	1	3.2	0.0047	114.5	4.1	0.1	0.0
	Sdw2.1+	2	11.1	0.0009	15.6	8.2	0.2	2						
	Sdw3.1+	3	19.6	0.0009	16.8	17.2	0.3	3	3.1	0.0094	25.8	4.2	0.1	0.1
	Sdw4.1+	4	4.8	0.0009	4.7	3.3	0.1	4	6.6	0.0009	58.3	11.7	0.2	0.0
	Sdw6.1+	6	2.5	0.0075	61.2	1.6	0.1	6						
	Sdw7.1+	7	22.9	0.0009	21.6	20.9	0.4	7	5.3	0.0009	63.4	18.5	0.2	0.2
	Sdw8.1+	8	6.7	0.0009	20.3	4.6	0.2	8	8.2	0.0009	29.9	8.8	0.1	0.0

Table 7 (Continued)

		$BC_1F_{1:2}$						$F_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Sdw10.1+	10						10	8.7	0.0009	23.3	10.0	0.2	0.0
SDT	*Sdt1.1+*	1	6.8	0.0009	123.9	6.4	0.1	1	12.6	0.0009	120.3	11.3	0.2	-0.1
	Sdt2.1+	2						2	9.0	0.0009	70.7	7.5	0.2	0.1
	Sdt3.1+	3	11.8	0.0009	0.0	10.5	0.2	3	21.4	0.0009	16.4	26.4	0.3	0.1
	Sdt4.1+	4	8.7	0.0009	3.8	7.7	0.1	4	5.5	0.0009	38.7	3.8	0.1	0.0
	Sdt6.1+	6	4.2	0.0009	39.4	5.3	0.1	6	5.6	0.0009	91.4	3.9	0.1	0.0
	Sdt7.1+	7	8.5	0.0009	39.4	7.5	0.1	7	14.2	0.0009	43.0	15.2	0.3	0.1
	Sdt8.1+	8	10.1	0.0009	20.4	9.5	0.1	8	12.1	0.0009	29.7	7.8	0.2	0.1
	Sdt9.1+	9	2.6	0.0009	51.4	2.1	0.1	9						
	Sdt10.1+	10	14.0	0.0009	38.6	13.8	0.2	10						
	Sdt11.1-	11	4.9	0.0009	29.2	4.2	-0.1	11						
PDL	*Pdl1.1+*	1	21.9	0.0009	81.3	12.1	5.4	1	22.3	0.0009	86.5	14.3	3.1	-1.2
	Pdl2.1+	2	2.2	0.0112	45.6	0.9	1.5	2						
	Pdl3.1+	3	13.0	0.0009	1.8	6.6	4.0	3	22.8	0.0009	15.1	19.9	3.6	1.8
	Pdl4.1+	4	6.2	0.0009	33.2	2.9	2.7	4	2.4	0.0280	38.7	0.9	0.7	0.7
	Pdl5.1+	5	9.6	0.0009	64.9	4.5	3.3	5	7.0	0.0009	49.9	3.1	1.5	0.0
	Pdl7.1+	7	42.4	0.0009	26.5	31.0	8.7	7	31.6	0.0009	33.9	26.9	4.4	0.1
	Pdl8.1+	8	15.7	0.0009	20.4	8.5	4.6	8	12.3	0.0009	23.6	6.9	2.1	0.9

Table 7 (Continued)

		$BC_1F_{1:2}$						$F_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Pdl9.1-	9	3.0	0.0019	29.6	1.3	-1.8	9						
	Pdl11.1+	11	23.0	0.0009	39.0	14.4	5.9	11	22.6	0.0009	24.4	10.4	1.7	-3.1
PDW	*Pdw1.1+*	1	17.8	0.0009	111.0	12.1	0.1	1	14.7	0.0009	105.8	10.7	0.0	0.0
	Pdw2.1+	2						2	5.5	0.0009	39.6	3.1	0.0	0.0
	Pdw3.1+	3	16.7	0.0009	1.9	10.6	0.1	3	29.7	0.0009	14.2	31.1	0.1	0.0
	Pdw4.1+	4	11.0	0.0009	23.5	6.4	0.1	4	12.1	0.0009	24.2	7.3	0.0	0.0
	Pdw6.1+	6	1.7	0.0290	73.4	1.0	0.0	6						
	Pdw7.1+	7	35.1	0.0009	23.2	31.2	0.1	7	15.5	0.0009	42.9	11.0	0.0	0.0
	Pdw8.1+	8	20.3	0.0009	24.9	14.0	0.1	8	21.1	0.0009	24.6	16.4	0.1	0.0
	Pdw11.1+	11	5.9	0.0009	32.1	3.5	0.0	11	3.3	0.0009	24.4	1.5	0.0	0.0
PDSBS	*Pdsbs1.1+*	1	17.3	0.0009	75.5	11.2	4.6	1	14.5	0.0009	64.0	8.8	1.2	-1.6
	Pdsbs2.1+	2						2	9.2	0.0009	64.0	5.1	1.2	0.6
	Pdsbs3.1+	3	6.3	0.0009	3.3	3.5	2.6	3	21.4	0.0009	12.9	21.6	2.4	1.1
	Pdsbs4.1+	4	6.2	0.0009	37.8	4.3	2.9	4	11.0	0.0009	0.5	5.2	1.1	0.9
	Pdsbs5.1+	5						5	8.8	0.0009	71.7	4.5	1.2	0.0
	Pdsbs7.1+	7	30.4	0.0009	34.2	25.0	6.9	7	6.2	0.0009	9.0	5.1	1.2	0.2
	Pdsbs8.1+	8	8.1	0.0009	9.6	5.4	3.2	8	16.2	0.0009	13.5	14.4	2.0	-0.9
	Pdsbs9.1+	9						9	7.7	0.0009	66.5	4.5	1.1	-0.4

Table 7 (Continued)

		BC$_1$F$_{1:2}$						F$_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Pdsbs11.1-	11	31.4	0.0009	32.3	28.4	-7.4	11	17.4	0.0009	18.1	10.8	-1.1	2.0
STT	*Stt1.1-*	1	4.0	0.0019	87.5	7.6	-0.6	1						
	Stt2.1+	2	1.7	0.0465	75.1	3.1	0.4	2	3.1	0.0102	86.9	4.3	0.6	0.1
	Stt3.1+	3	2.8	0.0056	78.1	5.2	0.5	3	6.4	0.0009	83.2	9.9	0.9	0.2
	Stt4.1+	4						4	4.1	0.0019	30.8	5.9	0.7	0.0
	Stt6.1+	6	1.8	0.0262	23.6	3.0	0.4	6	3.7	0.0028	56.0	5.8	0.6	0.5
	Stt7.1+	7						7	5.8	0.0009	42.0	13.0	1.0	0.4
	Stt9.1-	9	2.0	0.0112	19.5	4.6	-0.5	9						
	Stt10.1-	10	2.2	0.0112	63.2	3.7	-0.4	10						
	Stt11.1-	11	2.3	0.0065	35.5	4.1	-0.4	11	4.7	0.0009	23.8	6.6	0.7	-0.4
LFPL	*Lfpl3.1+*	3	2.0	0.0234	29.7	3.2	0.2	3						
	Lfpl6.1+	6	2.0	0.0168	79.5	3.1	0.2	6						
	Lfpl7.1+	7	10.9	0.0009	28.7	20.9	0.5	7						
	Lfpl8.1+	8	1.7	0.0475	62.4	2.5	0.2	8						
	Lfpl11.1+	11	4.9	0.0009	0.0	8.2	0.3	11	3.9	0.0009	21.4	10.0	0.3	0.1
LFPW	*Lfpw1.1+-*	1	3.1	0.0019	101.5	4.8	0.1	1	6.1	0.0009	6.7	9.7	-0.2	0.0
	Lfpw2.1+	2						2	5.5	0.0009	53.8	7.6	0.1	-0.1

Table 7 (Continued)

		BC$_1$F$_{1:2}$						F$_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Lfpw3.1+	3	3.8	0.0009	26.6	5.9	0.2	3	6.0	0.0009	89.3	9.6	0.2	0.0
	Lfpw6.1-	6	2.9	0.0009	8.6	5.0	-0.1	6						
	Lfpw7.1+	7	7.3	0.0009	28.6	13.1	0.2	7	3.0	0.0112	0.0	5.4	0.1	0.0
	Lfpw8.1+	8	2.6	0.0084	79.4	4.4	0.1	8						
	Lfpw9.1-	9	1.8	0.0215	38.9	2.7	-0.1	9	3.8	0.0009	41.3	6.8	-0.1	0.0
	Lfpw11.1+	11	1.4	0.0393	5.7	2.3	0.1	11	5.6	0.0009	20.2	9.5	0.2	0.0
ECL	*Ecl2.1-*	2	2.0	0.0206	25.6	1.9	-0.2	2						
	Ecl4.1+	4	6.1	0.0009	42.2	8.3	0.4	4						
	Ecl6.1-	6	6.8	0.0009	34.9	9.9	-0.4	6						
	Ecl7.1+	7	3.1	0.0009	41.0	3.0	0.2	7						
	Ecl9.1-	9	6.1	0.0009	33.2	6.9	-0.3	9	2.3	0.0178	49.7	5.2	-0.3	0.2
	Ecl10.1+	10	22.9	0.0009	63.2	28.7	0.7	10	3.2	0.0056	59.8	7.6	0.2	-0.6
STL10	*Stl101.1+*	1	2.8	0.0056	104.6	2.0	8.8	1						
	Stl102.1-	2	7.9	0.0009	56.9	6.3	-15.9	2						
	Stl103.1+	3						3	7.5	0.0009	32.1	8.1	7.9	-1.5
	Stl104.1+	4						4	4.7	0.0009	81.6	4.1	4.1	-5.4
	Stl105.1-	5	5.7	0.0009	54.7	4.2	-12.9	5	6.0	0.0009	67.2	5.5	-6.5	1.3
	Stl106.1-	6	9.5	0.0009	60.1	7.1	-16.8	6	7.9	0.0009	94.9	8.6	-7.3	5.2

Table 7 (Continued)

		$BC_1F_{1:2}$						$F_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Stl107.1+	7	14.6	0.0009	29.5	11.6	21.5	7	17.6	0.0009	36.9	19.7	10.5	-9.4
	Stl108.1-	8	2.8	0.0009	68.2	1.9	-8.7	8						
	Stl109.1-	9	4.0	0.0009	51.4	2.6	-10.2	9	4.6	0.0009	64.3	3.8	5.3	-1.7
	Stl1010.1+	10	30.2	0.0009	59.5	33.9	36.7	10	16.1	0.0009	59.8	16.0	10.5	-5.3
	Stl1011.1+	11	4.8	0.0009	5.7	3.4	11.6	11						
STLW	*Stlw1.1+*	1	4.2	0.0009	62.9	4.6	19.3				NI			
	Stlw2.1-	2	5.4	0.0009	51.0	6.4	-22.7							
	Stlw3.1-	3	2.1	0.0158	3.3	2.3	-13.6							
	Stlw5.1-	5	2.7	0.0056	35.7	2.9	-15.3							
	Stlw6.1-	6	6.0	0.0009	61.2	6.7	-23.3							
	Stlw8.1-	8	4.2	0.0009	39.0	4.6	-19.3							
	Stlw10.1+	10	19.0	0.0009	59.9	28.6	48.1							
BRN					ND$^{2/}$									
											NI			
FLD	*Fld1.1+*	1						1	3.7	0.0009	17.0	4.4	1.2	-0.8
	Fld2.1+	2						2	15.7	0.0009	65.5	17.6	2.5	-0.6
	Fld4.1+-	4	1.9	0.0177	3.1	2.6	1.4	4	3.7	0.0056	81.6	2.9	-1.0	-0.4
	Fld5.1-	5						5	7.8	0.0009	50.3	6.4	-0.6	2.0

Table 7 (Continued)

Trait	QTL name	$BC_1F_{1:2}$						$F_{2:3}$						
		LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
	Fld8.1+	8	5.3	0.0009	36.1	7.9	2.4	8	5.1	0.0009	55.1	4.8	1.3	-0.4
	Fld10.1+	10	3.0	0.0056	47.8	4.0	1.7	10	13.1	0.0009	39.2	15.6	1.7	-2.4
	Fld11.1-	11	11.4	0.0009	40.4	19.6	-3.8	11						
PDDM	*Pddm1.1-*	1						1	5.5	0.0009	28.3	9.2	-1.0	-0.4
	Pddm2.1+	2						2	2.7	0.0206	78.3	5.5	0.8	-0.2
	Pddm3.1+	3						3	4.4	0.0009	2.0	17.1	1.4	-0.1
	Pddm4.1+	4	2.7	0.0009	52.9	6.6	0.9	4						
	Pddm6.1+	6	2.5	0.0037	57.7	5.1	0.8	6						
	Pddm7.1+	7	3.8	0.0009	59.7	7.9	1.0	7	2.8	0.0178	13.5	5.5	0.3	-1.0
SDTWT	*Sdtwt2.1+*	2			ND			2	3.2	0.0019	37.1	4.5	4.6	43.5
	Sdtwt4.1+	4						4	2.7	0.0122	50.0	4.7	28.4	20.0
	Sdtwt5.1+	5						5	2.8	0.0168	49.9	3.6	27.0	-10.2
	Sdtwt6.1+	6						6	3.9	0.0028	52.3	5.1	9.4	45.0
	Sdtwt7.1+	7						7	8.0	0.0009	48.5	23.1	64.5	39.9
	Sdtwt10.1-	10						10	4.1	0.0009	38.6	6.6	-22.5	42.9

Table 7 (Continued)

		$BC_1F_{1:2}$						$F_{2:3}$						
Trait	QTL name	LG	LOD	P	Loci (cM)	PVE (%)	Additive effect	LG	LOD	P	Loci (cM)	PVE (%)	Additve effect	Dominant effect
SDNPPD	*Sdnppd7.1+*	7						7	10.6	0.0009	45.9	12.8	1.5	-0.4
	Sdnppd11.1+	11	48.1	0.0009	36.5	70.1	5.4	11	31.6	0.0009	26.6	45.7	1.7	-3.2
PDTN	*Pdtn1.1-*	1	3.9	0.0009	91.6	5.7	-3.9	1						
	Pdtn2.1-	2						2	4.0	0.0028	73.7	1.8	-50.3	47.6
	Pdtn3.1-	3	3.3	0.0019	0.0	4.7	-3.5	3						
	Pdtn4.1-	4	2.4	0.0047	64.0	3.6	-3.1	4						
	Pdtn6.1+	6						6	11.0	0.0009	10.6	57.1	270.1	-283.0
	Pdtn7.1-+	7	3.5	0.0009	28.0	5.2	-3.7	7	7.1	0.0009	50.2	8.1	69.6	149.3
	Pdtn8.1-	8	2.2	0.0178	70.1	3.2	-2.9	8	10.3	0.0009	10.2	6.7	-107.2	59.8
	Pdtn9.1-	9	1.8	0.0122	6.9	3.5	-3.0	9						
	Pdtn10.1-	10						10	3.9	0.0009	38.6	2.0	-57.9	33.9
	Pdtn11.1-	11	9.4	0.0009	35.8	15.6	-6.4	11	12.5	0.0009	3.5	6.8	-114.6	26.1

[1/]NI; not investigated, [2/]ND; no significant QTL detected

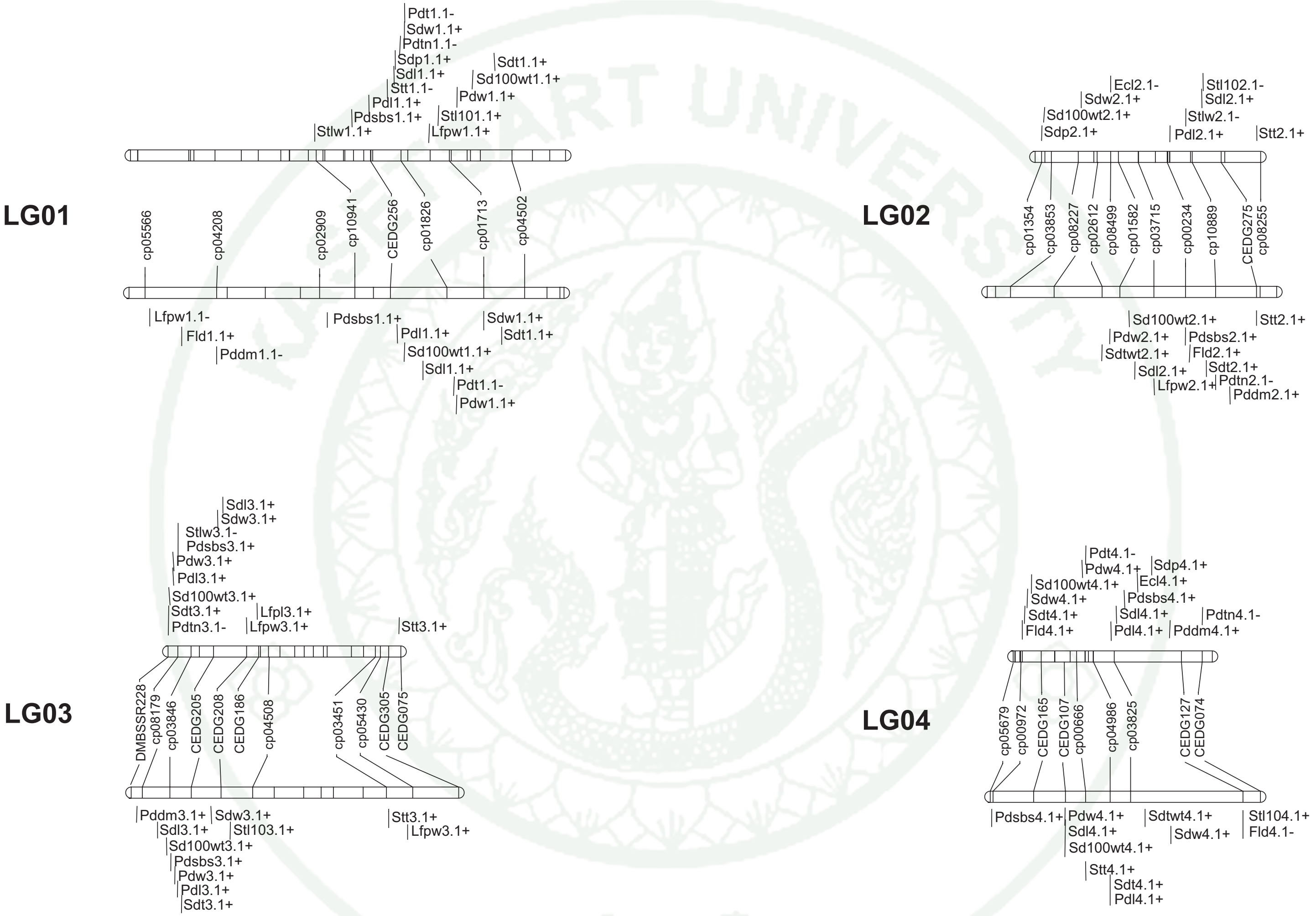

Figure 3 QTLs detected in BC_1F_1 (upper) and F_2 (lower) populations from the cross between cultivated yardlong bean and wild cowpea. The effect of the cultivated parent is indicated after each QTL name. For explanation of trait abbreviation, see Table 1.

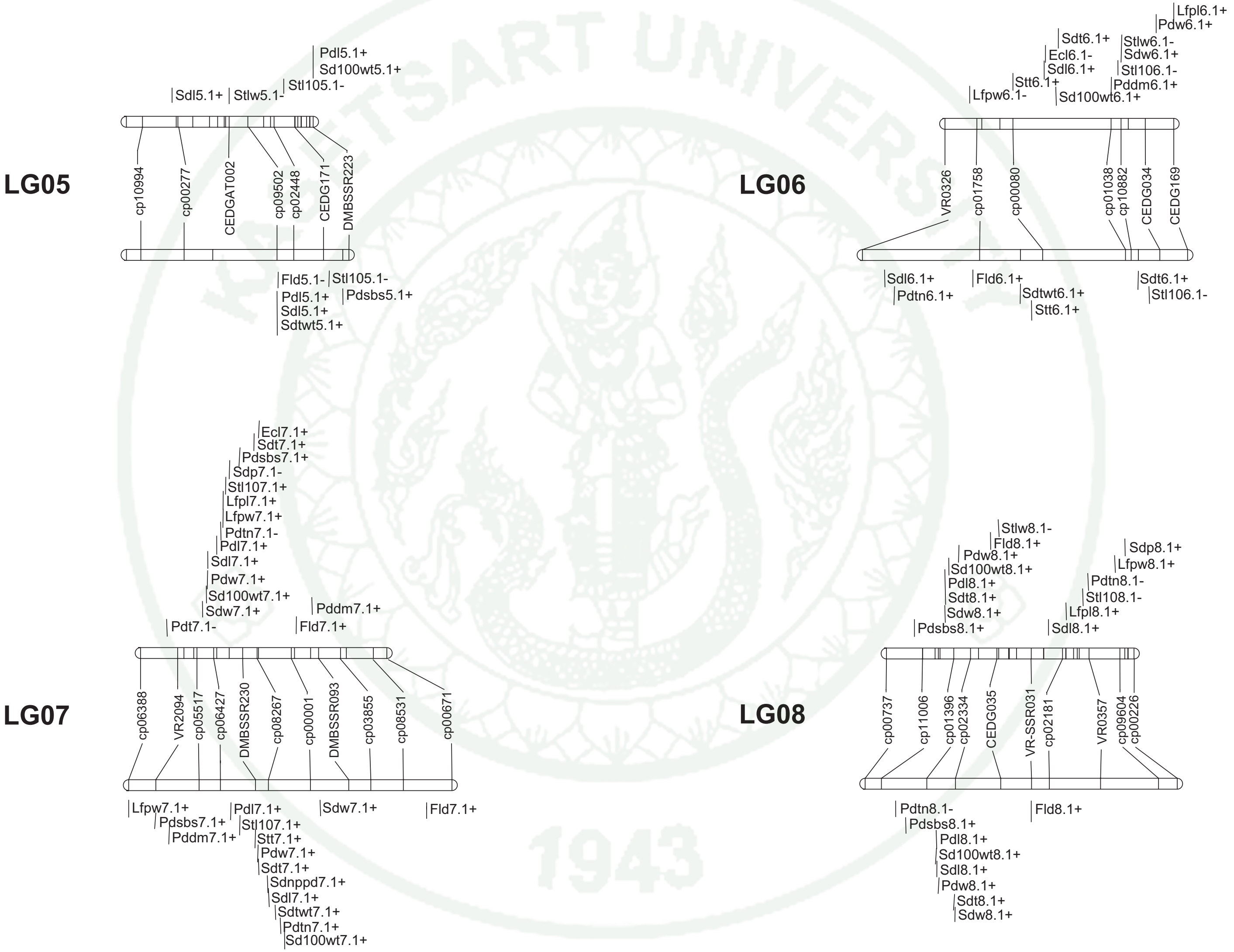

Figure 3 (Continued)

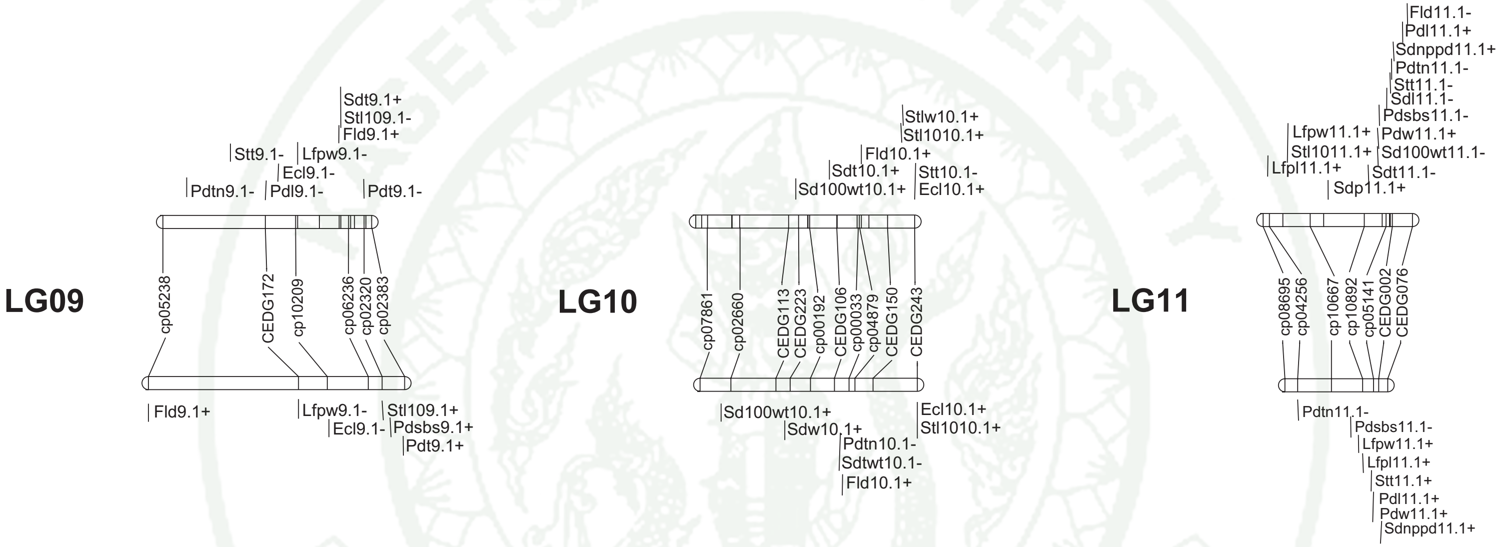

Figure 3 (Continued)

Table 8 Observed and expected numbers of QTLs and their chi-square values on each linkage groups

LG	Length (cM)	BC_1F_1				Length (cM)	F_2			
		detected QTLs	Expected QTLs	χ^2			detected QTLs	Expected QTLs	χ^2	
LG1	147.3	14	20.9	2.3		140.4	11	15.2	1.2	
LG2	76.4	8	10.8	0.7		93.4	12	10.1	0.3	
LG3	78.8	10	11.2	0.1		105.7	11	11.5	0.0	
LG4	67.4	14	9.6	2.0		87.2	11	9.5	0.3	
LG5	64.9	7	9.2	0.5		73.7	6	8.0	0.5	
LG6	79.5	10	11.3	0.1		107.7	7	11.7	1.9	
LG7	85.6	15	12.1	0.7		107.1	16	11.6	1.7	
LG8	86.0	14	12.2	0.3		103.4	10	11.2	0.1	
LG9	60.3	8	8.6	0.0		70.5	6	7.6	0.4	
LG10	63.2	7	9.0	0.4		59.8	7	6.5	0.0	
LG11	43.1	14	6.1	10.2	**	28.3	9	3.1	11.5	***
Total	852.4	121	121	17.5	***	977.1	106	106	17.8	***

Asterisks indicate significant differences between the observed and expected numbers of QTLs in each linkage group at ** *P* = 0.01 and *** *P* = 0.001.

DISCUSSION

The genetic linkage map developed in this study resolves the 11 chromosomes corresponding to the haploid number of yardlong bean. It contains 226 SSR markers from three *Vigna* species of two different origins, Africa (cowpea) and Asia (azuki bean and mungbean). Among the SSR markers used in this study, 55.9% of azuki bean (Wang *et al.,* 2004) and 57.6% of mungbean (Somta *et al.,* 2009; Tangphatsornruang *et al.,* 2009) could amplify one locus each in *V. unguiculata*, revealing high transferability of the SSR markers between Asian and African *Vigna*. In this study, 113 SSR markers derived from cowpea, azuki bean and mungbean were chosen from previous map constructed from a BC_1F_1 population, and were integrated into the F_2 genetic map. The LG and marker orders on each LG have been confirmed. Therefore, these yardlong bean maps are useful for understanding genome synteny among *Vigna* species, as well as for identifying QTLs for useful traits.

Regardless of the populations and marker types used in mapping of cowpea, the length of the current map (852.4 cM) is comparable to the maps previously reported by Muchero *et al.* (2009b) (643 cM), Ubi *et al.* (2000) (669.8 cM), Menendez *et al.* (1997) (972 cM), and Xu *et al.* (2011) (745 cM) but not to that of Ouédrago *et al.* (2002) (2,670 cM). However, the same mapping population (524B x IT84S-2049) used by Ouédrago *et al.* (2002) was again used by Muchero *et al.* (2009a) as one of the six RIL populations to construct EST-SNP consensus map. The SNP map from each population covered the length of 600 to 665 cM, giving the consensus map of 680 cM.

Some genetic barriers exist in inter-subspecific cross between cowpea and its wild progenitor. Ng (1995) reported partial incompatibility between cultivated cowpea *V. unguiculata* ssp. *unguiculata* and wild cowpea *V. unguiculata* ssp. *dekindtiana*. In our study where yardlong bean and wild cowpea was crossed, 27 plants in the F_2 population did not set pod, 15 had a very low pod set (≤15) or shriveled seeds. In the BC_1F_1 population, one plant failed to produce any pods and nine plants produced a small number of pods (≤10). Those plants were excluded from the mapping population.

Segregation distortion is a common phenomenon observed in wide intra-specific and inter-specific crosses of many plants (Jenczewski *et al.,* 1997; Xu *et al.,* 1997; Song *et al.,* 2006). In *Vigna* species, distorted segregation of markers has been reported in genetic maps of mungbean, cowpea and azuki bean with the percentage of distorted markers ranging from 12.0 to 30.8% (Kaga *et al.,* 2005). Although our linkage map was developed from an inter-subspecific cross between wild cowpea and cultivated yardlong bean, the percentage of distorted markers in our map (9.7%) was lower than that previously reported (24.7% and 13.8%) in a recombinant inbred line (RIL) population derived from a cross between cultivated yardlong beans (Xu *et al.,* 2010, 2011). This may partly be due to the effect of the population structure. Backcross (BC) populations usually have relatively fewer segregation distortions than other population types (Song *et al.,* 2006). Based on azuki bean (*Vigna angularis*) maps developed from the same parents, the percentage of distorted markers was 3.9% in BC_1F_1 (Han *et al.,* 2005) and 28.0% in F_2 populations (Isemura *et al.,* 2007).

All of the 13 markers on LG11 showed distorted segregation, directed towards the homozygous yardlong bean genotype. Similarly, Isemura *et al.* (2010) reported in a BC_1F_1 linkage map of cultivated x wild rice bean that all the 26 markers on LG11 exhibited segregation distortion. The distortion was directed towards the heterozygous genotype, resulting in a significantly higher transmission of cultivated alleles. Based on the common SSR markers on our linkage map and that of Isemura *et al.* (2010), LG11 of the two maps are the same linkage group. Thus, some common genetic factors associated with strong segregation distortion may be located on LG11 of both *V. unguiculata* and *V. umbellata*. The strong segregation distortion loci (SDL) may cause all the markers in the same LG to be distorted (Zamir and Tadmor, 1986; Vogl and Xu, 2000; Lu *et al.,* 2002). Further research is necessary to clarify the genetic factor(s) causing segregation distortion on LG11. Xu *et al.* (2011) reported segregation distortion regions on two LGs exhibiting skewed genotypic frequencies toward the female genotype. Unfortunately, we could not compare the region between our map and that of Xu *et al.* (2011) due to no common marker available between the two maps.

Markers showing segregation distortion were observed on some LGs on both the F_2 and BC_1F_1 maps. The F_2 map had more distorted markers than the BC_1F_1 map (*P*< 0.05, 48.7% vs. 9.7%). Particularly the markers located on LG11, where 7 out of 9 and 13 out of 14 of F_2 and BC_1F_1 maps showed distorted segregation. The direction of skewed markers on LG11 in both maps was toward the homozygous yardlong bean genotype. The same results have been found in an F_2 interspecific linkage map of *V. umbellata* x *V. nakashimae* (Somta *et al.,* 2006), and in a BC_1F_1 linkage map of *V. umbellata* (Isemura *et al.,* 2010). They reported that 9 out of 10 markers and all markers on LG11 of the respective linkage maps were highly distorted. The cluster of distorted markers may result from the presence of gene(s) controlling sterility and/or compatibility (Zamir and Tadmor, 1986). Therefore, gene(s) on LG11 may play an important role in genetic differentiation, both within the yardlong bean (cowpea) and among *Vigna* species.

Highly distorted segregation may affect inheritance of nearby traits, even with simply inherited trait. In mungbean, Lambrides *et al.* (2004) found that segregation ratio suggested dominant and recessive epistasis controlling seed coat color in mungbean. However, this was rejected and single-gene model was accepted based on molecular marker data. They eventually mapped seed coat color locus to a distorted genomic region with an understanding that the aberrant segregation ratio was due to the effect of segregation distortion. In our study on the seed coat color, segregation ratios suggest either dominant and recessive epistasis or a single gene control, although gene mapping supported the latter case.

Comparative mapping between yardlongbean map with azukibean and ricebean

Previously, SSR markers have been used to construct linkage maps of azuki bean (Han *et al.,* 2005) and rice bean (*Vigna umbellata*) (Isemura *et al.,* 2010). Based on common SSR markers, we compared our yardlong bean linkage map with those maps. Although the number of common SSR markers are not high, comparative mapping between our yardlong bean map and those maps showed high genome conservation at linkage group and marker order levels (Figure 4). All of the markers were mapped to the same linkage groups with the exception to 3 markers (cp02661, cp09102 and cp10093) that located in different linkage groups between yardlong bean and rice bean maps. All marker orders between yardlong bean and azuki bean or rice bean map are identical. High genome homology between African *Vigna* (cowpea) and Asian *Vigna* (mungbean) has been demonstrated by Fatokun *et al.* (1993) and Isemura *et al.* (2010). In addition, the three maps were constructed from a BC_1F_1 population between domesticated and wild accessions and QTLs for variation in pod length were detected. Currently, seven, four and three QTLs were detected in yardlong bean, azuki bean (Isemura *et al.,* 2007) and rice bean (Isemura *et al.,* 2010), respectively (Table 9). Among them, three QTLs of yardlong bean and azuki bean were located on the same LGs (LG7, LG9 and LG11) and two QTLs of yardlong bean and rice bean were located on the same LGs (LG3 and LG4). The QTLs on LG7 of yardlong bean and azuki bean had the largest effect on pod length. It will be interesting to clarify whether these QTLs found on the same LG were the same genes contributing to pod length elongation during the domestication process of different *Vigna* crops. However, detailed comparison of the detected QTLs is difficult because the number of common SSR markers is insufficient.

The genetics of domestication–related traits

Cowpea was domesticated in Africa from its wild progenitor while yardlong bean was domesticated from cultivated cowpea in Asia (Ng and Maréchal, 1985). This “divergent domestication” can result in a severe bottleneck and reduced genetic diversity in yardlong bean. In fact, genetic similarity among landraces of yardlong bean from China is 75.5% (Xu *et al.,* 2010), compared to 44.0% genetic similarity among IITA cowpea breeding lines (Li *et al.,* 2001), although number of alleles detected per SSR locus in the two reports was very similar (4.4 vs. 4.7). Thus cowpea germplasm are considered to harbor significantly higher levels of genetic variations compared with that of yardlong bean, and could be used to broaden the genepool in yardlong bean (Ehlers and Hall, 1997).

In yardlong bean the majority of domestication-related traits are controlled by one major QTL and several minor QTLs and a few are controlled by single major genes. Results from this study revealed that yardlong bean differs from other *Vigna* crops in the genetic control of some simply inherited traits. For example, pod dehiscence which is controlled by a single gene in azuki bean (Isemura *et al.,* 2007 and Kaga *et al.,* 2008) and rice bean (Isemura *et al.,* 2010), is controlled by several genes/QTLs in yardlong bean. Nonetheless, in a related crop, common bean (*Phaseolus vulgaris* L.) pod dehiscence is also governed by several genes (Koinange *et al.,* 1996).

In most of the domestication-related traits measured, two to eleven QTLs on two or more linkage groups were detected. QTLs for many related traits such as pod size, seed size, leaf size and stem-related traits were co-located on the same position on the same linkage groups. The same findings were reported for azuki bean (Kaga *et al.,* 2008; Isemura *et al.,* 2007) and rice bean (Isemura *et al.,* 2010). Co-location of QTLs for these traits may be due to the effect of developmental allometry (Smartt, 1976).

Genomic regions and distribution of QTLs for domestication-related traits

The QTLs controlling domestication traits are generally not randomly distributed across the crop genome (Gepts, 2004). The non-randomness of the domestication QTLs may be related to ‘cultivation magnetism’ phenomenon and should be considered under ‘protracted transition paradigm’ of crop domestication (Allaby, 2010). Domestication-related traits have been studied in several legume crops such as common bean (Koinange *et al.,* 1996), azuki bean (Isemura *et al.,* 2007 and Kaga *et al.,* 2008), rice bean (Isemura *et al.,* 2010) and soybean (Liu *et al.,* 2007), the results revealed that domestication traits are controlled by several QTLs/genes with each trait controlled by a few large QTLs or by a major QTL and several minor QTLs.

The results from our study demonstrated that for most traits the latter case exists in yardlong bean. For example, one QTL with large effect and 3-9 minor QTL control SDP, SDT, SD100WT, PDL, PDW, STL10 and STLW.

The distribution of domestication-related QTL across the yardlong bean genome is shown by co-location of the QTLs on several narrow genomic regions on almost all linkage groups, especially LG3, LG7, LG8 and LG11 (Figure 3). LG3 is associated with QTLs for increased organ size, such as seed, pod, and leaf, that were detected in a limited region of 16 cM. LG7 is associated with pod dehiscence, organ size (seed, pod, stem and leaf), stem length and yield potential. Major QTLs for organ size were principally located on this LG. In addition, the QTLs for days to first flower and days to maturity of first pod are closely linked in a region of 6 cM on LG7. LG8 is associated with increased seed and pod size. LG11 is associated with organ size (seed, pod, stem and leaf), earliness and yield potential traits.

QTL clusters for domestication are reported in several crops. Clustering of QTLs is due to either close linkage or pleiotropy or both. Single mutations can have pleiotropic effects on various organs. In common bean, the determinacy gene (*fin*) has pleiotropic effects on the number of nodes on the main stem, the number of pods, and the number of days to flowering and maturity (Koinange *et al.,* 1996). In maize, QTLs related to change in inflorescence sex, and number and length of internodes in lateral branches and inflorescences are distributed within a narrow genomic region (Doebley *et al.,* 1995). Change in these traits is explained by the pleiotropic effect of a single *tb1* gene. In tomato, the seed testa color mutant gene *bks* decreases seed weight and increases fruit pH (Downie *et al.,* 2003). In sunflower, the unbranched allele (*B*) decreases seed oil content and increases capitula diameter and seed weight (Bachlava *et al.,* 2010).

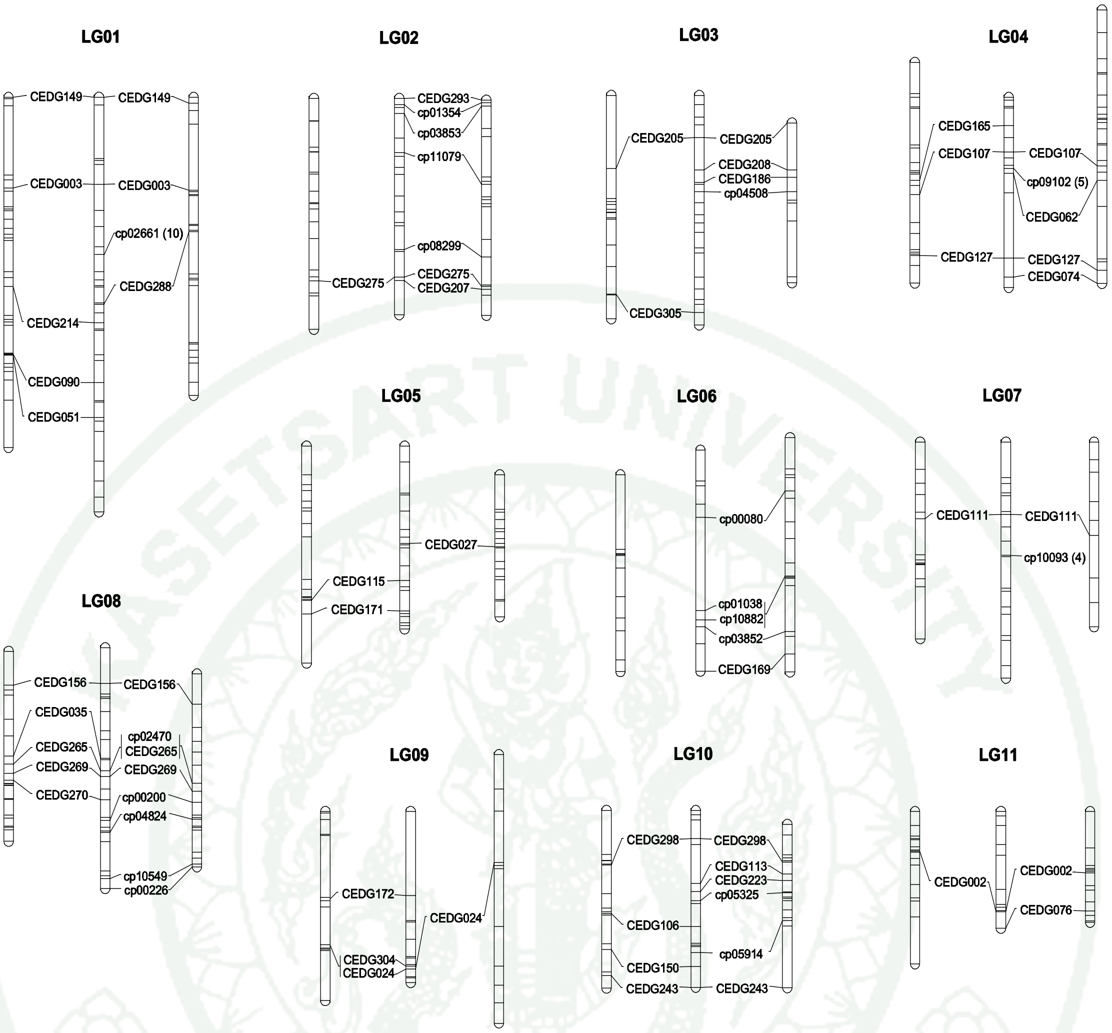

Figure 4 Comparative linkage maps of yardlong bean (*Vigna unguiculata* ssp. *unguiculata* cv.-gr. *sesquipedalis*), azuki bean (*V. angularis*) and rice bean (*V. umbellata*) based on common SSR markers. Linkage group of yardlong bean is at the middle, and azuki bean and rice bean maps are aligned on the left and the right, respectively. Lines connect common markers between linkage groups. The numbers in parentheses of the yardlong bean map indicate locations of the markers on the rice bean linkage groups.

Table 9 Comparison of pod length QTLs detected from 3 different *Vigna* species using BC$_1$F$_1$ populations derived from wild and domesticated parents

Species	Pod length (cm)		Magnitude of pod length increase	Population analyzed	No. of QTLs detected	Percent of variation explained (PVE) of each QTL on each linkage group (LG)											Ref
	wild	domesticated				LG01	LG02	LG03	LG04	LG05	LG06	LG07	LG08	LG09	LG10	LG11	
V. unguiculata ssp. *unguiculata* cv.-gr. *sesquipedalis*	8.5	64.4	7.6 times	BC$_1$F$_1$	7	12.1		6.8	3.2	4.5		**30.5***	9			14.4	present study
V. angularis	7.2	9.2	1.3 times	BC$_1$F$_1$	4							**39.1**	22.1	9.6	10.7		Isemura *et al.*, 2007
V. umbellata	4.5	11.8	2.6 times	BC$_1$F$_1$	3		16.7	6.9	**23.1**								Isemura *et al.*, 2010

*QTL with the highest contribution in each species is indicated with bold letter

Comparison of domestication QTLs between yardlong bean and related *Vigna* and other crops

QTLs with larger effect (PVE: phenotypic variation explained ≥ 20%) are considered here.

Comparison with azuki bean

Distribution of the main QTLs between the two species showed a marked difference. In yardlong bean, large effect QTLs were detected on seven linkage groups; LGs 1, 3, 6, 7, 8, 10 and 11. In azuki bean, large-effect QTLs were found on only five linkage groups – LGs 1, 2, 7, 8 and 9 (Isemura *et al.,* 2007; Kaga *et al.,* 2008). Marked difference between yardlong bean and azuki bean were found on LG9 and LG7. Several QTLs with large effect were detected on LG9 of azuki bean, whereas only one QTL was detected on this LG in yardlong bean. Major QTLs for domestication-related traits were abundant on LG7 in yardlong bean, but only a few QTLs were detected in azuki bean.

Seed size. Seed weight of the mapping parents used in this study had an 8-fold difference. The 100-seed weight of cultivated yardlong bean was 19.4 g while that of the wild parent was 2.5 g. Ten QTLs were detected for 100-seed weight and QTL with the largest effect (PVE = 21.1%) was found on LG7 for yardlong bean whereas eight QTLs for this trait were detected in azuki bean and QTL with the largest effect (PVE = 31.3%) was located on LG2. QTLs for 100-seed weight in yardlong bean and azuki bean on LG1, LG10 and LG11 may be common. QTLs for seed size in both crops on LG1 were linked to markers CEDG090 and CEDG051, on LG10 were between marker interval CEDG113-CEDG106 and on LG11 were linked to marker CEDG076.

Pod dehiscence. Reduction of seed dispersal is one of the first steps in crop domestication (Ladizinsky, 1979). Pod dehiscence is due to the presence of fibers surrounding the vascular bundles in the pod walls and to a fibrous parchment layer lining the pod cavity (Roth,1977). Pod twisting is caused by the oblique orientation of the fibers in the parchment layer. Four QTLs were detected for pod dehiscence in yardlong bean. The QTL with relatively high contribution (48%) was found on LG7 whereas a single QTL for this trait with a higher contribution (90.5%) was detected on the same LG7 in azuki bean (Isemura *et al.*, 2007). The pod size QTL with the largest effect in azuki bean and yardlong bean were both found on LG7.

Pod length. Among the pod-related traits, length of pod is the main distinguishing trait of yardlong bean. The distinct pod character of yardlong bean may be because it has been domesticated solely for edible young pods. The domesticated yardlong bean parent used here had pod length of 78.1 cm, while domesticated azuki bean had pod length of 9.2 cm (Isemura *et al.,* 2007). The yardlong bean and wild cowpea parents in this study showed an 8-fold difference in pod length, but azuki bean and its wild relative have similar pod length (Isemura *et al.,* 2007). Nine QTLs were involved in pod length in yardlong bean. Five were associated with the same trait in azuki bean. Largest effect QTLs for the two species were detected on LG7. These QTL and the QTL on LG1 were considered to be common in both *Vigna* crops.

Seed dormancy. Physical seed dormancy is generally caused by the presence of water-impermeable layers of palisade cells in the seed coat (Finch-Savage and Leubner-Metzger, 2006). The water absorption occurs through the structure of the strophiole (parenchymatous tissue) adjacent to the hilum in *Vigna* species (Gopinathan and Babu, 1985). Six QTLs were identified for seed dormancy-related traits in yardlong bean whereas two to five were identified in azuki bean. The largest effect QTLs for dormancy-related traits in yardlong bean and azuki bean were both found on LG1 and were likely to be common since they are linked to the same markers CEDG256 and CEDG214.

Comparison with rice bean

In rice bean, large effect QTLs were found on three linkage groups (Isemura *et al.,* 2010). LG2 and LG4 for seed and pod size, LG4 for water absorption by seeds (seed dormancy), and LG7 for pod dehiscent and growth habit (stem length).

Differences between yardlong bean and rice bean were found on LG4 and LG7. Several QTLs with large effect were detected on LG7 of yardlong bean, whereas a few QTLs were detected on this LG7 of rice bean. In contrast, major QTLs for domestication-related traits were found on LG4 of rice bean while no major QTL was detected on LG4 of yardlong bean.

Seed size. A QTL for 100-seed weight on LG1 of rice bean and azuki bean was common (Isemura *et al.*, 2010), thus this QTL of rice bean may be the same as QTL for seed weight in yardlong bean (see above discussion regarding yardlong bean and azuki bean).

Pod dehiscence. A major difference between the yardlong bean and rice bean is pod dehiscence. Only a single major QTL (42.4% PVE) controlling pod dehiscence was found on LG7 in rice bean, but one major and six minor QTLs were detected for this trait in yardlong bean. The major QTLs in both rice bean and yardlong bean were located on LG7, and are possibly the same. Marker CEDG111 is linked to both QTLs on LG7 of rice bean and yardlong bean (18.5 and 17.2 cM, respectively).

Pod length. As in azuki bean, pod length of rice bean and its wild relative was only slightly different (Isemura *et al.*, 2010). Nine and three QTLs were detected for pod length in yardlong bean and rice bean, respectively. QTLs on LG2 and LG4 were considered common in both species. The two QTLs were linked to markers cp08299 and CEDG062, respectively. However, largest effect QTL in yardlong bean was on LG7 while that of rice bean was on LG4.

Seed dormancy. Six seed dormancy-related QTLs were detected in yardlong bean. Of these, five were detected in rice bean. Two QTLs for yardlong bean each on LG4 and LG8 appear to be common to rice bean. The QTL mapped on LG4 of yardlong bean and rice bean were both linked to marker CEDG062 (22 and 19.7 cM, respectively). The QTL on LG8 of the two species linked to marker cp10549 at 1.2 and 6.1 cM in that order.

Comparison with cowpea and mungbean

Seed size. Seed weight is an important trait related to yield in domesticated *Vigna*. QTLs for seed weight of cowpea and mungbean have been reported by Fatokun *et al.* (1992) in which two QTLs were detected for cowpea and four QTLs were found for mungbean. LGs ii and vi in the cowpea map correspond to LG1 and LG4, respectively in the azuki bean map (Isemura *et al.,* 2007), which also correspond to the same linkage groups in yardlong bean map. LGs i, ii, iii and vi in the mungbean map correspond to LGs 9, 1, 8 and 10, respectively, in the azuki bean map (Isemura *et al.,* 2007) and yardlong bean map in our study.

In this study, a QTL for seed weight was detected on LG1 at the location corresponding to that of a QTL for this trait on LG ii in cowpea and mungbean. Other QTLs for seed weight were also detected at similar locations on LG4 of yardlong bean and LG vi of cowpea, as well as LG8 and LG10 of yardlong bean and LG iii and vi of mungbean. Although the QTL with largest effect for seed weight was detected on the LG7 in yardlong bean, no QTL was detected on the corresponding linkage groups in cowpea and mungbean.

CONCLUSIONS

This is the first report of QTLs for domestication-related traits in yardlong bean/cowpea. Twenty-four domestication-related traits (3 qualitative and 21 quantitative traits) were investigated in the F_2 and BC_1F_1 populations. The 21 quantitative traits were dissected into 153 QTLs. The difference between wild cowpea and cultivated yardlong bean were found to be controlled by several major QTLs. Major QTLs for unrelated organs were distributed in clusters on LGs 3, 7 and 11. This study revealed that domestication-related traits are controlled by a few major and many minor QTLs. For pod length which is the unique character distinguishing between yardlong bean and wild cowpea, one major and six minor QTLs were identified. The largest effect was found on LG7. Comparing these results with other domesticated *Vigna* showed similarity to azuki bean but difference from rice bean. High genome synteny in the genus *Vigna* enabled QTL comparison between yardlong bean and azuki bean, rice bean, mungbean and cowpea. Major QTLs in yardlong bean were found on LG7, whereas those of azuki bean were on LG9 and for rice bean were on LG4. Some genomic regions for seed dormancy, pod dehiscence and seed and pod size are conserved between yardlong bean and azuki bean and/or rice bean. Some genomic regions for seed size are conserved between yardlong bean cowpea and/or mungbean. The results in this study provide a foundation for marker-assisted selection of domesticated-related QTLs in yardlong bean and enhance understanding of domestication in the *Vigna.*

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APPENDIX

Appendix Table 1 Information of cowpea SSR primer pairs used in this study

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
1	cp07296	tggagtggaaatttaggttgtggtc	tctatcgatcgctcgctcatacac	M	M		288
2	cp08497	accaaaacgccaaagtagagacct	tgggccttttcctgaacaactaga	286	-		300
3	cp01357	ctcttgagtttgatagccttagagttg	tcctcgcacatgttagagacagac	202	163		300
4	cp09620	ttgttagtccaagcacccaccata	catccatatgcatacagccacttc	-	-		288
5	cp04983	atatgattgatggtggtgtagccg	gcacttcacttatcgtcgcttgtg	161	160		284
6	cp05586	ggtgcttttgatgtactttgcttga	ggcaggaatttgacaaaagaaatgg	223	223		277
7	cp01304	tcaccgcttaataaaggctcctca	gagaatatcaagggtgaaaccgaca	228	287		247
8	cp00685	ttttccagaaaatatgctcagggg	gaatcacgttatgtttctcatgcc	258	223		254
9	cp02071	cactctcgtgcagggacattatga	ctggctcaaacaatctcagcactc	126	-		277
10	cp04129	acagggctcttgataccagcttga	aaccgtgcttgtctccctagtgtt	146	151		289
11	cp03344	gcgtcaacctcttctcccaattta	cctagccttgaacaaacctacgga	M	170		266
12	cp10075	gtactgaaatgtgaaccgttttgac	cgtgagtgagagcacgctttctt	-	-		287
13	cp02383	aggctctcagagttggaggtgaag	taccctcacctctttgtccctctg	214	222	9	286
14	cp06398	tgaacttagtcctgcaataagcatacg	tgtacacattacagatactgatgcctg	302	216		280
15	cp01428	accccaactctcctaactcccctt	tcagcaaggtggtcttaagttacgc	-	-		294
16	cp09781	ctgacgcattcagcattttacagc	ggaaatacggttgcgtccatgtat	283	262		283

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
17	cp09619	ccacaccacactttcattcatttca	cccacactctcacatttatcacgc	M	M		254
18	cp04478	atccgtgacaacagtaagtgcagc	ttgggtacctaaagcaactgggtc	M	M		300
19	cp05611	caatagaggcaagacttacgccat	gggttgatttgcaaagatggatttg	314	213		300
20	cp03505	cgtgagaggagagtgtgactaagga	atggtgtcagcaccccaattttat	M	356		242
21	cp01088	cttcgccacattgctttcttctct	ttattggggaaagttggtgaatgg	203	164		208
22	cp01196	tcttacagagatgagttcccagtcca	ctgtcccaaggaatatcacccatc	287	244		252
23	cp10454	gtgatgctttggaacaaggaacat	tgcacccacgttagtaggaaaaa	327	327		300
24	cp01257	tcttttccaaattaagtggggg	atgcatgcgtgtatgtctctgtga	-	-		251
25	cp05031	caaacacaagcagatagggtgagc	cgcacgtgttctacaagttatggc	218	218		295
26	cp08288	ccaaacttatccatagaccaactca	tcaggcgaggttaagcattggtat	262	228	1	280
27	cp04674	gcgattgccgatgatataaccagt	cataaatacccgatatcccaccga	M	M		293
28	cp10690	cttcccctgcttaaattggtttcc	aattggagcagagttggttgcttc	270	266		259
29	cp05922	accttcttgcatgcttcttccaac	caagccaaaaccttgaacaattcag	154	176		197
30	cp05440	gaagttaggttgaaatgggagcca	tgttcttgcgactcaaagccatta	435	165		180
31	cp08516	ggctgaaattagtcatcaacacctca	aaagaaagaaagcaccccaagtgc	234	232		220
32	cp04859	cctttcacgaaactatgaataaggtgg	tgagatggcgagattgatttcgta	129	129		207
33	cp07801	gttgcagccatttatgcttttcct	gtggtcattcgactcctctcgttt	129	129		228

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
34	cp08712	tgaatggttttctggaagaatgtgg	cactcacgcccttcaacacataat	226	122		296
35	cp00787	tggcaacaaggctctagataccaac	tttgggtgtatgtatctagtctaaggg	275	274		258
36	cp10898	ttggaaacaacggccttattcaac	caccatgaatgctcaaggcttatt	175	191		209
37	cp11058	acgtagccaccaaccaccttattc	gatcagtggaatcaccaccatcac	238	252		243
38	cp04463	tggcatgcattctgcactactttt	cattggggcatatgaacgaatatag	-	-		267
39	cp10190	cttcaacgtctctaatcaacgcga	ggatcgttccaaagcgattgatct	189	153		262
40	cp09452	tatcgattccacttgcatccacac	tcacccaatttgagtctccatcaa	-	-		260
41	cp03444	cagtgcgaaattgcggatttaatg	cggattctgtgccataatctggtt	239	280		233
42	cp04328	ctggattgttagattgattagcgtagg	gggtgttgagcaaagcatgaagag	M	M		180
43	cp05834	tttcgtgttcctactcgtccacaa	tcggtgacggatatgcagtacaat	185	185		209
44	cp00773	tgtgatgctttaaaagtagtctgggga	tttgatgcttttgcacctgaactc	221	194		206
45	cp02673	accctcgaggttcttcagttcctt	tcgagatattcagaacctaaacagg	211	201	5	232
46	cp08204	tgtgattattgccctcacggtatg	ttttgtcatgctctccctgaaaca	277	190		228
47	cp06108	attgccctgaagggctttacaatc	tgacgcatccagatacagacacaa	284	250		283
48	cp02447	agcactctgtggtttctgggaact	gtcatacaattggttctgcttatgg	259	241		256
49	cp00823	cgtcattatcgtagtcatacactccaa	gaaatagtgggtcctattccttcac	296	291	5	300
50	cp04558	ttctggtgagtcttggacagagga	ggactcatcaattgcgcagacata	258	279	2	235

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
51	cp00806	cattaaccaaacaactttcggc	acccaccccagaacgttagttgta	283	320	7	287
52	cp00361	taaatgctaatactcgccgccttg	actgtgtccgttcgtccttttctc	263	277	3	290
53	cp05202	aacctaaatcacaagggtgggtga	gattttcagattgtctcgcctacg	-	-		173
54	cp09101	tgtgctgaattaaagtcgtgtcact	cagtgatacttaatgggcaaaagca	220	221		274
55	cp00462	gtttttcaaacgagtacggaaacca	cagaggaaggttcactccaacttca	243	303		221
56	cp08015	tggtagaaagaaaattggaccaggc	tgatagagggagctgaacagagga	235	211	5	256
57	cp03770	ccaaagataaattctcctaggctca	cgaaagcctagcttttgaaagagga	252	284		283
58	cp04208	gaccgtgtatcagagaagctgcaa	aatcgcgaagcttacctgttgtgt	251	162	1	251
59	cp09955	gggttagagcaccctcaaatgtttc	ttgcgaggagaacaacgtaaacaa	226	269		234
60	cp05446	agcaaaccttctttcacagcaagc	caatcttcaatgacagtgagccgt	233	292		254
61	cp05134	ggtgcacctatgcatcctcatgta	gagacgagcacggatctgttttcta	160	169	5	158
62	cp08616	tcttcccttctaccaatccaagaa	ggttggattcacgtcatactccct	271	222	1	281
63	cp00091	aggaacactcccttgacacctgac	gcttttggacagagaagtatgtgc	236	180		251
64	cp00001	ccagacaaaacaaaaactctcggt	cgaggatcacatactcacacgtcta	287	228	7	300
65	cp10549	catttgccttcctcatgccaatac	ccattcagaccaccaatcacgtt	270	254	8	260
66	cp02612	aagagaactaaaatgcctccacgc	gcaagcgacagtttttgagtggta	205	252	2	218
67	cp06421	tgagctccccgaggtaacataaaa	gcattggtgtcttctgatttgactg	-	-		298

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
68	cp09444	tcatcccgaaaatgtgtttacc	tctacattcgtatatccatccccc	M	M		175
69	cp06397	ggagctcagataacagaagcggtg	ttctgtcccacaaacaatcttcca	267	276	5	277
70	cp10384	tggatgacaaccattgttcaagcta	agaaggaaattgctcacgcttgtt	274	278	1	286
71	cp01987	ggatgtcagataaatgtgaagacatcg	tggattggtctaagagctatgatgag	319	282		300
72	cp03967	tttatctgtgtcagcaaaagctgc	ttggatgctacaatcaccaacaca	194	144		202
73	cp06274	cgatgtgagacttccaacacatcc	agtgagatttgtgtttgttgggca	M	M		202
74	cp09557	cgaaaaggtcagatccaaaacacaa	actcccattaaatgtttctggccc	M	M		173
75	cp10936	ttagtgccgaatatttgtttgggg	catgacttgtgaagccgagaattg	186	186		200
76	cp03811	catttgccgtctttgccgtatatt	gtccctcccgctccttgtataatc	221	221		247
77	cp05599	cactgaatgttaagcgaccaaaaac	cttcttgcagaagctggtccttgt	M	M		198
78	cp08763	cacgaaagcttgctttttagttcg	ttagtttcacgtccagctatgcca	291	286		287
79	cp04700	gcccttagtttagtgcttaggatctga	tgtgcacattttcacagtctccct	207	207		269
80	cp08023	cctcactcttttctttcggttagca	tgtccacctctgctcttaaatgtt	257	264	4	274
81	cp06146	ttatttgctgatatgggggtgtcc	tctcacaagagattcagctccagg	-	-		290
82	cp08729	aggctgattcactcacatacaggc	cacatatcagggaagcacaagcac	259	307	5	280
83	cp08791	tttgaggtagtcatcgcataagaag	aacttagtgtggtcaaggtctccaaa	297	303		287
84	cp01363	ttgagcaatggatgaaacatcgtc	ctagcgagcaaactcacacccc	331	331		255

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
85	cp10986	ccatttggaacccaaactcatcat	gcaggtatacgtaccccaaagcaa	232	315		268
86	cp02448	tcactttggtttccttcgtcagaa	gaggatcaggattagcacatgttgg	158	141	5	191
87	cp11079	gaaccgtgttaaaagcacagaggg	attacctggccacctgcttctgta	261	212	2	262
88	cp06157	tgcttgtgattcaaagttgatcgg	cattcttcatgggacaatactcgtg	-	-		279
89	cp08777	tcaggacgacatggataaagcaag	tgtgaccttaagtaatgcttcacgc	274	M		264
90	cp10915	gatgacccaaattcttccacaagc	aagggggcaacaactgactttagg	266	304	11	250
91	cp08299	cacacccatctcaactccttgatg	gtggtcctgtctctgcctcagaat	229	186	2	213
92	cp03533	gtaccctacccgccctgttgacat	gaaaggtggtggagggaagaaaat	308	273		292
93	cp08654	gtgctaaaatagttttggcggatag	tttttaagttcagtccagcacgca	-	-		275
94	cp08255	ggcaaacgtttcttatcatgtccc	tcacttgggtctgaaaactggtca	300	273	2	264
95	cp08002	gttgatatcgtggttgaaggaaaag	tgaaatgtggacacaaaagaggga	180	179		154
96	cp08365	gtggtggttgtgcaacatttttga	tgtgaactccattgaattgtgggt	192	192		207
97	cp04479	ggttctcaaattcaactacaatcggc	tctggatctctgtctctgtgtctgtg	M	-		222
98	cp03562	acatttgcagaaaagctttggagg	cgccgcttaaattatcacaggtct	M	M		220
99	cp06173	tccactgaatactctccgcttttg	ggtccgttcttgctcctttctttt	289	294	10	289
100	cp00654	tggatcctttgccttctaccaaaa	ctcaaatcatggagtaaaccaaagtgg	M	M		254
101	cp00337	ggtttgaaaggacaacaatttggg	gagggagcggtaggtggtgtttat	M	M		184

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
102	cp05430	tccaagattcactgaaagtcaaaggac	ttactccctttcaaacccatgtgc	299	325	3	289
103	cp05517	tctaatcctaaaacttgcgcggat	attctccctccgagaccaagtacc	190	154	7	193
104	cp08499	aacctgaggccgacctagaattg	gttcaggtttcaccctcaccctaa	305	240	2	300
105	cp00347	gcaaaatcgaggattaggacatgc	tgttgcttcactgatagttctcaca	163	189	6	147
106	cp10138	aaatagtgtcggggtggttagggt	accgcacataaataagcaggcact	171	171		191
107	cp00449	acatgcgaggcatgatcatacaaa	gcttcactacctgaaattccaagcata	206	206		214
108	cp04550	ggtatagttccctcggctcttcgt	ggtgtgaaagtctctgatgggttt	263	M		256
109	cp06042	ggtacattcccctatgtccaacga	gcttggctgaatttcacgtttagg	279	335		259
110	cp05238	cgcatataaaccttccacctccaa	caccggaaaactgtgctagaaagg	267	245	9	262
111	cp00754	tcatcttcaaactctagtgccacg	tcctttggatcacacttccttgtg	285	322		283
112	cp10260	atgcaaccaaatctatgaaccgct	tttgtctacactccatggtgcctc	172	173	8	219
113	cp08017	cagatcattcaatccaaaggatgc	tcctgtttaaggaaaaataggtcgc	237	272	3	246
114	cp11006	ttggttaagatcttcaggacggga	gcaacttattgactcatccatttcg	186	166	8	147
115	cp00226	acatttggaggctcaagttggaaa	ggcctattactacagtctggaggg	221	256	8	231
116	cp00671	gagaaatgatccacgatggaaagc	atttgggtggattcgacaagaatg	298	284	7	297
117	cp02345	atgggcataaaccaattctcaacg	atgcacttacaaggacacgcacac	-	-		291
118	cp05566	agcatctatgccagcaatccaaat	tcgaggttagacatgttatgagtgc	173	146	1	204

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
119	cp07791	gaatcatcaaagaatgatgccgtc	gatcgaaaggggttcaatccaaat	201	258	1	222
120	cp03852	gcccaacaccatctcctagcttct	aacaaccttgttgcggtgtgagta	240	224	6	220
121	cp11089	ataacttcgccctccgatcatttt	tggtgtcatataaactatgtgtctggg	395	-		149
122	cp04232	ccaccctaaagttagaaaggggga	acctcggataaaccaaagctcaaa	217	173	3	217
123	cp08357	agctagaattttggaacgtggagg	aatcatagtgtccctgtagaaggagtt	M	M		209
124	cp04620	cgttcccattatccatttcccttt	tcgtgagcctttgagcttctcttt	281	244	1	285
125	cp08571	caaaaaggtgagtgagtgattccaa	cctcagttggcaaatcggttttag	315	297		295
126	cp01506	ccatggctgttttaggttttcatca	acacctcccctcactttcactcac	M	M		189
127	cp00737	gcaagggtgtgcaaataaaagtca	atctgatgcaacactcaatctggg	168	188	8	220
128	cp05799	cagctctatgcctcaccagattca	tggtggcaagacatcttgaggtaa	251	252		290
129	cp05941	agtggttgcatgtccaaatctcaa	tcttgggtcaacaactgattttctg	237	251	7	233
130	cp10209	tggtatccaaacaatggtgtttctc	tggtgaactcgtacatgtcggata	221	224	9	274
131	cp10882	ggcaatgtatggtccaaatcaaac	ggagatggtgttcgtaaatttccat	281	294	6	300
132	cp01787	caaaagccgttcttaaatcttgcg	cgggagttgtatccgggtctatatc	-	255		162
133	cp07847	ctgaatgaggtttattgatgccga	acggaaacatgcaaataacccaag	224	349		213
134	cp10517	ccaatggggacaaatgaagaacat	agatagcgacggatcttccagaaa	264	294		285
135	cp01142	agccacgagatagacacttctcca	gcaaatcctttcctacaagctaaac	179	154	3	195

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
136	cp07984	atccgttcttaaccggagacaatg	aaggcgattctgctacacaacaag	M	-		172
137	cp09766	tctaaaagcggcatgattgaaagg	ccgccccatatttctactcacact	M	M		263
138	cp03655	ggtactttcacctctttctcaaattgc	ctgcaacattccagaagtgcaaag	248	255	8	283
139	cp04502	cgtgacttctaagaacctccttccc	aaatgaatttaacgctcctcgctg	215	271	1	245
140	cp03113	gagtggatcaggtaagcgagttcc	ttttgggatttgagattggagacc	304	250		289
141	cp05137	ccgattgtagatgatcccgattgt	tgatgattgctgtggggaaatatg	280	265		271
142	cp03696	accatggtacatgtcaagccataa	gacatcccgcaaatcctagtcttg	246	240	11	250
143	cp10734	atcaccatgagaaggaagtccacc	tgtatgctcttcctctttctaagtgc	312	M		289
144	cp05970	caatgttcaacttggcctccataa	cgtgctcttggaagtctatttcag	285	289	11	297
145	cp05146	gaactcatcaatttcaaatccggc	tgtagtggactaattccgcttaatg	M	M		282
146	cp05311	gactcattcagattgaacccgtga	gctgttttagttaggctcctttgg	M	M		297
147	cp11111	caaaacagaagtctttggttcgaca	gcaattgtaccagccgaaggtaag	-	-		266
148	cp10667	ccaaggattcattcctcacaaacc	tattggccactgagaaacccaact	222	255	11	226
149	cp08559	tggaaaataagtgtcacacggtgc	tcgtgtttatttcatgtacccca	255	254		292
150	cp07863	gcttgtccagcctgtgctttatgt	ccaagaatgcaaggccttaaacag	264	285	7	264
151	cp02010	tgcaccaagattcgcgtatgatta	cactgacatgagtgaaagtgtagaaca	260	287		287
152	cp00455	ccctctgagcagattctccttgat	taactgtaaatccccggctgagaa	95	83	4	133

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
153	cp02660	ctcgtatttgatcaagacttgccg	gccttcaatgaaaatgcaggaatc	221	196	10	208
154	cp03658	ccccactgccatctaatcttaaacc	tgggatcatgaaagtgagaaaggg	253	-		254
155	cp02661	tgagtttggctacttgaaacgcac	ggtgagtgacttatgcttggtgct	244	230	1	217
156	cp08952	atactggttggtggcgagaatgat	ctgtcgagctctcccattcaaagt	M	M		211
157	cp04540	cgggatattttacgaactcgtaggg	cagtcctcaatgtaccggtgtcaa	211	212		297
158	cp03422	caactggattctcaaatctgtggc	ttgtcttggaaatcccacattcac	-	-		275
159	cp00195	ttgacctcaacgtgctctttatttc	gtgtcctgcatttgtggttagcct	167	167		203
160	cp08008	attgtagggcttgtacgctaacga	actcaattttcctccaattgctgc	298	298		293
161	cp10211	cattcaacgctgtgtaaggtccag	ttgcagaagagctttgtgatgagg	250	251		291
162	cp00431	ggagtggaagttaagttggcaatga	gcataactgcgtgtgattccattc	298	233		278
163	cp04111	ttggtgattacgtgaaggtgatgg	ccacgtcaatctagaaactctttccg	157	157		204
164	cp04193	tggcctaaactactgcatgaaggc	aagaggaaaagggaagggaataga	305	305		300
165	cp03673	gagagggggccaatagtgattacc	ggcttcttttacatgctaattcgtg	274	261	2	288
166	cp08819	ttgtttttgcctcttcctctcagc	tcaagagtaaccgagcaagatgaca	114	114		299
167	cp03923	accaacaagccagtccatcaactt	tgcgttgcgtactacacctttcac	M	240		275
168	cp05271	ttcccacttcctcgctttattcaa	cgtgaatttcttgagggattgatgt	243	260	10	279
169	cp02468	cagcagagcgggactgtacctact	ctaaattgtagcctgctgccatcc	242	289		238

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
170	cp04967	caaatcctcaccctaacgcaagtt	tctgcgctcatacactgatggagt	284	271		237
171	cp08554	ccgaagagtatttcatcagcctca	ctgctattaggacgatccactccg	M	M		273
172	cp01180	gctgttgaataacaaaaatggccc	gggtgaagagtccctctcatgtgt	M	M		133
173	cp08188	ttatcttcagcttgcacaacccaa	gggacctcatttctgtaaaatggttg	246	150		195
174	cp05939	gggagctttaaccgtgcttagaca	tgtggatctcaaaatgtcataacgg	258	262	2	228
175	cp04173	acttggcgactcttaatcttggca	cctttcagccaaaggtatacacgg	M	M		299
176	cp06240	gggtctgcatccataaccaaaaat	tgagcccatgttatgacctcaaga	282	302		276
177	cp00464	atggttggtagttaaaggtggggg	caaaatgtccaatcctgatggatg	168	167		218
178	cp00972	attttaagcattgccaccagagga	cagggataagattgagatttattgggg	236	197	4	225
179	cp00420	agcatgacccgataaggttttgaa	cagcagtgtagcaggtgcatcttt	230	-		212
180	cp01783	ttcgaatgtccatgaaacagaacac	ttcatacgaagggttgtctccaaa	277	288		271
181	cp04937	cctttttgcactgacgtagaattac	tcacgctgaatctggcgtataaaa	96	97		150
182	cp02915	agttggcttgacctgtccattttc	ggacaacatgtggggttggatatt	292	328		300
183	cp00099	tggcgggatcacataagaagaaat	ggagccaaatatctccctgaattg	256	272	9	262
184	cp08506	gcataactcatgaatgcctctcaca	cggaaaggtgaaataggagattgg	240	282		276
185	cp03451	gcattttcctttttcacttgccac	aatggggcttcagacattagtgtag	283	301	3	271
186	cp03855	gtgtctttgcctcgttctttgctt	gcaaccattttgtactacgcacga	308	317	7	299

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
187	cp10258	tgatacaccactcacagatatgcaa	gggaagtttctgcaaatcaaaaagg	276	216		246
188	cp05915	ccaatactgctggtccaaatgaaa	tgaaatccaggtcaggatatcgaa	269	306		299
189	cp00801	gccatttagtagctttggcattcg	aatcatggcatatcaaaccaaccc	215	219	2	257
190	cp05402	ctttttccactgtgctggaaaggt	agcgcatgtcaaaactcattagca	280	242		189
191	cp08316	ccaaaattcatgcatctctgcaaac	tgacactgtttagagatggattcg	237	245	5	208
192	cp04371	ttattgtagtgctggtgcatggaa	cgggcctatagctgtgagacttgt	267	M		224
193	cp04824	tgggcatttgatagaggtttaaagg	tcccaaaactctcatcattcagcc	274	262	8	246
194	cp06102	ataaccaatgggattggattgcg	tggttgtgaaaagtctgtgatccct	M	M		240
195	cp06216	cattatttgtgaccacttgtgcaac	atggagggaaacatgcaccttaga	222	226		262
196	cp06377	gtgttggaagcagaggaaggtgat	gcaagttgagaaccggcattaaaa	221	M		264
197	cp05133	tgaagctcgcatatgaagaagcag	ttcttggaagacttggagtcaggg	M	M		280
198	cp10853	gtaaagttcgattgcggcataaca	gccagtttcgcacacttcctagtt	194	211		226
199	cp04143	tcgttcccatacctgccaaaatac	ggtttattggatacggcaggaaca	316	M		275
200	cp00180	ggtggtatgtcctgcaaatatggg	tctttcttccttaagccctctaacca	297	327	8	300
201	cp08803	tgtgacctattaagacgggagaaaa	tcaaaacctacacagtacctccagc	215	M		219
202	cp07735	gcgtgttgaagctaactttttggc	ggaaagcattaacagggataaaggg	234	235		280
203	cp00069	taatggtgggttaatcttcgaggc	aaatggatccctactcaatttctcc	251	251		278

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
204	cp10615	ggccaaccaaattacgatagggag	atcaccccaaatttctggccttag	141	113		139
205	cp10941	tgatcctctgcattcaatgttggt	tttttcagaccagatgacacccct	267	245	1	259
206	cp00307	accacagtgaacttctttagttcca	tcgtgaggagggatgtctttaaaat	257	314		277
207	cp04704	tgaccctgtgaattgtgttattgagg	ggtcgaggatttgttgttcaccat	301	393	11	298
208	cp02884	ggcaatccaagaccattggaataa	tccatcacatcattgaacaatcgc	-	249		291
209	cp10903	tccaatccaaacaaaccctaagtga	ctttggagaatgacccatgaaaaga	157	157		298
210	cp08018	tccaacatttaaacctcccttgttg	ctgtcacgcatgagacaaatattga	161	157		187
211	cp01538	aggacaaattgaaactgctttgcc	tctcactccagcataaaatcaggc	-	-		209
212	cp05498	tgctatgcctcactagcatcacct	gaacacggatagcatgatggtgac	215	215		237
213	cp06388	ggtaaggacaaggtttttgtcccc	ggaaagaaagaaaacacggtgtgg	197	169	7	183
214	cp10757	tgacgaatctgtcatttgtggatg	agaggagcatgtttggtgcttttc	263	261		268
215	cp11035	ggatttgttgatttggaatggtgc	aacgtcaattccctccacaacaat	M	M		249
216	cp09502	cgtgtttgtggccatagaattcag	atcgcttgaacgaacgaacacaac	198	164	5	159
217	cp08486	gaggggacatacttgagatttggc	tcagaaacacagaagaagggtgaa	M	M		175
218	cp07971	ctgcaatttatccaatccaaccgt	ccatctggccagttcttggtagtt	173	167	5	159
219	cp07766	gcgtggaacaactatagctctggg	tcatggacggtggatgattgttat	222	238		221
220	cp01354	gcaaagtttggagaaaggaacaga	gacccaccagaaataaaagctccc	223	252	2	227

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
221	cp10086	atagttgcaagcagacacgctttg	gaccaaaaagcaaaactgccaaac	136	136		171
222	cp04488	tgctagtgttcatgccaatcaatg	tgtgggcatcagcttttaaactcc	228	204	11	234
223	cp04471	caaaagaccacaaccttatgcgtg	tccagtgattttatgcgagctaca	208	224		215
224	cp08467	tctttctcctttaactgtttctccc	ccataaccgtactggttttaaggca	M	M		290
225	cp00127	cggtagtgagtgagatgacaaaagaa	caaaactgagtcatgccaccgata	302	302		285
226	cp05255	cgaagttttagacagagacgaattcca	gagactcctttgctgaacgttggt	174	204		178
227	cp09979	ggcgggatacagacttaaacaaatg	ggaaaatattgtttgggaccaccc	319	342		298
228	cp04728	aatcttcaccactcataccaaacac	ttaggccatgggcgcttttatatt	281	301	10	275
229	cp04120	tatttgcgaggacatctggttcag	caccctcatgcaaccacctttaat	225	280		247
230	cp08824	tgaggttatccagtttttgggatg	tgtcctatgtgaagttattccacca	251	252		289
231	cp02392	cttgtaaggtgagatttgcaccca	cgacttgtaaggtgaggtttgcac	196	195		209
232	cp05762	gggtttgaagtccttaggttgtaggc	gagcaaaagcaagtgtattgatagg	272	286	1	300
233	cp08227	cctcatgtcatatcttaacttcagtgg	gcagctaaaatcccctcattctaa	329	270	2	300
234	cp00680	catgtgggcgaaactaatattcctg	ggtggttagcatttgcagctatca	288	M		298
235	cp08780	atcaaacctatcccctcctgcatt	ttgtccgtcatctttttcaaccac	204	220		199
236	cp00623	tgtttctgggaccatacatgtgct	gggtgatgcaggaaggtttgtaaat	308	M		284
237	cp10648	atcacaccaaggccaagtctaagc	tccaagggaggaagtcacgaataa	138	189	1	126

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
238	cp05247	tcagaatcaaacacaaggtttgtcg	tatttgatgggatcaatagagggg	M	-		261
239	cp05095	tgctagtgtagagtctgcccctcc	tgtggcatgaaatcagttcaggat	M	M		234
240	cp00234	tgggtctctttgctgattcagatt	ttccttaagcctctattgtatgcca	161	156	2	148
241	cp03777	agttttcatgcaatggctccttct	aacgaaaaagggttctgtattcacc	231	231		219
242	cp02142	gggccaaaggagaaaaataactgg	cagaagttcctcccaaaactatagca	260	303		245
243	cp05011	ggataaagtcaagacgtggttttcg	cacattatctgtcgtcgcaggaac	173	131		156
244	cp01918	cccctaggctctccctcatacaag	tgcagctttcttacagtgtgatgtg	324	296	9	292
245	cp10443	tgaaatgggtaccacttttaacgca	ttgttagaagggtgagatggttatg	271	321		290
246	cp05467	tccacatagtgtttgtgccttcgt	gattctgccattgaaattgccttag	M	M		249
247	cp04518	tcaaaatatcagggactcgtgacaa	ttgacgaaactcaaatcacgga	M	M		137
248	cp05677	aaccatggcaaaagacaacgtgta	atgaaccaaggcccattcttttct	M	M		300
249	cp09551	gatctcgaagaccttctctgctgg	aagagaaagtgcagggggtggt	236	230	8	223
250	cp06223	gaaagcttgaactttgccgttgtc	accagcacatgtagaaagccacaa	201	273		191
251	cp10994	cggtcttcataaaaatgaatccagc	acctcaaacgcttcgattatctgc	213	165	5	190
252	cp05382	tttaggatgacacgagctagcttaca	tccgtgttaatgatctgaaccatca	M	M		213
253	cp00500	gaacccagcacctgtactaagcgt	tatcatggctcaattggttgttgg	237	261	2	229
254	cp10271	aaggagttcactgtgttgagtgtgg	ggactctttccctctttctgcctc	240	220	4	214

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
255	cp08092	taaatgtcgttgaatgggcacaac	gccaaattggtacgttcatcattg	303	261	1	296
256	cp10612	aggctggatactttggctgtcttg	tgtcagacactgctacacccatga	-	291		289
257	cp10313	aagaagataaaagctgtccccaca	tcgaataaggcagtaagataggagtga	M	269		300
258	cp02936	gcggtttggacctcaagaatatga	gactggtcatccccaaaactgcta	295	-		268
259	cp01713	gctcccactaacctccattcgttt	ctgctctgctccttcaagtcactg	301	270	1	290
260	cp00416	cattcgttgtggactaatcatcct	ctattcatcccactaccaccctcg	274	321		267
261	cp04516	aattcgtgacatttacggcctttg	tccctttaccttgttcaagcattag	-	-		187
262	cp04849	cttcgaatttgtccatcgtcgagt	ccttttatttctctaacacgcgcc	258	283		298
263	cp02181	tggttagattggaagtcaatgcaca	tcgaacaaggagaatggtgtcaga	324	309	8	298
264	cp10639	cggtcaaaacgtgatgacagaaga	gggcagcacagaactaaaagagga	207	111		281
265	cp03989	caagaaagccatttttgttgggttc	caagtgtgagtcaaaaagttccaca	-	186		264
266	cp02530	tgcaaaagaagaacacaacacatgc	tccccacgtaatttagttacaccc	M (multiple bands)	M		281
267	cp11025	tgcaaaccatggtctatgttgagg	ttcagtgtacgacagcagcaatga	210	M		217
268	cp01038	ttttgacagaagaaacgtggtgga	ggggtatgtctgaaagttcaacgc	277	273	6	277
269	cp03482	ctatcgttttgttggccgaatcat	ctggagcctgagtagagcttggac	264	272	5	246
270	cp04908	ggacaaacggaatcattaacacca	atgacccgttttgtcacccttaga	231	247	9	226

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
271	cp07861	accgaaagtcacagatgacaacga	cgtttaggggattttggtgatgaa	261	233	10	248
272	cp04311	ctccagaagaagcaagctaaggtg	atgtcactgacgtctcaaggatgg	M	M		290
273	cp05103	cgagtcgagtcgagactacgaaactt	catgtatttgtttgccacgaacca	203	-		204
274	cp02334	ttatatgccccaacttgagaggga	ggaaccaatgcaataaaagaggaga	195	186	8	187
275	cp05087	ttctccattccaccgtagaggaac	tatgcctcattgaaaccttgctca	327	333		299
276	cp07842	tggttgaattggaagattgtgggt	gctatcacatgggagtatatggaatca	264	M		230
277	cp04934	catgacaacatgtttaaggatgcc	gtttcaaccggccaaccaatact	232	262	8	239
278	cp05783	aacgcaattacacagttggggaac	caaaactcgtcagatgcaacttca	245	M		255
279	cp05141	ggctgtaagttatgtaccttgccact	tgggcaaaattcagtgtcttcaact	195	163	11	172
280	cp08576	aaatctgacccacgtgaacaaggt	gtgtcgcatttggctccttttatt	131	113	8	147
281	cp06069	cttcattagttgctttagctagttccc	tatgcgcatgcattttctgtctct	-	353		222
282	cp00697	acatggttgcatgtgctcgattta	gatgctgccttggtttagatttttg	212	179		188
283	cp01951	gtccatgtcaaatatagaagtaggtgc	ggtctcgcttgaccctgtacacat	M	M		238
284	cp05393	aacattcaaaatttagggggcacg	cccatggtcaagggacatattgtt	250	285		228
285	cp03981	caggccagaacattgtcacttttg	tctttttctgaggttgtggcatga	229	260	4	234
286	cp01225	tcatcagctcataagccactggta	tgagtatttaacctgcgacaggca	M	M		237
287	cp01105	tgcgttggaagtcccacattttat	cctcaccctctcacagtgtcaaac	M	M		205

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
288	cp01469	tgaaatgtggacacaaaagaggga	ttaatttttctcgagagggccaca	-	-		281
289	cp10322	acaattagccatggtgcatttgac	tgatgttggcttctgatgaatggt	244	206	7	226
290	cp05689	tggacttatagtgtcacgttcaaga	tcacccatgcatataaagagatgga	194	194		232
291	cp00647	tatccactaagacacgccacaacg	gcaaattgattgcttcatccacttc	246	245		275
292	cp01564	tggtcttctgaccaaacgaacttt	tgctgattgtattggtggaaaagg	M	M		221
293	cp10892	caagatgtgacgagtgagttaaccaaa	gacttactccaacctcccaccaaa	279	220	11	257
294	cp06155	catggttagccaacccgtgtaaat	tggcacgaaagttggattacatttc	300	292		292
295	cp07857	ccgtaaaagaatttgtggcaaacc	tgaacccacctactttcttaaacacg	298	M		283
296	cp05723	tcaaattactgtcacagacacccga	cttatttaaggtgtgatgggggca	314	-		218
297	cp00177	ccggcggaatagtatgtttgaca	cactctcaccctcttactctcatcca	110	254		268
298	cp05660	tactcccaatcattcatgagccaa	ggtgtttttcactgcagtcaccc	158	172	10	172
299	cp03925	cactgctcgaaaaattgaatgcct	cacatgtaaaagtagggttgggca	296	304	3	298
300	cp02951	agctcctaccccacactctgtcac	ggtggtgcaagattacgagaatca	272	318	1	288
301	cp03115	tggagatgcgaatgagtttcaaga	tatgaggatgtacatatccccgcc	179	179		215
302	cp04570	acaagcatgcctcctcgtaacttc	cagaggtcaaaccgtttagaaatga	205	203	1	220
303	cp02534	catgagtgctgattggggatacac	acccgaatttaaaaatctgcaccc	288	-		290
304	cp08695	attagtggcggctcacaacagttc	acggcctgttgtcggatagaataa	260	255	11	285

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
305	cp04037	acacatgctgtgcaccttttatgg	gtcttgcaaggaaaacatcatacac	280	270		288
306	cp08116	cttttcatggttgtttgggcttgt	cttacgtggttgcttttctggctt	120	121		155
307	cp01037	catacatctgttcctcacaacatca	aaggacaaagttcatgccgggtta	252	270	4	274
308	cp08239	gctatgcatcgctgtatcttttga	tcatgagtttggtttggctcttca	192	164	9	180
309	cp03836	caccattgtcttaaggttttggggt	ctttgaaccatcggctctgatacc	M	M		189
310	cp05096	aatagttgcacagcccaaaaggaa	cagaaaacggagacgactgtctga	260	264		245
311	cp00277	aattacaaccgctgccaagggtat	cgtccacaagtgatgtgcctttag	305	269	5	300
312	cp02320	tgaatgctaaggtgactttgaacca	cctggaataattgcacattgggaa	223	243	9	235
313	cp09667	ccaggttcaaaagacgacaccttta	ggaaaactggggttgcaaaatcta	303	275		300
314	cp00200	aaccatatgcttccccgctagaat	tgccaatggatcaggtaacctaactt	230	194	8	227
315	cp03681	ggcccttgtacaaaaacttccttt	ttcaaatgtcttccccgagaaact	230	M		218
316	cp06190	cgagaaaaggaaatacagtgagaagg	atctccggggttgtaatcctcaat	M	M		242
317	cp00363	gtctttatggttggaagtctcacatca	aagcatatgcatctcgtatgtaggtc	M	228		216
318	cp02694	tgttcagtcctactattcatacctgcc	acagtgcaacgtgtccatgtcttt	M	M		277
319	cp03715	aggagctctttctttggatggagg	gtcattatcttgtgttttagacctgcc	214	245	2	187
320	cp05298	aaaaccacccaatgttacacccat	tcagagtaatgtttctgtttaggagcg	M	M		173
321	cp02937	ttgtcacatgaattctatgccgttc	aatggcctgacaggaaatgaaaga	168	152		167

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
322	cp03853	atgctcacaaactctgcaatctgg	acaccaaccatacacggtcaacat	179	169	2	164
323	cp02662	cacttcagtttcttgctctcataggc	gctcaaactgcgagacattcaaga	M	207		227
324	cp09370	acagttgcgaagctatgctgtcaa	aggctcatactcaaccatacccca	253	251		221
325	cp02779	acagttcatggttgcttcaagacg	tgcacctccatctattattccatca	139	M		165
326	cp08102	ggatattccagataatgcagacacg	tgagacatgatcaaaagtcgctga	152	148		217
327	cp01984	attttgatgcgttgtctcttgcct	ttccatcatttcaagcggagaagt	231	-		264
328	cp11009	cgaacttcattcgttccatgttga	aatttcaggtgggtcgaggtgtaa	182	237		173
329	cp03868	gtaaagctttcaaatgcgtttcacg	agaggggaagagggaaaagaaagg	223	392	1	247
330	cp08503	ttatcagcattaatgaaccgaccg	aatgtctgggttttgcagaaagga	234	243	10	246
331	cp01869	ccaccatacatgttgatagcatagttc	tccacgccttgaaagattcgtagt	M	M		297
332	cp08267	atatgaacggaatacaaccacgca	gcatccatcgacaagagttaggct	285	298	7	273
333	cp01814	tatttcttcctccacctcgtgctc	tcctcgtcggtaactacacatcca	304	310		277
334	cp01826	ttcgacactttcaacccagtcaaa	ctataaacgaaatcccatccgcaa	294	330	1	281
335	cp08179	tgagtatttgctgcatgtgttcca	gccaatatgagcacacttttcacg	237	224	3	255
336	cp03825	cgacagtggccattttattgtttg	ttccatctctaaacattgacgccc	242	256	4	237
337	cp04879	ttaatccgtgactcgtgactgaca	tcaagctagcaggatattttgtctcac	238	230	10	245
338	cp00381	ggcagaggagcatttaccaaacac	cacatgttctgacgataggccaaa	279	M		271

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
339	cp00937	ggctgccaaatcagttggtacagt	atataggaatctttgtggcgtggc	389	-		296
340	cp03862	ctcttgccacaatatggaatttgc	caataacaacatgcccaacaagaga	255	266	3	252
341	cp10285	gatgggcacaaaacaacatgatcta	tacaaatcatcgaagggtcgtcaa	312	255	7	282
342	cp10507	gctgaaagagtggcaattacagaa	tatgacaactcggcctttgag	-	-		300
343	cp06178	ttgcacttgcatagcatagccaaa	gatatgctacccacagccaaaacc	273	M		276
344	cp10923	cctgttaaatctcgtctatgctaccga	cactggtgcaggattgcaatataa	270	305	5	290
345	cp04114	gggtgtgaagttcgaaatgtttgac	accgacaagaacatatcggcta	231	251		256
346	cp05388	ctttcacgtgccatgtctactgct	gagatcttgagtttttctcctctatgg	275	M		269
347	cp00328	tgatcacctaagaaggaatggacaca	ggatgacaatgagattcttgagatagc	309	322	1	297
348	cp00221	tatacgccggcaaagtgaaagagt	gccagatagactccatgttccacc	279	263	8	298
349	cp03762	gcaacattcttaacattactctgcc	tttggtcgcaagtttcactttctg	228	147		223
350	cp02556	cacctgagaagttatttttgtggaacg	cgggtattccaattttgatatggtg	M	206		237
351	cp06038	attggcacctactgttttgcatga	ccaattcgagtccaatgccttaaa	310	301	10	295
352	cp00206	tccgtgaagtgcttgctgaagtag	gtggttgtccacgaatatgtgtca	245	210		236
353	cp08585	tgaaatagatgaagcaagggca	tcattttccctttgaggttggag	296	316	1	290
354	cp01710	gtacatgcacctaatgttgtggcg	tgtctttgtgcctttgtcaattgtg	249	M		254
355	cp04986	ctagctctttgaagtgcagccacc	tactctcacctcctccacttgtgc	252	246	4	246

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
356	cp10412	ttttggtcttccgtcttctcattg	tgaactgcataaactcgcctatgtt	305	279		288
357	cp00674	tcagattggaaatggccacaacta	gcagctacaatggacacaaccaac	205	255		239
358	cp10527	tctttcactgttatggggcactca	ggttgtggttactggtcagtgtgc	313	267	6	289
359	cp04988	acgggacaaaccaatacattttgc	tgtttgcaaaagctatcactcacga	203	215		193
360	cp08531	gtgaagacactttttcgtttgggc	caagctgatacctttcttttgtggg	151	125	7	136
362	cp08734	caaaatcggaacaccaaatagctc	atcgctctcaaccattcctatcca	M	M		129
363	cp10423	catgtttcaaaggctcacaatgct	caaaaggatagtttggatggattgg	M	M		205
364	cp02606	gtggacgacattggacaacattct	cttctttctacacaaacgacttcca	305	303		219
365	cp03494	atgtggtgcattaggagacaacca	tttaggatgaaagcatgaggacttc	208	192	2	184
366	cp09633	tatggtaacaaccgctaaacgtgc	cataactacacgacctcacgccag	431	M		122
367	cp06263	ggggctattttaattggcaaca	ggagtttttctcctaaagagcattc	495	471		266
368	cp06208	taagcatatggccagaccttgtcc	ctgtcgcgaattttatccacagat	195	M		184
369	cp01569	gatgtcatgtcttcattcatgggc	gttggcatacacatatggaaatgga	149	149		142
370	cp03802	atgttatgaatgcacctgcacgat	ctgcaagaagctagtgttgctcca	263	183	9	203
371	cp01020	ccaacaagtaaatgctcgccaagt	ggatggagttgaccattgagaaaa	216	206		218
372	cp03876	cccttatgccctagctacatgcac	cgtgtgcgtgtttcgaattcttat	122	107	4	139
373	cp03218	ccccagaaaaattttatttccctcc	aggtgaagtttgacgaaggtgaag	M	180		220

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
374	cp01523	ttataatccgacttggctggaagc	ttagcgagcaaaggaaaagtccag	-	-		296
375	cp10247	agagattgaagaacgcgtggtagg	cgctcttcctcaaacttccctctt	148	M		164
376	cp03846	gatcaaacccgattccaccaataa	taggggaagaatgggaagaggaag	265	278	3	268
377	cp09367	ttcgtcttctgaccaatatgtgac	taagatgaccaggaagtttcgcgt	282	M		257
378	cp05592	gctgtttttgtgctttataacctcgg	tagccaatgctggatcaattaggg	311	306	7	277
379	cp03863	aaccattcaagtggctgaccaaat	cccaaggaaacttgactttttcca	M	M		268
380	cp02569	tcggagcgttccataacggtaata	tcactcccatgactccgtcaatta	327	331	4	300
381	cp04256	cagtacacatgcagtgttttggca	gacaactgatgtaggtgtgggtgg	229	237	11	231
382	cp03743	gagtgtatgttttccctaagtcattcg	cgcatgccgtctaacaaagttcat	260	-		263
383	cp00800	aacgcttgagaaaacaaaaggggt	gcaattggcttctccactgactct	298	278	5	279
384	cp05187	aaggaagtgccaccctcattgtaa	tgaaccctcagttgttcctttgtg	284	-		280
385	cp05125	caagatactgtcatacaattagggacg	aaagatgggaagggagaggaacag	M	M		292
386	cp02585	tgctaggaaacatgaccttgacgtt	tgttccacggtgattgaatcgtta	303	M		293
387	cp05679	ccactcagatcagaccaaaatagg	cattcagcgcgtcgtttagttct	182	168	4	180
388	cp05325	gtccaagtggaagaaagaaggggt	ggagctaccttttcagtgtggagc	206	208	10	200
389	cp03521	ttgaaaagggtttgggtaggtgtg	ctttaatggcacttcgcaattcct	254	275		284
390	cp10668	ggtttatgccaaaagagaactcacca	ggcaggtaacatatgaagacatcatcc	M	M		295

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
391	cp01961	ttgatccacctgttgcacaagtct	atggtgccaaaggagatggtaaaa	313	317		291
392	cp05914	aagatcgtctcctccgactcaatg	aagcttccagtgaaggttaaggca	274	290	10	258
393	cp01877	cttggtagaaaaatgcgtgtcgtg	tgtcatccaagatgagcaacacaa	M	220		227
394	cp04871	cacagggcttttgtgaggtttttg	actaacaggagcacaacccattca	-	-		294
395	cp11005	caggtgcaaagtctcacatttcaa	gctctatgggctactatcattgttcca	M	M		194
396	cp10093	atcatccgcaagctaaacctacca	aactcacatcacccacgtgtcatt	278	248	7	264
397	cp08786	agaaattgattgaggccataggca	ggccttgggatggctatgtttag	235	260	3	231
398	cp00765	ccttgaaagtggagactcttcagaaa	tcatgcgtaataggcagaggtgag	-	-		142
399	cp08304	acgttcattgttcctttccctctg	gtgctcgaagagttcgtcttcctc	236	248	4	221
400	cp00690	ccggtgttagaaactgatactgctttg	tggggtgttagccatattttga	163	179		157
401	cp10627	catgagattaagaatcaaggccca	tcgtcattaaaccacatgaccgtt	M	M		245
402	cp08861	tgctatcaaattgtatggtgcaacg	gtgcccttggcaagaaattatcac	M	M		229
403	cp02470	tcgtctctttctccgtctcactca	aaagctctcaacgagaatgaacgc	242	240	8	227
404	cp03676	tccatctaacatgaacatgccctt	catcgatactgaagtgttgccagg	222	224	8	180
405	cp05150	aatggtgaaggagaagaaacgtgc	ggcaatcttgctccgcttatgtta	123	129		262
406	cp04320	gttttccagttttctgcattccaac	aaccatcagctttcctttcagaca	M	M		284
407	cp02909	ccgttggagatctgcttcagtttt	tgcaagccattagagaatgacaca	293	299	1	279

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
408	cp10438	atgaattcaatgggtcagttcg	ctacttattcggtcggcttagggg	256	242	5	177
409	cp06116	tgcgttaagcaaacatacaaccaac	gaaaccaatggaaaagcaccaaca	298	142		298
410	cp08831	tcgtatcgtgtatcgtgttcatgc	agttatgctttccgcatccacatt	M	M		208
411	cp03103	aagtaatgtattcgggaaagcacg	agagtgacttgaaacggctagaaa	M	M		290
412	cp06180	gaagtctgaatccaacgggctaaa	tgccttggatttcctaccatagag	299	257		267
413	cp05370	tctcaccatgtggaattcctgaaa	gcctgtgatcccatcctaccatac	280	249		300
414	cp07831	tcccaagacaattttggataccgt	acctcttcgatgtggaatgataag	M	M		191
415	cp06554	gagaattcgaaagtagcaaccgga	tacgagtcaagtgcctctcgaatg	251	M		253
416	cp01953	ccaacttaagggggagcgttagat	aaattcagcaaaaggaaaggggag	317	175		299
417	cp04508	cctactgattcacgaacgtgacaaat	aaacagaaacggttcgggctagtt	144	151	3	176
418	cp10143	ctgcgccattttcaggtacctatc	ctccctatttcctacattcttccaa	289	216		261
419	cp05718	ggccttactcctgggaaagtgagt	tgatgttagttgtgcaacctgcaa	-	-		236
420	cp04220	agacgaaagcttggacacgagaag	tcactgtcctttaatgctcacgca	281	M		289
421	cp08042	ggccattctgagtaaagaaaaggc	tggtaacgtgtagcaatcatagca	-	244		249
422	cp00794	tcgcatccagcaaataaaaggact	aggaatcggaagaattgcaaggtt	-	-		300
423	cp05010	catgaaatggctgcatctacacct	caactgttcctgtggcatgtttgt	296	292		275
424	cp01758	gacgaacacaactatttttcctctgc	cccctattatccccgaccaactta	294	278	6	281

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
425	cp05294	taacctcgactctttctccaatcg	gcgagctcatcattacattgctca	294	300		274
426	cp04264	gcaagaagcaacaggctatcatca	gaggagagtctgtctcattgcaagtt	275	265	5	216
427	cp04561	tgcgtactgtacttgtgcctatgt	cgcgccgtcttcttagatattttg	M	M		273
428	cp00033	catgatcaaccaaatacctggcaa	gacccccttttcgctaaaatttcct	226	252	10	210
429	cp00192	ttacaacaaccctcctccctagca	ttcgtgtaccatttgaactgtccg	315	284	10	299
430	cp03506	ttacgcttacgttcctccctcaac	cacgttaaatgtgatgtgtcacgaa	282	264	8	292
431	cp00228	gatcgattgtttccgtgacctagc	caacttggcaactgtcatcagctt	217	241		270
432	cp05392	tatacgggctgggatatccaaatg	cgcttattaccgttacattcatgacg	160	166		112
433	cp00653	gtcaaatgcctttttcgttggttc	agttggacagttgatgccatttga	242	175	1	210
434	cp02069	tcactttatctcatgcctgaaccg	gatatggaagaaacaatcatcctcc	108	108		141
435	cp01396	cacgcccagattcttatctttgttg	tgtttcagctcccccaatttaagc	189	177	8	172
436	cp00152	cagttttgtctccaccagacagttg	tcggccttatttagctctccttcc	193	212		178
437	cp00004	ggtaagggctgcttgccttttt	gctctaacagaatacatgatacgatgg	264	274		244
438	cp08652	gggaaagcaaaacctttccatgag	agttatgggcaccgggattaagac	301	301		300
439	cp08382	tcgttaaggagatcgatgctagacag	gaagctaacatatctgagtccattgtg	M	M		272
440	cp02271	gcctccacccttctttatcctctt	aggacctcttcgaaggtccatctc	272	276		275
441	cp02584	cgctttgtatatgggatgaaacacc	tcctactccaaaactttgccatca	289	288		274

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
442	cp10917	tcagacacaactttgatttgctcg	tcggaaacaggttcacctactcct	306	-		296
443	cp02423	ccaggagattgacagaaaacctga	aagccagcaaagttttggagtttg	279	-		271
444	cp09565	catttcgctaaatccaaatcccct	tcactctcactctcaagtaaacactca	-	-		212
445	cp08769	tgtcttttacatattaacgggtgcc	tctcatagactcatagaatttccctcg	-	-		163
446	cp06328	aagaacaaccttgtaagcgccaaa	tcgagaattgtttcttcgaactgc	290	246	1	280
447	cp02429	tcccagtcaatcttaatccgaatg	gaaggcaccaccgcttttaatgta	262	258		254
448	cp03433	atttttccgtggaattgaaggagc	ccagctgcctaatcatggaatcta	306	263	1	290
449	cp09604	tgtaatggcacacttccctgtgat	tcatgttttccaacagggttctcc	271	261	8	250
450	cp00193	gatcagtttaagtggcagtaaggca	ttgaaaactatctactccaagccca	206	206		230
451	cp10889	caaccctcattgtcacccctaatc	tcgtggaagatggtatattggtttg	252	245	2	228
452	cp06039	cggtgaactcggtgtactctgcta	aaaaagtagaaaaccaaacgcggc	233	220	1	220
453	cp07770	tgcgtgttgcattagtcatgtttg	acccttgagacagaaaccgacaac	311	319	1	293
454	cp04279	catctgcaatgacgacgtttttaac	ggtcaaaatgaagtacacaaagcca	250	312		269
455	cp04185	aaaatcatctttggagttgccctc	tctgtgcgtaaaatggatccttca	263	M		265
456	cp03429	ttgctagataatggtgcatggtgg	gtgcagtgccaataaattgtcgag	M	M		290
457	cp03436	gagcacaaaacacaacctgcaaac	tggtgacctgtaaatcgaagagaa	204	-		217
458	cp05500	cccaatcttacacggcccttttat	ggaacgtttatctaagtaaggggaagc	277	-		299

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
459	cp00037	tccagtgacatcatttacacgtagac	gggaagaagtgatggaagatcgaa	331	-		299
460	cp08513	gcaggaagaaagtcgtttactgcaa	gatatcaaggaccttcctccccac	314	-		294
461	cp09687	tgttgggagtgctacaggctacag	tcaacttccacaccaagaccagaa	M	M		299
462	cp05018	ctccctctcaaaatctctgctcca	aaaagatgcctgtgttgttggtga	257	-		279
463	cp01615	catggtaagccgcacttgttctaa	ctcgtgggttcgacactcctactt	291	-		295
464	cp01784	cttagaccagtccaggctgaaagc	atttgggtcagttcagagggttga	276	-		272
465	cp09985	tctttgctttgtaatcatgcccct	tggtaaagttcacgtcaagaacagc	268	268		257
466	cp00148	taagcaaagactttcgtggtgcag	ttcagattttcctcgttctggagg	303	304		267
467	cp06427	ttttgcacgtataaaggggtagca	gtgccaagcttttcacaggctaac	166	162	7	148
468	cp02928	gccaattaggggaaccactaccat	gcttacctatttcattaagtggggttg	239	259	11	227
469	cp09102	caagaatttgctccaatccctttttc	aagggtgaccacaaataaggcaga	103	129	4	114
470	cp06236	gtctcttttgaaccccaattgtgaa	gaatgaaattttgcaggacttgcc	194	216	9	177
471	cp02873	agtccggtgttgactgctaaccat	ggactgggatggtatgttcctcac	292	319	8	275
472	cp00080	ggcaacctgcacaaagtaatctga	atcaaggacaaagttccgccctat	131	147	6	116
473	cp10971	ttttaccctctccttctgtcaagtg	aggtgaccacaagatgaaaggctc	207	296		291
474	cp00451	agttggagcaaaaccggagtatca	atatctttgacgcaacccaaaacg	244	M		230
475	cp00106	ttcccttctctttctcgcctaacc	cattaagatcaacacagtcgagaca	268	260	4	276

Appendix Table 1 (Continued)

	Marker name	Forward primer	Reverse primer	Cultivated yardlong bean (JP81610) (bp)	wild cowpea (TVnu457) (bp)	Yard long bean linkage group	Expected product size(bp)
476	cp07768	catgctgcggagttgtaagtgtct	gctttcaaaatctacactgaaggttgg	-	-		172
477	cp01582	gcaaagaaccacaaccctgtgata	tgctcaagatggcacttctcattc	132	143	2	137
478	cp00666	tcacctaaattcgggaccagaaaa	aaaacagtttagccccctgcaagt	213	262	4	218
479	cp10311	tgagcaatcatccactaaccacct	atgatagtgcagaattcaaggccc	243	281		223
480	cp02070	agttcccatctcatatcttagtcagg	ccggaaacgagtctctgggtatc	M	-		174

Appendix Table 2 The SSR markers showing segregation distortion in the BC_1F_1 population of the cross between yardlong bean JP81610 and wild cowpea TVnu457

Marker	LG	Genotypic frequency[a]			χ^2	
		A	H	-		
cp10648	1	80	107	3	3.9	*
cp03451	3	110	79	1	5.08	*
cp05430	3	106	79	5	3.94	*
CEDG305	3	111	74	5	7.4	**
CEDG075	3	98	60	32	9.14	***
cp08015	5	66	104	20	8.49	***
VR0326	6	73	101	16	4.51	*
cp00180	8	68	95	27	4.47	*
cp04728	10	79	106	5	3.94	*
cp08695	11	114	73	3	8.99	***
cp04256	11	108	69	13	8.59	***
cp03696	11	101	70	19	5.62	*
cp10667	11	114	70	6	10.52	***
cp04488	11	118	72	0	11.14	***
cp10892	11	108	75	7	5.95	*
cp04704	11	118	71	1	11.69	***
cp05141	11	115	72	3	9.89	***
cp05970	11	118	70	2	12.26	***
cp10915	11	117	70	3	11.81	***
cp02928	11	118	72	0	11.14	***
CEDG002	11	116	71	3	10.83	***
CEDG076	11	108	77	5	5.19	*

[a] A, H and – are homozygous JP 81610 genotype, heterozygous genotype and missing data, respectively

*, ** and *** indicate significance at 5%, 0.1%, and 0.01% levels, respectively

Appendix Table 3 The SSR markers showing segregation distortion in the F_2 population of the cross between yardlong bean JP81610 and wild cowpea TVnu457

	markers	LG	Genotypic frequency						χ^2	
			A	H	B	C	D	-		
1	CEDG149	1	44	103	29	0	0	3	7.67	*
2	CEDG003	1	63	62	35	0	0	19	17.9	***
3	cp08585	1	58	68	32	0	0	21	11.62	***
4	cp02909	1	58	69	31	0	0	21	11.76	***
5	cp10941	1	54	73	29	0	0	23	8.65	*
6	cp07770	1	62	91	24	0	0	2	16.46	***
7	CEDG256	1	70	88	21	0	0	0	26.88	***
8	cp01826	1	74	75	16	0	0	14	42.14	***
9	cp01713	1	58	97	24	0	0	0	14.17	***
10	cp04502	1	51	86	25	0	0	17	8.96	*
11	CEDG293	2	23	106	50	0	0	0	14.23	***
12	cp01354	2	21	105	51	0	0	2	16.32	***
13	cp03853	2	29	101	49	0	0	0	7.42	*
14	cp08227	2	23	74	51	0	0	31	10.59	**
15	cp02612	2	46	91	27	0	0	15	6.38	*
16	DMBSSR228	3	25	138	16	0	0	0	53.47	***
17	cp08179	3	23	136	18	0	0	2	51.27	***
18	cp03846	3	39	114	26	0	0	0	15.3	***
19	CEDG205	3	45	102	30	0	0	2	6.66	*
20	cp05430	3	51	73	19	0	0	36	14.38	***
21	CEDG305	3	39	0	1	0	139	0	112.2	***
22	cp05679	4	30	87	62	0	0	0	11.58	***
23	cp00972	4	27	85	62	0	0	5	14.17	***
24	CEDG165	4	25	103	48	0	0	3	11.12	***
25	cp04986	4	31	94	54	0	0	0	6.36	*
26	CEDG127	4	47	70	54	0	0	8	6.19	*
27	cp00277	5	40	106	31	0	0	2	7.84	*

Appendix Table 3 (Continued)

	markers	LG	Genotypic frequency						χ^2	
			A	H	B	C	D	-		
28	DMBSSR217	5	55	92	31	0	0	1	6.67	*
29	CEDG171	5	58	80	37	0	0	4	6.33	*
30	VR0326	6	6	61	83	0	0	29	84.28	***
31	cp01758	6	29	98	50	0	0	2	7.02	*
32	cp06388	7	59	100	20	0	0	0	19.46	***
33	VR2094	7	49	112	18	0	0	0	22.05	***
34	cp05517	7	71	78	23	0	0	7	28.28	***
35	cp06427	7	78	74	27	0	0	0	34.43	***
36	DMBSSR230	7	72	86	20	0	0	1	30.58	***
37	cp08267	7	69	81	22	0	0	7	26.27	***
38	cp00001	7	56	98	21	0	0	4	16.52	***
39	DMBSSR093	7	56	101	19	0	0	3	19.4	***
40	cp03855	7	64	76	24	0	0	15	20.39	***
41	cp00671	7	30	111	15	0	0	23	30.81	***
42	cp00737	8	39	108	30	0	0	2	9.51	**
43	cp11006	8	36	96	30	0	0	17	6	*
44	VR0357	8	44	110	25	0	0	0	13.42	***
45	cp00226	8	50	67	31	0	0	31	6.2	*
46	CEDG172	9	44	104	27	0	0	4	9.53	**
47	cp02660	10	35	73	59	0	0	12	9.54	**
48	cp00192	10	41	76	60	0	0	2	7.61	*
49	cp08695	11	57	97	24	0	0	1	13.67	***
50	cp04256	11	50	90	26	0	0	13	8.12	*
51	cp10667	11	57	89	32	0	0	1	7.02	*
52	cp10892	11	55	92	30	0	0	2	7.34	*
53	cp05141	11	58	90	31	0	0	0	8.15	*
54	CEDG002	11	58	89	31	0	0	1	8.19	*
55	CEDG076	11	58	86	27	0	0	8	11.25	***

Appendix Table 4 Correlation coefficients among traits in the BC$_1$F$_1$ (or BC$_1$F$_{1:2}$) population of the cross between yardlong bean JP81610 and wild cowpea TVnu457

	ECL		LFPL		LFPW		STLW		STL10		STT		PDL		PDW		PDT		SDNPPD	
LFPL	0.28	***	1.00																	
LFPW	0.29	***	0.64	***	1.00															
STLW	0.64	***	0.21	**	0.23	**	1.00													
STL	0.55	***	0.32	***	0.36	***	0.65	***	1.00											
STT	-0.04		0.22	**	0.08		0.13		-0.12		1.00									
PDL	0.11		0.41	***	0.41	***	-0.01		0.23	**	-0.02		1.00							
PDW	0.03		0.27	***	0.33	***	-0.14		0.24	***	0.08		0.64	***	1.00					
PDT	-0.02		-0.28	***	-0.29	***	-0.05		-0.25	***	0.02		-0.58	***	-0.47	***	1.00			
SDNPPD	0.10		0.18	*	0.13		0.11		0.09		-0.01		0.45	***	-0.13		-0.12		1.00	
PDTN	0.00		-0.04		-0.03		0.25	***	-0.10		0.54	***	-0.43	***	-0.33	***	0.20	**	-0.27	***
SDTWT	0.15	*	0.27	***	0.20	**	0.37	***	0.09		0.69	***	0.08		-0.06		-0.03		0.25	***
FLD	-0.09		0.06		0.09		-0.32	***	0.05		-0.14		0.01		0.37	***	-0.11		-0.47	***
PDDM100	0.01		0.16	*	0.17	*	-0.06		0.05		0.00		0.32	***	0.21	**	-0.15	*	0.14	*
BRN	0.07		0.07		0.07		0.31	***	0.05		0.43	***	-0.06		-0.18	*	0.01		0.14	*
SD100WT	0.10		0.27	***	0.30	***	-0.03		0.25	***	0.09		0.56	***	0.82	***	-0.37	***	-0.19	**
SDL	-0.05		0.12		0.11		-0.23	**	-0.02		0.08		0.48	***	0.64	***	-0.34	***	-0.18	*
SDW	-0.04		0.26	***	0.31	***	-0.14		0.16	*	0.04		0.67	***	0.73	***	-0.50	***	-0.05	
SDT	0.11		0.15	*	0.20	**	0.03		0.24	***	0.08		0.31	***	0.63	***	-0.13		-0.20	**
PDSBS	0.04		0.19	**	0.24	***	-0.06		0.19	**	0.01		0.39	***	0.67	***	-0.39	***	-0.61	***
SDP	-0.01		-0.13		0.01		0.03		-0.03		-0.26	***	0.33	***	0.04		-0.16	*	0.18	*

Appendix Table 4 (Continued)

	PDTN		SDTWT		FLD		PDDM		BRN		SD100WT		SDL		SDW		SDT		PDSBS
LFPL																			
LFPW																			
STLW																			
STL																			
STT																			
PDL																			
PDW																			
PDT																			
SDNPPD																			
PDTN	1.00																		
SDTWT	0.68	***	1.00																
FLD	-0.19	**	-0.34	***	1.00														
PDDM100	-0.18	*	0.01		-0.10		1.00												
BRN	0.53	***	0.57	***	-0.38	***	-0.05		1.00										
SD100WT	-0.29	***	0.00		0.38	***	0.20	**	-0.20	**	1.00								
SDL	-0.25	***	-0.09		0.21	**	0.14		-0.17	*	0.70	***	1.00						
SDW	-0.33	***	0.00		0.21	**	0.24	**	-0.20	**	0.79	***	0.74	***	1.00				
SDT	-0.24	***	-0.02		0.31	***	0.10		-0.19	**	0.79	***	0.59	***	0.61	***	1.00		
PDSBS	-0.09		-0.16	*	0.49	***	0.14		-0.14	*	0.66	***	0.55	***	0.59	***	0.44	***	1.00
SDP	-0.26	***	-0.14	*	-0.19	*	0.15	*	-0.10		0.06		0.18	*	0.29	***	0.02		0.06

*, ** and *** indicate significance at 5%, 0.1%, and 0.01% levels, respectively.

Appendix Table 5 Correlation coefficient among traits in the F_2 (or $F_{2:3}$) population of the cross between yardlong bean JP81610 and wild cowpea TVnu457

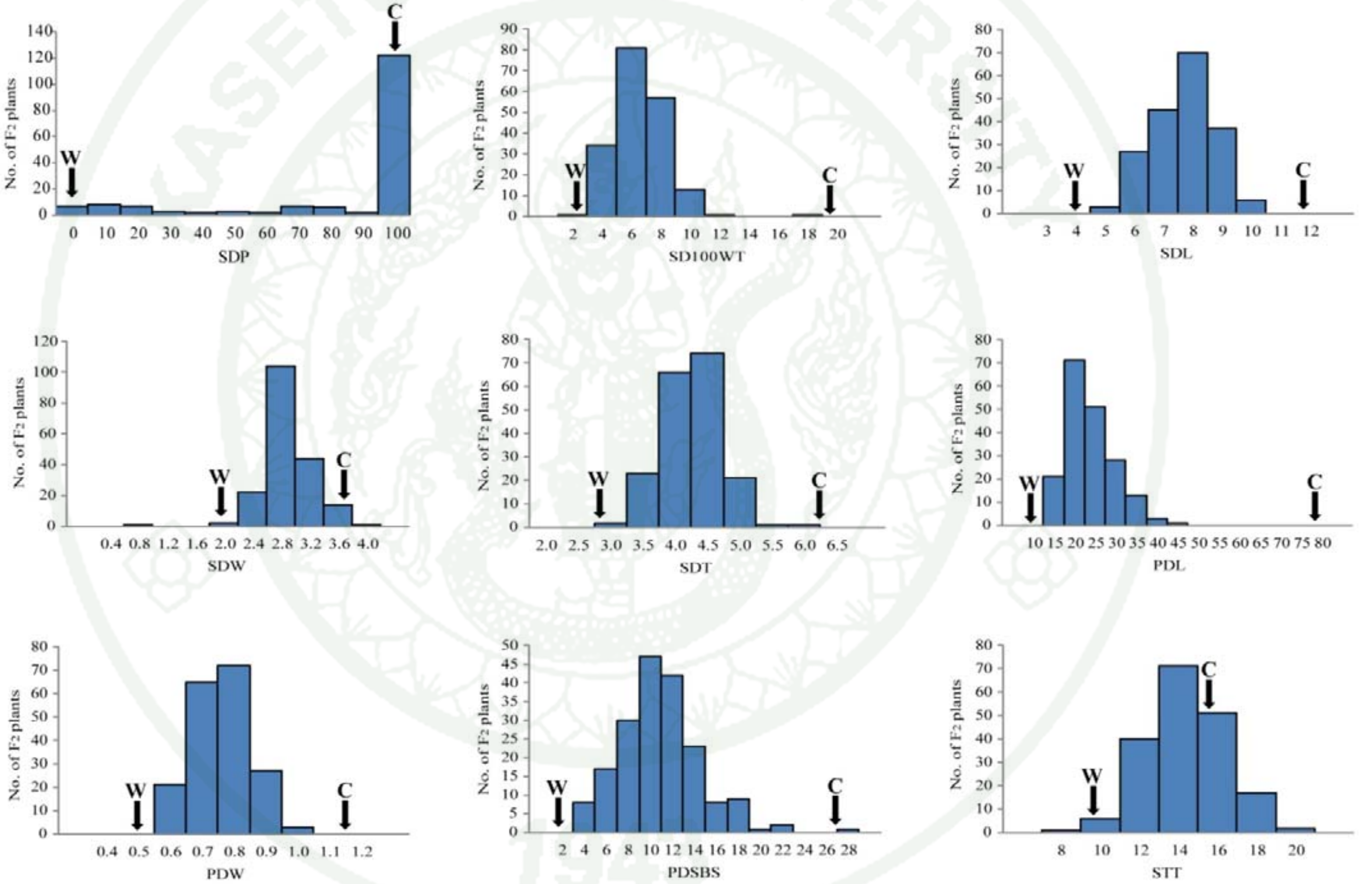
	STT		STL10		ECL		PDDM		SDNPPD		PDL		PDW		SDTWT		SDL	
STL10	-0.00		1.00															
ECL	-0.00		0.05		1.00													
PDDM	0.17	*	0.14		0.05		1.00											
SDNPPD	0.26	***	0.05		0.04		0.01		1.00									
PDL	0.21	**	0.21	**	0.04		0.17	*	0.5	***	1.00							
PDW	0.14		0.19	**	0.05		0.16	*	0.14		0.74	***	1.00					
SDTWT	0.27	***	0.03		0.02		-0.01		0.21	**	0.17	*	0.11		1.00			
SDL	0.12		0.17	*	0.02		0.12		0.08		0.73	***	0.77	***	0.22	**	1.00	
SDW	-0.00		0.08		0.08		-0.02		0.03		0.35	***	0.51	***	0.03		0.47	***
SDT	0.13		0.12		0.00		0.12		0.08		0.62	***	0.77	***	0.18	*	0.82	***
PDTN	0.10		-0.05		0.03		-0.09		-0.22	**	-0.30	***	-0.26	***	0.35	***	-0.10	
LEPL	0.37	***	0.20	**	-0.10		0.06		0.15	*	0.18	*	0.18	*	0.10		0.18	*
LEPW	0.32	***	0.21	**	-0.10		0.18	*	0.12		0.28	***	0.27	***	0.06		0.31	***
FLD	-0.10		0.03		-0.20	*	-0.08		-0.04		0.14		0.15	*	-0.25	***	0.12	
SD100WT	0.02		0.22	**	0.10		0.15	*	0.03		0.63	***	0.77	***	0.11		0.75	***
PDSBS	-0.10		0.12		0.01		0.16	*	-0.51	***	0.43	***	0.57	***	-0.13		0.52	***
SDP	-0.00		0.11		0.09		-0.02		0.06		0.15	*	0.18	*	0.03		0.24	***

Appendix Table 5 (Continued)

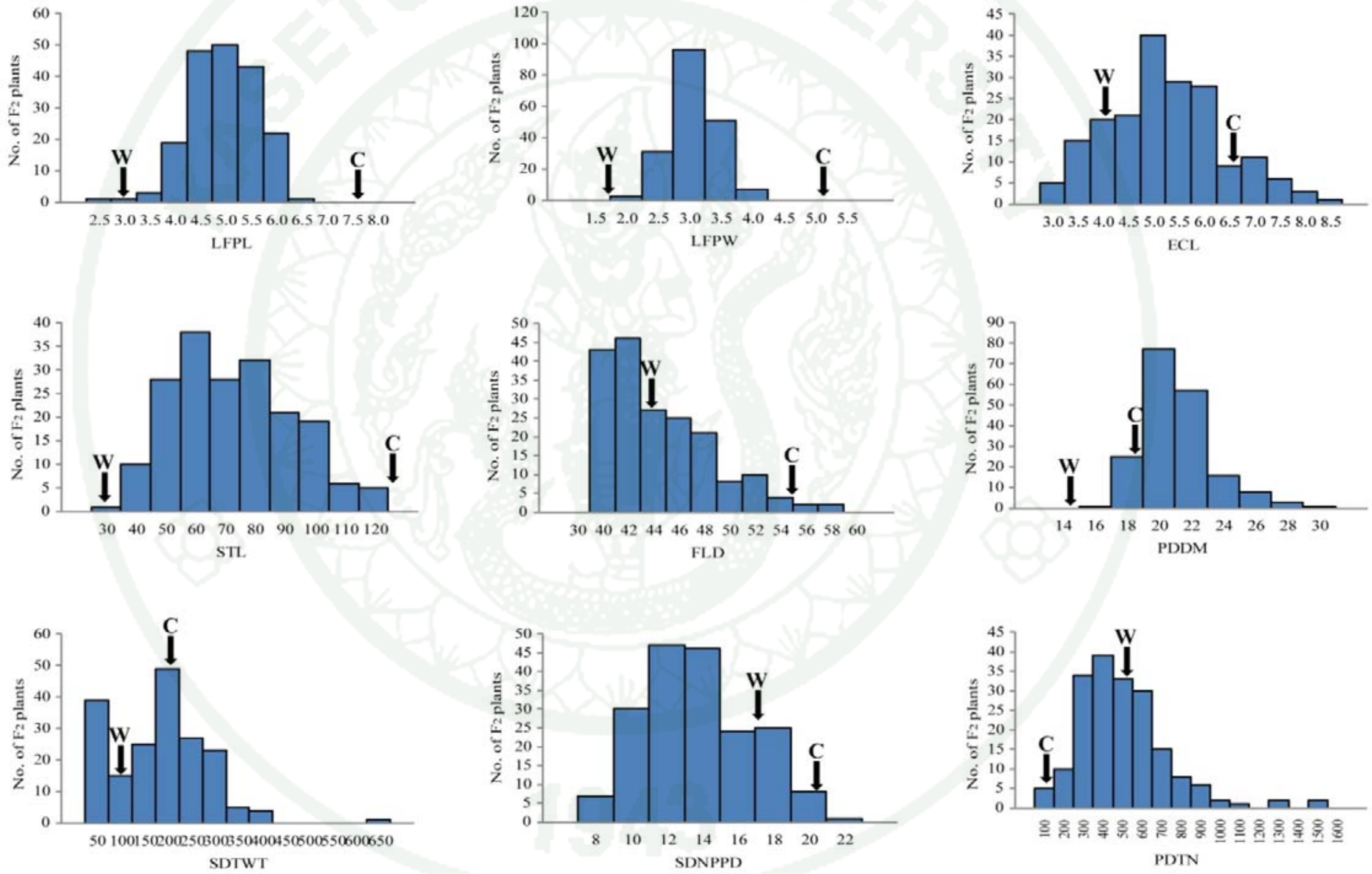
	SDW		SDT		PDTN		LEPL		LEPW		FLD		SD100WT		PDSBS
STL10															
ECL															
PDDM															
SDNPPD															
PDL															
PDW															
SDTWT															
SDL															
SDW	1.00														
SDT	0.50	***	1.00												
PDTN	-0.00		-0.10		1.00										
LEPL	0.13		0.17	*	0.10		1.00								
LEPW	0.15	*	0.34	***	-0.06		0.75	***	1.00						
FLD	0.23	**	0.13		-0.24	***	-0.11		-0.02		1.00				
SD100WT	0.49	***	0.67	***	-0.22	**	0.17	*	0.23	**	0.30	***	1.00		
PDSBS	0.29	***	0.45	***	-0.18	*	-0.06		0.06		0.20	**	0.50	***	1.00
SDP	0.24	***	0.19	**	0.06		-0.01		-0.04		0.00		0.10		0.05

*, ** and *** indicate significance at 5%, 0.1%, and 0.01% levels, respectively.

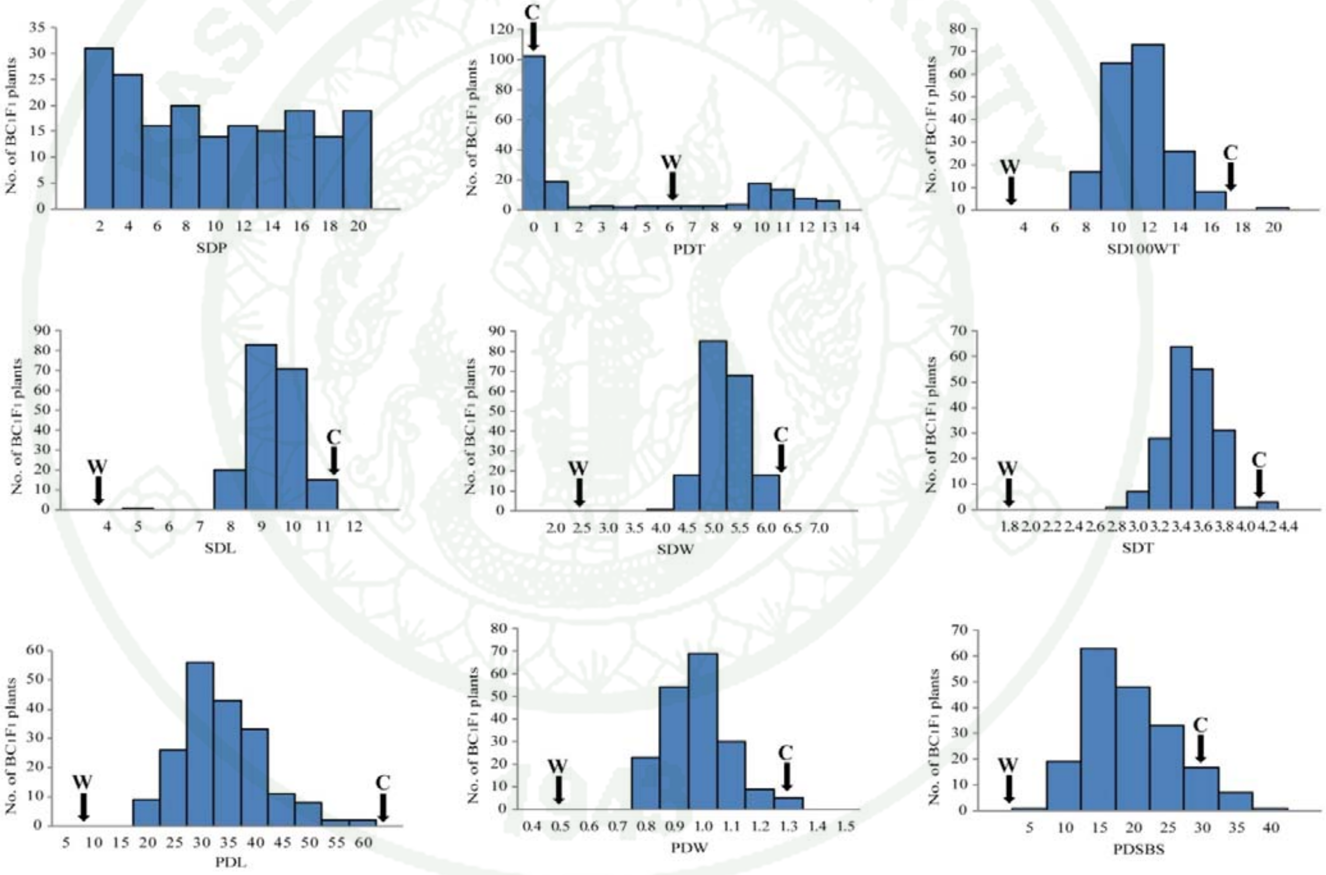
Appendix Figure 1 Domestication-related traits examined in F_2 population (JP81610 x TVnu457). JP81610 is a cultivated (c) accession and TVnu457 is a wild (w) accession.



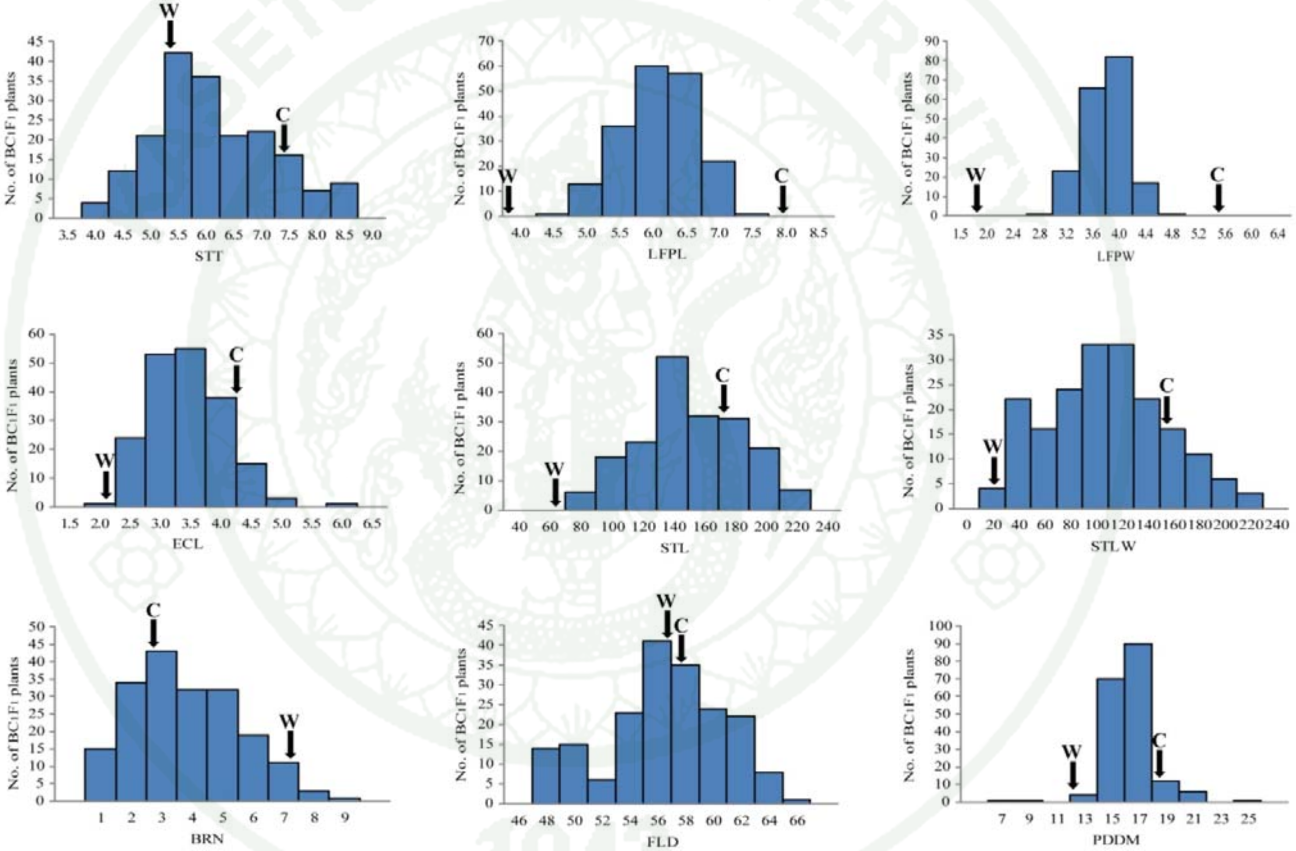
Appendix Figure 1 (Continued)



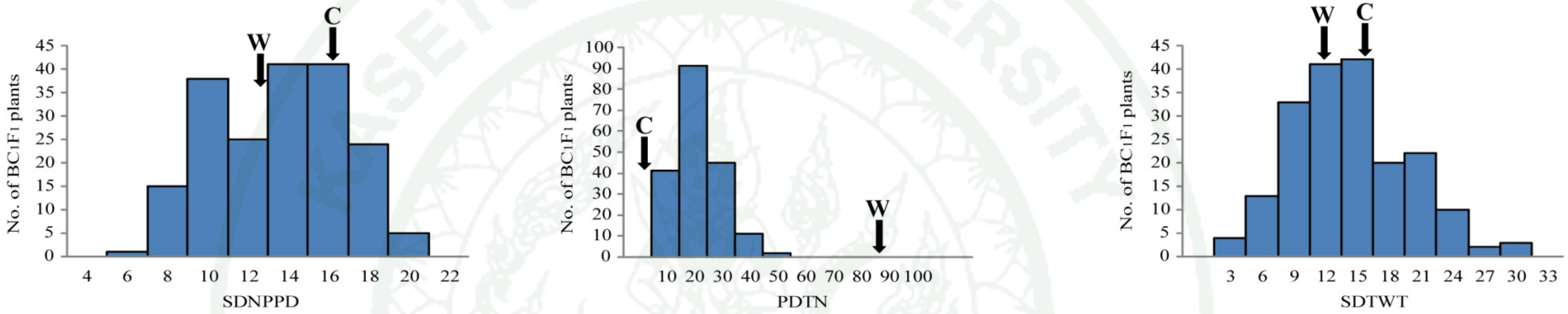
Appendix Figure 2 Domestication-related traits examined in BC$_1$F$_1$ population [(JP81610 x TVnu457) x JP8160]. JP81610 is a cultivated (c) accession and TVnu457 is a wild (w) accession.



Appendix Figure 2 (Continued)



Appendix Figure 2 (Continued)



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Kongjaimun, A., P. Somta, A. Kaga, N. Tomooka, D.A. Vaughan and P. Srinives. 2011. The QTL analysis of hard-seededness in wild cowpea using F_2 population. **Khon Kaen Agr. J**. 39 supplement 3: 328-334.

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