Genome Dissection of Traits Related to Domestication in Azuki Bean (Vigna angularis) and Comparison with other Warm-season Legumes

TAKEHISA ISEMURA1,†, AKITO KAGA1,†, SAEKO KONISHI2, TSUYU ANDO2, NORIHIKO TOMOOKA1, OUK KYU HAN3 and DUNCAN A. VAUGHAN1,∗

1National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba 305-8602, Ibaraki, Japan, 2Society for Techno-innovation of Agriculture, Forestry and Fisheries 446-1 Ippai-zuka, Kamiyokoba, Tsukuba, Ibaraki, 305-0854, Japan and 3Winter Cereal and Upland Crop Division, Honam Agricultural Research Institute, NICS, Rural Development Administration, #381 Songhak-dong, Iksan 570-080, Seoul, Korea

Received: 29 September 2006 Returned for revision: 28 November 2006 Accepted: 23 May 2007 Published electronically: 29 August 2007

INTRODUCTION

Most domesticated legumes belong to two distinct phylogenetic clades, the inverted-repeat-lacking clade that includes many of the temperate or cool season legume crops, and the Phaseoleae clade that includes many of the tropical or warm-season legumes. In the Phaseoleae a large group of closely related domesticated species is present in the genera Phaseolus (five species domesticated in the Americas) and Vigna (eight species domesticated in Africa and Asia). In addition, the Phaseoleae includes the most important domesticated legumes in terms of production, the soybean [Glycine max (L.) Merr.]. All of the domesticated species in the Phaseolus–Vigna group are diploid except one, the tetraploid species Vigna reflexo-pilosa Hayata. The close phylogenetic relationship of the Phaseolus–Vigna group suggests that at the genome level there is likely to be a high degree of synteny among the domesticates of this group.

The genus Vigna is naturally distributed in the New World, Africa, Asia, Australia and the Pacific and has been divided into six subgenera. Most of the Vigna species that occur in Asia belong to the subgenus Ceratotropis and are known as the Asian Vigna. In the subgenus Ceratotropis six species have been domesticated. These six species are azuki bean [Vigna angularis var. angularis (Willd.) Ohwi and Ohashi], mungbean [V. radiata (L.) Wilczek], black gram [V. mungo (L.) Hepper], rice bean [V. umbellata (Thunb.) Ohwi and Ohashi], moth bean [V. aconitifolia (Jacq.) Maréchal] and creole bean [V. reflexo-pilosa var. glabra]. Among them mungbean, azuki bean and black gram are the most important in terms of production (Tomooka et al., 2002).

Azuki bean (also known as adzuki) is a traditional legume grown across East Asia and northern South Asia (Zong et al., 2003). The presumed wild ancestor of cultivated azuki bean is V. angularis var. nipponensis (Yamaguchi, 1992). This wild species is distributed across a wide area of Japan, the Korean peninsula and China, including Taiwan, Nepal and Bhutan (Vaughan et al., 2004). Recently, a new species, V. nepalensis Tateishi and Maxted, from Nepal and Bhutan has been described (Tateishi and Maxted, 2002). Vigna nepalensis resembles V. angularis var. nipponensis in morphological characters and is genetically closely related to cultivated azuki bean with which it is cross compatible.
They are considered to belong to the same species complex (Tomooka et al., 2006). Since V. nepalensis is within the primary genepool of azuki bean it was used in a cross with azuki bean to develop a molecular linkage map of azuki bean. Azuki bean was the first Asian Vigna with a molecular linkage map that has resolved the 11 linkage groups corresponding to the haploid chromosome number of the diploid Asian Vigna (Han et al., 2005).

Azuki bean shows numerous differences in morphological and physiological traits associated with domestication compared with its closely related wild relatives. Domestication of azuki bean has resulted in a conspicuous increase in seed and pod size, non-twinning growth habit, and loss of seed dormancy and seed dispersal ability. In addition, seed colour variation that is not found in its wild relatives is present in azuki bean cultivars. Among the domesticated Asian Vigna and their presumed wild ancestors, seed size, seed colour and life history traits differ markedly. For example, cultivated mungbean generally has green seeds that are about five times the size of wild mungbean, while cultivated azuki bean usually has red seeds that are more than eight times the size of the wild azuki bean (Tomooka et al., 2000).

Since the Phaseolus–Vigna group includes many domesticated species, comparative genome studies of the domestication syndrome in this group may shed light on cryptic species-specific genes for morphological and physiological variation (Hawkes, 1983; Hammer, 1984). The genetic control of some domestication-related traits has been characterized in some of the warm-season legumes including mungbean of the Asian Vigna and New World Phaseolus (Boutin et al., 1995; Koinange et al., 1996; Menacio-Hautea et al., 1993; Papa et al., 2005).

The cross between V. nepalensis and V. angularis that was used to develop the molecular linkage map of azuki bean (Han et al., 2005) was the basis for the research presented here. The overall objectives of this study were to provide a foundation for comparative analysis of the domestication syndrome across the Asian Vigna and other warm-season legumes. The specific objectives in this study were (a) to develop a molecular linkage map based on the F2 population of a cross between V. nepalensis and V. angularis, the same cross for which a molecular linkage map of azuki bean based on the BC1F1 population has been published (Han et al., 2005); (b) to dissect into QTLs the morphological and physiological traits associated with the domestication of azuki bean; and (c) to infer commonality of QTLs for domestication-related traits based on information available from other crosses involving warm-season legumes.

MATERIALS AND METHODS

Mapping population

The segregating populations studied were derived from the cross between the wild species Vigna nepalensis (female) and the cultivated azuki bean (male and recurrent parent for backcross) and used to construct molecular linkage maps and for trait measurement. Vigna nepalensis (JP107881) was collected in Nepal and the cultivated azuki bean (JP81481) is a landrace from Tokushima Prefecture, Japan. These accessions were obtained from the Ministry of Agriculture, Forestry and Fisheries, Gene Bank, Tsukuba, Japan.

The BC1F1 population, consisting of 187 individuals (from 200 seeds), was planted in pots in a vinyl greenhouse of the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan, 36°2’N 140°8’E, from June to November 2002, and used for DNA extraction to construct the molecular linkage map. The F2 population, consisting of 141 individuals (from 144 seeds), was grown from June to November 2003 under the same conditions as the BC1F1, used for DNA extraction to construct a molecular linkage map and evaluated for domestication-related traits (Table 1). BC1F1:2 lines each consisting of ten individuals per line were planted in the field in June 2003 and 2004 at NIAS and were evaluated based on mean value for each trait per line. The F2:3 lines each consisting of ten individuals per line were planted in the field in June 2004 at NIAS and were evaluated in a similar way. Ten plants of each parent were grown together with these populations. In total, four sets of data for seedling and vegetative domestication-related traits were obtained from BC1F1:2 (2003, 2004), F2 and F3 generations except for seed and pod size traits.

Information on climate (monthly average temperature, rainfall and daylight hours) for 2002–2004 when the segregating populations were grown is provided (see Supplementary Information 1, available at online).

Trait measurement

Thirty-three domestication-related traits were evaluated. These traits related to the size of various plant parts (seeds, pods, leaf and stem), plant habit (stem and branch), life cycle (flowering time), pod dehiscence, seed and epicotyl colour and seed germination (Table 1). These traits are all known to differ between wild and cultivated azuki bean and, therefore, are considered to be directly or indirectly the result of changes associated with domestication. Of these, 30 were treated as quantitative traits and three – epicotyl colour, seed coat colour and black mottle of seed coat – were treated as qualitative traits. The seedling traits (epicotyl length, primary leaf length and width) were recorded when the 1st trifoliate leaf opened. The vegetative traits (1st to 10th internode length, stem diameter, leaf length and width, number of branches and growth habit) were recorded when the 10th trifoliate expanded completely. When the uppermost internode showed twining extension, the growth habit characteristic was recorded as ‘1’; when the uppermost internode was non-twinning it was recorded as ‘0’.

The seed and pod traits were investigated using the seeds and pods from BC1F1 and F2 plants that were harvested in 2002 and 2003, respectively. Length, width and thickness of five seeds were measured using digital callipers. Seed germination was investigated under two different conditions. The first experiment was carried out in the field from 15 June in 2003 and 2004 using seeds from BC1F1.
and $F_2$ plants, respectively, stored at room temperature after harvest. Ten unscarified seeds were sown in the field and the number and date of germinated seeds up to 21 d after planting were recorded. Germination was recorded as the proportion of germinated seeds to total surviving seeds, including germinated seeds and seeds recovered from the field soil. The second experiment was carried out in the laboratory. Ten unscarified seeds were placed on wet filter paper and incubated in the dark at 25°C for 21 d. The number of seeds that expanded after imbibing water was recorded as a percentage. For pod traits, number of twists along the length of the naturally shattered pod dried at 32°C for 2 weeks was recorded on ten pods, and then their length and width were measured after soaking in water to reconstitute their shape. As an index of pod dehiscence, the ratio of twist number to length of pod was used.

**Map construction and QTL analysis**

A molecular linkage map based on the $F_2$ population was constructed using framework SSR markers that were distributed throughout the $BC_1F_1$ genetic linkage map (Han et al., 2005). Total genomic DNA from $F_2$ individuals was extracted from 100 mg of fresh leaf tissue using the

---

| Table 1. Domestification-related traits examined, their trait and QTL abbreviations and evaluation method |
|---|
| **Organ** | **General attribute** | **Trait** | **Trait abbreviation** | **QTL/gene** | **Evaluation method** | **Evaluated population** |
| Pod | Pod size | Pod length (cm) | PDL | $Pdl$ | Length of straight pod | $BC_1F_1$, $F_2$ |
| Pod | | Pod width (mm) | PDW | $Pdw$ | Width of pod at widest part | $BC_1F_1$, $F_2$ |
| | | Number of twist (count) | PDT | $Pdt$ | Number of twists along the length of the pod | $BC_1F_1$, $F_2$ |
| Seed | Seed size | Seed weight (g) | SD100WT | $Sd100wt$ | Weight of 100 seeds | $BC_1F_1$, $F_2$ |
| | | Seed length (mm) | SDL | $Sdl$ | Maximum distance from top to bottom of the seed | $BC_1F_1$, $F_2$ |
| | | Seed width (mm) | SDW | $Sdw$ | Maximum distance from hilum to its opposite side | $BC_1F_1$, $F_2$ |
| | | Seed thickness (mm) | SDT | $Sdt$ | Maximum distance between both sides of the hilum | $BC_1F_1$, $F_2$ |
| Seed colour | Black mottle color | Seed coat colour | SDC | $Sdc$ | Tan or red | $BC_1F_1$, $F_2$ |
| | | Seed coat colour black mottle color | SD CBM | $Sdcbm$ | Present or absent | $BC_1F_1$, $F_2$ |
| Seed germination | Germination in field (%) | Seed – Germination in field (%) | SDG | $Sdg$ | Germination percentage at 21 d after sowing in field | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | | Coat permeable (%) | SDP | $Sdp$ | Percent of imbibed seeds at 21 d after sowing at 25°C in incubator | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Stem | Epicotyl colour | Epicotyl colour | ECC | $Ecc$ | Red or green | $F_2$ |
| | | Epicotyl length (cm) | ECL | $Ecl$ | Length from cotyledon to primary leaf | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | Stem – Intermodal length (1st to 10th) (cm) | ST1-10I | $St1-10i$ | Length from primary leaf node to each node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | Stem length (cm) | STL | $Stl$ | Length from primary leaf node to 10th trifoliate leaf node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | Stem thickness (mm) | STT | $Stt$ | Stem diameter below the primary leaf | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Growth habit | Stem – twining (%) | STTW | $Sttw$ | Stem twining beyond the main stem 10th internode | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Leaf | Leaf size | Leaf – Primary leaf length (mm) | LFPL | $Lfp$ | Distance from pulvinus to leaf tip | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | | Leaf – Primary leaf width (mm) | LFPW | $Lfpw$ | Widest part of primary leaf | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | | Leaf – maximum leaflet length (mm) | LFML | $Lfml$ | Length of the largest terminal leaflet on a leaf between 1 st trifoliate leaf node and 10th trifoliate leaf node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| | | Leaf – maximum leaflet width (mm) | LFMW | $Lfmw$ | Width of the largest terminal leaflet on a leaf between 1 st trifoliate leaf node and 10th trifoliate leaf node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Branch | Number | Branch number (count) | BRN | $Brn$ | Number of branches on main stem from 1 st trifoliate leaf node to 10th trifoliate leaf node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Position | Branch – Position of 1st branch (if node) | BRP | $Brp$ | Position of the first branch on main stem from 1 st trifoliate leaf node to 10th trifoliate leaf node | $BC_1F_{1.2}$, $F_2$, $2:3$ |
| Flower | Flowering time | Flower – Days to first flower (days) | FLD | $Fld$ | Number of days from sowing to 1st flowering | $F_2$ |
RESULTS

Establishment of the linkage map

The BC\textsubscript{1}F\textsubscript{1} map constructed by Han et al. (2005) consisted of 381 markers (205 SSR, 94 RFLP and 187 AFLP markers) across 11 linkage groups. The linked markers span a total distance of 832.1 cM.

The F\textsubscript{2} map constructed here used 74 SSR markers covering 649.7 cM. This was equivalent to 78.1 \% of the BC\textsubscript{1}F\textsubscript{1} map. For the F\textsubscript{2} map, segregation distortion was observed in 28 \% (21 out of 74) of markers. All 21 markers were distorted in favour of alleles from the wild female parent and 14 of these were found on linkage groups 4, 6, 8 and 11. The order of the SSR markers on each linkage group was consistent in the two maps, BC\textsubscript{1}F\textsubscript{1} and F\textsubscript{2}, although there were small differences in the distance between the markers (see Supplementary Information 3, available online).

Field data analysis

The means, standard deviations and broad sense heritabilities in 2003 and 2004 of traits in the parental lines and F\textsubscript{2} and F\textsubscript{2.3} populations are shown (Table 2). These populations showed a high degree of morphological and physiological variation. The means of lines (or plants) in BC\textsubscript{1}F\textsubscript{1}, BC\textsubscript{1}F\textsubscript{1.2}, F\textsubscript{2} and F\textsubscript{2.3} populations generally fell between the means of cultivated and wild parents for all traits except the position of the first branch. The seed germination (\%), pod dehiscence and size of organs such as leaf, stem, seed and pod differed markedly between the parents. For internode lengths, the 1st to 3rd internodes in the cultivated parent were longer than those in the wild parent, whereas the 4th to 10th internodes in the cultivated parent were shorter than those in the wild parent. For qualitative traits, in the cultivated parent seed coat colour was red and the epicotyl was green, while the wild parent had a black mottled over tan seed coat and red epicotyl.

The traits analysed showed nearly normal distributions among lines (or plants) in these populations. In 2004, each internode length showed a normal distribution in both the BC\textsubscript{1}F\textsubscript{1.2} and F\textsubscript{2.3} populations (see Supplementary Information 2, available online). However, for some internodes mean values were distorted [BC\textsubscript{1}F\textsubscript{1.2} (2004) internodes 1–3, F\textsubscript{2.3} internodes 1 and 4–7] and some lines showed transgressive segregation [BC\textsubscript{1}F\textsubscript{1.2} (2004) internodes 4 and 5, F\textsubscript{2.3} internode 1].

The F\textsubscript{2} mean for seed germination percentage in the field showed a distribution distorted towards the wild parent type. A binomial distribution was observed for twist number in the pod suggesting that a single gene controls this trait. A binomial distribution was also observed for twining versus non-twining growth in the BC\textsubscript{1}F\textsubscript{1.2} population in 2003. However, in the BC\textsubscript{1}F\textsubscript{1.2} (2004) and F\textsubscript{3} populations the mean number of lines with twining growth was distorted towards the wild parent. Generally the traits measured showed high broad sense heritability (exceeding 0.6; Table 2). The segregation ratios for the three qualitative traits, seed coat colour, black mottle on the seed coat and epicotyl colour, matched the ratios expected for control by a single gene in both populations.
Table 2. Trait mean, standard deviation and heritability values for parents, the BC₁F₁:2 (2003, 2004), F₂ and F₂:₃ populations of the cross between V. nepalensis and cultivated azuki bean

| Trait                         | BC₁F₁:2 population (2003) | V. nepalensis | V. angularis | BC₁F₁:2 | Heritability (%) | BC₁F₁:2 population (2004) | V. nepalensis | V. angularis | BC₁F₁:2 | | | |
|-------------------------------|---------------------------|----------------|--------------|---------|------------------|----------------------------|----------------|--------------|---------|---------|---------|---------|---------|
| PDL (cm)‡                    | 7.2                       | 0.85           | 9.2          | 1.03    | 9.0              | 1.21                       | No investigation | | | | | | |
| PDW (mm)‡                    | 4.8                       | 0.15           | 10.2         | 0.30    | 8.5              | 0.59                       | No investigation | | | | | | |
| PDT (count)†                 | 3.0                       | 0.12           | 0.7          | 0.19    | 2.6              | 2.10                       | No investigation | | | | | | |
| SD100WT (g)§                 | 1.7                       | 0.18           | 16.3         | 0.14    | 9.1              | 1.61                       | No investigation | | | | | | |
| SDL (mm)§                    | 3.7                       | 0.17           | 7.3          | 0.14    | 6.1              | 0.44                       | No investigation | | | | | | |
| SDW (mm)§                    | 2.5                       | 0.11           | 5.6          | 0.07    | 4.6              | 0.32                       | No investigation | | | | | | |
| SDT (mm)§                    | 2.0                       | 0.06           | 5.2          | 0.04    | 4.2              | 0.33                       | No investigation | | | | | | |
| SDC                          | Tan                       | Red            | T : R = 94 : 91 (x² = 0.049) | No investigation | | |
| SDH§                         | Present                   | Absent         | P : A = 96 : 89 (x² = 0.265) | No investigation | | |
| SDG (%)                      | 2.5                       | 5.59           | 70-1         | 5.40    | 50-1             | 34.07                      | 97-4           | | | | | | |
| SDP (%)                      | 0.0                       | 0.00           | 75-0         | 3.71    | 31-1             | 32.53                      | 97-6           | | | | | | |
| ECC                          | No investigation          | No investigation | | | | | | | | | | | |
| ECL (cm)§                    | 1.0                       | 0.06           | 2.1          | 0.40    | 1.3              | 0.46                       | 60.5           | | | | | | |
| STI (cm)                     | 0.2                       | 0.06           | 2.0          | 0.59    | 1.6              | 0.58                       | 49.1           | | | | | | |
| STI (cm)                     | 0.4                       | 0.10           | 2.4          | 0.87    | 2.1              | 0.75                       | 31.8           | | | | | | |
| STI (cm)                     | 0.6                       | 0.15           | 3.0          | 0.43    | 2.7              | 0.93                       | 88.2           | | | | | | |
| STI (cm)                     | 0.7                       | 0.31           | 3.1          | 0.51    | 3.0              | 1.19                       | 87.7           | | | | | | |
| SDL (mm)                     | 1.0                       | 0.15           | 3.3          | 0.77    | 3.5              | 1.72                       | 89.6           | | | | | | |
| ST7 (cm)                     | 1.3                       | 0.20           | 3.3          | 0.68    | 3.7              | 2.18                       | 94.7           | | | | | | |
| ST8 (cm)                     | No investigation          | No investigation | | | | | | | | | | | |
| ST9 (cm)                     | No investigation          | No investigation | | | | | | | | | | | |
| ST10 (cm)                    | No investigation          | No investigation | | | | | | | | | | | |
| STL (cm)                     | 4.3                       | 0.76           | 19.2         | 3.22    | 17.9             | 6.67                       | 87.7           | | | | | | |
| STTW (%)                     | 100.0                     | 0.00           | 0.0          | 0.00    | 26.0             | 31.71                      | 100.0          | | | | | | |
| STT (mm)                     | 2.9                       | 0.47           | 5.0          | 0.36    | 4.4              | 0.68                       | 62.7           | | | | | | |
| LFPL (mm)                    | 22.4                      | 1.93           | 40.8         | 3.08    | 38.3             | 4.72                       | 70.3           | | | | | | |
| LFPW (mm)                    | 17.5                      | 1.32           | 40-7         | 1.97    | 35-7             | 4.07                       | 83.1           | | | | | | |
| FLML (mm)                    | No investigation          | No investigation | | | | | | | | | | | |
| FLMW (mm)                    | No investigation          | No investigation | | | | | | | | | | | |
| BRN (count)                  | 3.7                       | 0.58           | 2.3          | 0.47    | 2.2              | 0.74                       | 49.7           | | | | | | |
| BRP (ith)                    | 1.0                       | 0.00           | 1.7          | 0.20    | 1.8              | 0.58                       | 94.0           | | | | | | |
| FLD (day)                    | No investigation          | No investigation | | | | | | | | | | | |

Continued
| Trait                  | V. nepalensis | V. angularis | F2 | Heritability (%) | V. nepalensis | V. angularis | F2 | Heritability (%) |
|-----------------------|---------------|--------------|----|------------------|---------------|--------------|----|------------------|
| PDL (cm)‡             | 7.30          | 10.1         | 0.37| 8.0              | 145.0         | 93.3         |    | No investigation |
| PDW (mm)‡             | 5.2           | 9.5          | 0.29| 7.0              | 0.62          | 80.1         |    | No investigation |
| PDT (count)‡          | 5.9           | 0.47         | 0.04| 3.4              | 1.80          | 96.5         |    | No investigation |
| SDL100WT (g)†         | 1.8           | 0.07         | 0.23| 5.0              | 1.20          | 97.9         |    | No investigation |
| SDL (mm)‡             | 3.4           | 6.4          | 0.18| 5.0              | 0.47          | 90.7         |    | No investigation |
| SDW (mm)‡             | 2.5           | 4.9          | 0.08| 3.7              | 0.32          | 95.6         |    | No investigation |
| SDT (mm)‡             | 2.2           | 4.5          | 0.11| 3.2              | 0.34          | 93.9         |    | No investigation |
| SDC†                  | Tan           | Red          |     |                  |               |              |    | No investigation |
| SDG (%)               | No investigation |                     | 3.3| 5.77          | 76.7          | 11.55        | 25.8| 26.46         | 88.1 |
| SDP (%)               | No investigation |                     | 0.0| 0.00          | 80.0          | 14.14        | 14.0| 23.47         | 81.8 |
| ECC                   | Red           | Green : G = 108 : 33 (χ² = 0.191) |     |                  |               |              |    | No investigation |
| ECL (cm)              | 1.7           | 0.18         | 1.07| 4.1              | 0.94          | 32.5         |    | No investigation |
| ST11 (cm)             | 0.2           | 0.03         | 0.27| 0.6              | 0.20          | 4.8          |    | No investigation |
| ST21 (cm)             | 0.2           | 0.06         | 0.53| 1.1              | 0.51          | 43.8         |    | No investigation |
| ST31 (cm)             | 0.8           | 0.24         | 0.52| 2.0              | 1.05          | 85.3         |    | No investigation |
| ST41 (cm)             | 1.7           | 0.31         | 0.40| 4.4              | 3.67          | 99.1         |    | No investigation |
| ST51 (cm)             | 3.5           | 0.77         | 0.70| 9.2              | 6.75          | 98.8         |    | No investigation |
| ST61 (cm)             | 8.5           | 2.04         | 5.2 | 14.0             | 8.51          | 96.5         |    | No investigation |
| ST71 (cm)             | 15.3          | 2.48         | 6.7 | 1.08             | 19.0          | 8.43         | 94.8| No investigation |
| ST81 (cm)             | 22.8          | 2.72         | 10.1| 22.0             | 7.04          | 83.1         |    | No investigation |
| ST91 (cm)             | 25.2          | 2.59         | 14.6| 22.7             | 6.68          | 82.0         |    | No investigation |
| ST101 (cm)            | 25.1          | 1.92         | 16.9| 2.88             | 22.6          | 6.62         | 86.3| No investigation |
| STL (cm)              | 103.2         | 8.60         | 67.7| 8.60             | 117.5         | 38.16        | 94.9| No investigation |
| STTW (%)              | No investigation |                     | 100.0| 0.00          | 0.00          | 73.5          | 33.93| 100.0         |
| STT (mm)              | 3.7           | 0.18         | 4.6 | 0.32             | 3.7           | 0.45         | 68.3| No investigation |
| LFPL (mm)             | 28.2          | 2.20         | 57.6| 6.12             | 47.3          | 4.56         |    | No investigation |
| LFPLF (mm)            | 22.5          | 1.75         | 56.1| 5.33             | 44.3          | 3.81         |    | No investigation |
| FLML (mm)             | 125.2         | 6.81         | 131.0| 9.56             | 135.0         | 14.88        | 68.9| No investigation |
| FLMW (mm)             | 80.7          | 6.07         | 103.4| 6.25             | 92.6          | 13.43        | 79.0| No investigation |
| BRN (count)           | 9.2           | 0.42         | 1.9 | 0.74             | 6.8           | 240.0        | 91.1| No investigation |
| BRP (ith)             | 1.8           | 0.42         | 1.1 | 0.32             | 2.8           | 1.35         | 92.4| No investigation |
| FLD (day)             | 96.3          | 1.06         | 49.1| 0.99             | 79.2          | 7.30         | 98.0| No investigation |

1 Trait abbreviations are shown in Table 1.
2 The BC1F1 generation was evaluated for these traits.
3 s.d., Standard deviation.
* Significant at 5 % level.
except that seed coat colour in the $F_2$ population showed distorted segregation significant at the 5% level. Red seed coat colour and green epicotyl colour of the cultivated parent are recessive. Black mottle and tan seed coat colour, and red epicotyl colour are dominant.

In general, similar or related traits showed significant positive correlations at $P \leq 0.05$ in all populations (see Supplementary information 4, available online). For example, there were significant positive correlations between stem length and each internode length and also significant positive correlations between lengths of each internode. Traits related to seed size were positively correlated with pod length and stem thickness but negatively correlated with the days to flowering in the $F_2$ population. Seed germination percentage was positively correlated with seed coat colour but not with traits related to seed size.

Detection of putative QTL

The results of the QTL analysis for each trait in each population are shown in Table 3. Only QTLs with a probability of $\leq 0.001$ were considered. Although many other QTLs were detected when a probability level of $\leq 0.01$ was used, these are not reported to ensure a high level of confidence in the QTLs named. One to nine QTLs were detected for each trait except for branch position for which no QTL was detected. For 22 out of the 30 quantitative traits two to six QTLs were detected.

Seed germination (SDG and SDP). In azuki bean domestication has resulted in loss of dormancy (or high percentage seed germination). For seed germination in the field, six QTLs were detected on linkage groups 1, 2, 4 and 9. Alleles from the cultivated parent at two of these QTLs on linkage group 1 involved a strong positive effect on germination percentage. Unexpectedly, the four remaining QTLs from the cultivated parent had a negative effect on germination percentage in the field. Based on the laboratory test for permeability of the seed coat to water, five QTLs were detected on linkage groups 1, 4 and 9. At two QTLs on linkage group 1, alleles of the cultivated parent had the effect of increasing the percentage of seeds with a permeable seed coat. Based on their location, these two QTLs are probably the same as those found on linkage group 1 in the field test and are the only QTLs detected from the cultivated parent that promote water absorption by seeds and germination.

Pod dehiscence (PDT). Azuki bean domestication has involved a loss of pod dehiscence reflected in reduced ability for the pod wall to twist. The frequency distribution for pod dehiscence (reflected by the number of twists on the pod after opening) suggests that a single recessive gene controls loss of pod dehiscence. One QTL with a high heritability (above 96%) was located on linkage group 7.

Increase in organ size. Seed size, pod length and width and stem thickness were considered.

Seed size (SD100WT, SDL, SDW and SDT): five to eight QTLs for traits related to seed size were located on linkage groups 1, 2, 3, 5, 7, 8, 9 and 11 (Table 3). At all QTLs, alleles from the cultivated parent increase the size of each trait. The QTLs with the largest phenotypic contribution for all four traits were located on linkage group 2 (from 16% for seed length to 31% for seed weight).

Pod length and width (PDL, PDW): five QTLs were detected for pod length, on linkage groups 1, 7, 8, 9 and 10. The QTLs located on linkage group 7 had the largest effect. This region on linkage group 7 coincides with the QTL region for pod dehiscence. Of the five QTLs detected for pod width, the QTL with largest effect was also located close to the QTL region for pod dehiscence.

Stem thickness (STT): three QTLs for stem thickness were detected on linkage groups 4, 6 and 9.

Growth habit. Despite very different weather conditions in 2003 and 2004 (see Supplementary Information 1, available online) many common QTLs were detected for stem length, internode length and twining habit. The QTLs with large effects were located on linkage groups 1, 2, 7 and 9. QTLs affecting length of stem and lower internodes were located on linkage groups 1 and 2. Alleles from the cultivated parent result in longer lower internodes and shorter upper internodes.

For twining habit (STTW) three QTLs were detected, on linkage groups 4, 7 and 9. The two regions on linkage groups 7 and 9 with QTLs for twining habit are the same as those having QTLs of the cultivated parent for shorter stem and internodes.

Leaf shape. Nine QTL for maximum leaf length and width were found. However, different QTLs were detected in the $B_C_1F_{1:2}$ population from those found in the $F_2$ and $F_{2:3}$ populations.

Flowering time. One QTL was detected on linkage group 4, with the allele of the cultivated parent resulting in earlier flowering.

Epicotyl traits (ECL and ECC). Three QTLs having alleles of the cultivated parent for increased epicotyl length were detected on linkage groups 1 and 2. Location of two of these QTLs for epicotyl length is in a similar position to those for stem length and lower internode length.

The gene that controlled epicotyl colour was mapped on linkage group 4.

Seed colour. Seed colour traits were recorded as $a$) seed colour either red or tan (SDC) and $b$) presence or absence of black mottle (SDCBM) (Table 2). The genes that controlled the seed coat colour and black mottle on the seed were mapped to linkage groups 1 and 4, respectively. The gene for seed colour is closely linked to a QTL for seed germination on linkage group 1 and that for black mottle seed coat was closely linked to epicotyl colour on linkage group 4.

The independence and distribution of QTL related to domestication traits across the azuki bean genome and linkage groups

Assuming independent gene action, 65–85 QTLs for domestication-related traits were identified and, assuming
### Table 3. QTLs detected in the backcrossed (BC$_1$F$_1$,2) and self-pollinated (F$_2$ and F$_2$,3) populations

| Trait | QTL     | LG | LOD | P value | Loci position (cM) | PEV | Additive effect | PEV | Additive effect | LG | LOD | P value | PEV | Additive effect | PEV | Additive effect | D[A] | PEV | Additive effect | D[A] |
|-------|---------|----|-----|---------|-------------------|-----|-----------------|-----|-----------------|----|-----|---------|-----|-----------------|-----|-----------------|------|-----|-----------------|------|
| PDL*  | Pdl2-1.1+ | 1  | 7   | 18.63  | 0.00009 | 8.5 | 0.391 | 1.50 | 1 | 5.36 | 0.00009 | 88.5 | 0.177 | 1.1-16 | 0.17 | 0.48 | 0.015 | 0.15 | NI |
| Pdl3-1.1+ | 2  | 8   | 9.18 | 0.00010 | 48.1 | 0.221 | 1.12 | 1 | 4.80 | 0.00027 | 59.7 | 0.194 | 1.2-14 | -0.15 | -0.12 | 0.17 | 0.015 | NI |
| Pdl1-1.1- | 9  | 3.19 | 0.00050 | 5.5 | 0.096 | 0.73 | 1 | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
| Pdl1-1.1- | 10 | 3.45 | 0.00050 | 42.2 | 0.107 | -0.78 | 1 | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
| PDW*  | Pdw2-2.1. + | 2  | 7   | 14.96 | 0.00009 | 8.7 | 0.338 | 0.68 | 1 | 4.05 | 0.00045 | 41.9 | 0.194 | 0.68 | -0.03 | -0.05 | NI |
| Pdw1-7.1. + | 9  | 3.74 | 0.00040 | 10.6 | 0.107 | 0.37 | 1 | 9 | 7.18 | 0.00010 | 10.2 | 0.233 | 0.43 | -0.02 | -0.06 | 0.17 | 0.015 | NI |
| Pdw2-9.2. + | 11 | 3.91 | 0.00010 | 27.2 | 0.105 | 0.37 | 1 | 11 |  |  |  |  |  |  |  |  |  |  |  |  |
| PDT*  | Pdt1-7.1-  | 7  | 81.47 | 0.00010 | 11.3 | 0.905 | -3.99 | 1 | 7 | 27.42 | 0.00009 | 0.1 | 0.610 | -3.35 | 1.54 | 0.46 | NI |
| SD100WT* | Sd100wt2.1.1 + | 1  | 4.61 | 0.00010 | 91.2 | 0.133 | 1.14 | 1 | 6.35 | 0.00100 | 28.5 | 0.279 | 1.15 | -0.16 | -0.14 | 0.17 | 0.015 | NI |
| Sdl1.1.1. + | 2  | 11.63 | 0.00010 | 33.5 | 0.268 | 1.65 | 1 | 2 | 8.16 | 0.00009 | 45.5 | 0.313 | 1.85 | -0.14 | -0.08 | 0.17 | 0.015 | NI |
| SDL*  | Sdl2-1.1-  | 1  | 4.96 | 0.00010 | 96.6 | 0.132 | 0.31 | 1 | 7.76 | 0.00010 | 20.6 | 0.311 | 0.39 | -0.06 | -0.16 | 0.17 | 0.015 | NI |
| Sdl2-1.1+ | 2  | 6.26 | 0.00010 | 32.8 | 0.162 | 0.35 | 1 | 5.86 | 0.00009 | 43.9 | 0.245 | 0.62 | -0.05 | -0.07 | 0.17 | 0.015 | NI |
| SDL*  | Sdl1-1.1-  | 7  | 4.23 | 0.00018 | 21.7 | 0.117 | 0.30 | 1 | 4.57 | 0.00018 | 38.2 | 0.202 | 0.49 | -0.19 | -0.39 | 0.17 | 0.015 | NI |
| SDL*  | Sdl1-7.1+ | 1  | 3.78 | 0.00040 | 41.1 | 0.118 | 0.29 | 1 | 5.05 | 0.00009 | 43.4 | 0.195 | 0.52 | 0.09 | 0.18 | 0.17 | 0.015 | NI |
| SDW*  | Sdw2-1.1+ | 1  | 3.44 | 0.00010 | 93.1 | 0.093 | 0.18 | 1 | 7.86 | 0.00009 | 44.7 | 0.297 | 0.48 | -0.01 | -0.02 | 0.17 | 0.015 | NI |
| Sdw2-1.1+ | 2  | 8.11 | 0.00010 | 31.0 | 0.212 | 0.28 | 1 | 5.69 | 0.00030 | 8.3 | 0.212 | 0.20 | 0.00 | 0.02 | 0.17 | 0.015 | NI |
| SDT*  | Sdt1-1.1-  | 9  | 3.40 | 0.00050 | 10.3 | 0.100 | 0.27 | 1 | 4.36 | 0.00010 | 20.6 | 0.14 | 0.08 | -0.04 | -0.04 | 0.17 | 0.015 | NI |
| SDG*  | Sdg1-1.1+ | 1  | 2.19 | 0.00009 | 37.9 | 0.304 | 15.16 | 1 | 21.06 | 0.00009 | 11.0 | 0.040 | 12.48 | -0.84 | -0.08 | 0.040 | 12.84 | -0.84 | -0.08 | ND |
| SDP*  | Sdp1-1.1-  | 1  | 3.77 | 0.00010 | 36.1 | 0.374 | 13.83 | 1 | 10.87 | 0.00100 | 54.7 | 0.040 | 12.48 | -0.84 | -0.08 | 0.040 | 12.84 | -0.84 | -0.08 | ND |

Provided by Isomura et al. — Azuki Bean Domestication
| Study | Group | Treatment | ID | FDR | P-value | Q-value | Log2FoldChange | Log2FoldChange | Log2FoldChange | Log2FoldChange | Log2FoldChange |
|-------|-------|-----------|----|-----|---------|---------|----------------|----------------|----------------|----------------|----------------|
| ECL   | Ecl1.1- | 1       | 85.7 | 0.04 | 0.14 | 0.28 | 0.75 | 0.39 | 0.52 | 0.75 | 0.39 | 0.52 |
| Ecl1.1- | 2       | 13:11 | 0.00010 | 27.6 | 0.319 | 1.23 | 2 | 0.00010 | 45.0 | 0.579 | 2.07 | 0.12 | 0.06 | 0.575 | 1.72 | 0.13 | 0.07 |
| Ecl2.1- | 1       | 2       | 31:74 | 0.00010 | 44.0 | 0.386 | 1.67 | 0.28 | 0.17 | 0.371 | 1.80 | 0.15 | 0.08 |
| STII  | St11.1- | 1       | 11:47 | 0.00100 | 8.8 | 0.198 | 0.86 | 0.08 | 0.09 | 0.160 | 0.84 | 0.37 | 0.44 |
| St11.2- | 2       | 12:64 | 0.00009 | 93.0 | 0.138 | 0.31 | 0.210 | 0.37 | 86.5 | 0.46 | 0.02 | 0.05 | 0.94 | 0.04 | 0.05 |
| St11.3- | 3       | 13:89 | 0.00009 | 35.0 | 0.045 | 0.17 | 0.294 | 0.44 | 22:07 | 0.00010 | 44.0 | 0.386 | 1.67 | 0.28 | 0.17 | 0.371 | 1.80 | 0.15 | 0.08 |
| STIII | St11.1- | 1       | 11:47 | 0.00100 | 8.8 | 0.198 | 0.86 | 0.08 | 0.09 | 0.160 | 0.84 | 0.37 | 0.44 |
| St11.1- | 2       | 2       | 12:64 | 0.00009 | 93.0 | 0.138 | 0.31 | 0.210 | 0.37 | 86.5 | 0.46 | 0.02 | 0.05 | 0.94 | 0.04 | 0.05 |
| STIV  | St11.1- | 1       | 11:47 | 0.00100 | 8.8 | 0.198 | 0.86 | 0.08 | 0.09 | 0.160 | 0.84 | 0.37 | 0.44 |
| St11.1- | 2       | 12:64 | 0.00009 | 93.0 | 0.138 | 0.31 | 0.210 | 0.37 | 86.5 | 0.46 | 0.02 | 0.05 | 0.94 | 0.04 | 0.05 |
| STV   | St11.1- | 1       | 11:47 | 0.00100 | 8.8 | 0.198 | 0.86 | 0.08 | 0.09 | 0.160 | 0.84 | 0.37 | 0.44 |
| St11.1- | 2       | 12:64 | 0.00009 | 93.0 | 0.138 | 0.31 | 0.210 | 0.37 | 86.5 | 0.46 | 0.02 | 0.05 | 0.94 | 0.04 | 0.05 |

Continued...
| Trait | QTL | LG | LOD | P value | Loci position (cM) | PEV Additive effect | PEV Additive effect | LG | LOD | P value | Loci position (cM) | PEV Additive effect | PEV Dominant effect | D[A] | PEV Additive effect | PEV Dominant effect | D[A] |
|-------|-----|----|-----|---------|-------------------|---------------------|---------------------|----|-----|---------|-------------------|---------------------|---------------------|------|---------------------|---------------------|------|
| LFPL  | Lfp1-1-1+ | 1  | 5.26 | 0.00018 | 85.0 | 0.124 | 3.09 | 0.052 | 1.62 | 1 | 5.77 | 0.00340 | 45.1 | 0.043 | 0.19 | 0.12 | 0.60 | 0.022 | 1.13 | 0.08 | 0.07 |
|       | Lfp1-1-1- | 7  | 6.67 | 0.00030 | 12.6 | 0.054 | -1.15 | 0.123 | 1.82 | 7 | 9.86 | 0.00020 | 18.3 | 0.054 | 0.30 | -0.15 | -0.52 | 0.030 | 0.93 | -0.10 | -0.11 |
|       | Lfp1-1-2+ | 7  | 36.1 | 2.73 | 1.14 | 7 |
| LFPW  | Lfpw1-2-1+ | 2  | 1.26 | 0.00060 | 11.8 | 0.047 | -0.94 | 0.104 | 1.26 | 7 | 6.11 | 0.00030 | 10.9 | 0.086 | 0.43 | 0.14 | 0.33 | 0.059 | 1.02 | 0.10 | 0.10 |
|       | Lfpw1-2-1- | 7  | 37.6 | 2.29 | 1.11 | 7 |
|       | Lfpw1-2-2+ | 8  | 4.00 | 0.0100 | 37.3 | 0.086 | 1.95 | 0.043 | 1.21 | 8 |
|       | Lfpw1-9-1+ | 9  | 4.24 | 0.0073 | 14.4 | 0.061 | 1.77 | 0.064 | 1.58 | 9 | 5.61 | 0.00030 | 10.9 | 0.086 | 0.43 | 0.14 | 0.33 | 0.059 | 1.02 | 0.10 | 0.10 |
|       | Lfpw1-11-1+ | 11 | 1.11 | 0.00099 | 20.6 | 0.068 | 1.86 | 0.087 | 1.88 | 11 |
| LFML  | Lfml1-5-1+ | 5  | 3.62 | 0.00020 | 22.2 | NI | 0.112 | 4.92 | 5 |
|       | Lfml1-5-1- | 6  | 2.92 | 0.00100 | 29.2 | 0.088 | -4.31 | 6 |
|       | Lfml1-7-1+ | 7  | 4.93 | 0.00010 | 17.4 | 0.147 | 5.65 | 7 |
|       | Lfml2-8-1+ | 8  | 5.52 | 0.00070 | 46.6 | 0.170 | 0.82 | -0.02 | -0.03 | 0.152 | 0.38 | -0.01 | -0.02 |
| LFMW  | Lfms1-5-1+ | 5  | 4.51 | 0.00010 | 14.8 | NI | 0.123 | 4.34 | 5 |
|       | Lfms1-5-1- | 6  | 4.31 | 0.00010 | 29.1 | 0.110 | -4.08 | 6 |
|       | Lfms1-7-1+ | 7  | 3.44 | 0.00050 | 26.3 | 0.115 | 4.18 | 7 |
|       | Lfms2-8-1+ | 8  | 5.81 | 0.00040 | 39.0 | 0.172 | 0.83 | 0.18 | 0.22 | 1.40 | 0.60 | 0.02 | 0.03 |
|       | Lfms2-9-1+ | 9  | 6.07 | 0.00010 | 7.1 | 0.166 | 5.06 | 9 |
| BRN   | Brn1-10-1- | 10 | 5.90 | 0.0009 | 36.6 | 0.154 | -0.55 | 0.029 | -0.21 | 10 |
| BRP   | ND | ND | ND | ND |
| FLD   | Fld2-4-1- | 10 | 4.99 | 0.00009 | 57.6 | 0.239 | -9.94 | 1.03 | 0.10 | ND |

* The BC<sub>1</sub>F<sub>1</sub> generation was evaluated for these traits.
† NI, Not investigated.
‡ ND, Not detected.
pleiotropy or close linkage at all loci, 16 or 17 positions with possible pleiotropic effects were identified (Table 4). Overall $\chi^2$ values were 39.4–53.1 for independent gene action and 5.2 and 4.7 for pleiotropy or close linkage (Table 4). Thus the assumption of independent gene action was rejected and the assumption of pleiotropy or close linkage could not be rejected.

The observed number of QTLs in each 10-cM interval for each linkage group was compared with the expected number of QTLs. The null hypothesis that QTLs were randomly distributed was rejected for many linkage groups (Table 5). The uneven distribution of QTLs on linkage groups 1 and 2 was pronounced for both the $BC_1F_1:2$ and $F_2$ ($F_2:F_3$).

**DISCUSSION**

**Reproducibility of QTLs**

Despite the use of a stringent threshold ($P \leq 0.001$) for detecting QTLs in the two populations, about one-third are common (Table 3). For example, one-third of the QTLs (eight out of 24) for traits related to seed size were detected in both the $BC_1F_2$ (2003) and $F_3$ populations, although the seeds of these populations were harvested in different years. For pod length, two QTLs detected in the $F_2$ population were also observed in the $BC_1F_2$ (2003) although the heritability in the $BC_1F_2$ (2003) was lower than in the $F_2$ population, suggesting that environmental factors may influence pod length. Growth habit traits are sensitive to the growing environment (Koinange et al., 1996). Even though temperature, rainfall and solar radiation received varied greatly in 2003 and 2004, analysis of stem internode length, stem length and stem twining revealed 15 QTLs common to the $BC_1F_2$ and $F_2/F_3$ out of a total of 45 QTLs detected.

**The genetics of domestication-related traits**

In azuki bean a few domestication-related traits are controlled by a single major gene and most of them are controlled by a small number of QTLs. Results from this study reveal that azuki bean differs from related legumes in the genetic control of several simply inherited traits. For example, a few genes control pod dehiscence in common bean ($P. vulgaris$ L.) (Koinange et al., 1996) but in azuki bean a single gene controls this trait. While a single gene controls twining plant habit in common bean (Koinange et al., 1996), in this study three QTLs for twining habit (evaluated in a different way) were detected. For the majority of traits measured, between two and nine QTLs on two or more linkage groups were detected. QTLs for many related traits such as pod size, seed size and leaf.
shape and stem-related traits co-occur in the same location on the same linkage groups. This can be explained by developmental allometry (Smartt, 1976).

QTLs for internode length suggest gene association across the genome resulting in an effect that is best described as a cascade as they act at sequential stages in the plant life cycle. Most QTLs for lengths of the lower internodes (internodes 1–5) are located on linkage groups 1 and 2. For lengths of upper internodes (internodes 6–10) most QTLs are on linkage groups 4, 7 and 9.

Seed germination in azuki bean

Unexpectedly, at four QTLs, alleles of the cultivated parent were associated with lower seed germination. Koinange et al. (1996) reported that in common bean, at one QTL the allele of the cultivated parent was associated with a higher level of dormancy than the allele of the wild parent. There are several possible explanations for why cultivated azuki bean may have QTLs for lower germination. These may include prevention of premature germination in the field or the tendency of local farmers in Japan to use seeds from good harvests over several planting seasons in their home garden plots of azuki bean. Alternatively, some alleles that promote seed germination may be linked to traits not suitable for cultivated azuki, or QTLs for delayed germination in the wild parent used here may differ from QTLs in other wild germplasm of the azuki bean complex. Currently, hard-seededness in cultivated azuki bean is an occasional problem for azuki bean paste makers (Lumpkin and McClary, 1994). Azuki breeding which targets cultivated alleles that cause a lower germination percentage may help overcome this problem.

Distribution of QTL for domestication-related traits

The genes controlling domestication-related traits are not randomly distributed across crop genomes (Poncet et al., 2000; Doebley and Stec, 1991, 1993). For example, in common bean (Phaseolus vulgaris L.) three out of the 11 linkage groups, D1, D2 and D7, play an important role in growth habit, phenology, seed dispersal, dormancy, pod length and seed size (Koinange et al., 1996). The distribution of domestication syndrome QTLs departed significantly from random across the azuki bean genome (Table 4). Particularly important linkage groups with major QTL for domestication-related traits were linkage groups 1, 2, 4, 7 and 9 and are discussed below. For the linkage groups 1 and 2, domestication syndrome QTLs were non-randomly distributed across the linkage group in both populations studied (Table 5). The locations of QTLs on linkage groups 2 and 9 are shown in Figs 1 and 2 and figures for other linkage groups are also provided online (see Supplementary information 3).

Linkage group 1. On linkage group 1 the QTLs involved in loss of dormancy and increased size of organs such as seed,
pod, stem and leaf were detected in a limited region of 16 cM. This suggests either close linkage or pleiotropy of these domestication-related traits (Table 4). Close linkage or pleiotropy for domestication traits is reported in several crops such as maize (Doebley et al., 1997), rice (Cai and Morishima, 2002), eggplant (Doganlar et al., 2002) and common bean (Koinange et al., 1996). The best studied case is the single locus \( \text{tb}1 \) (teosinte branched 1) of maize that controls multiple traits (Doebley et al., 1997).

The genes or QTLs for seed colour and loss of seed dormancy in azuki bean are closely linked and there is a significant correlation between these two traits. This helps explain why in wild and weedy populations, individuals with a red seed coat are not found, since lack of seed dormancy is a detrimental trait in the wild. Unlike seeds with a red seed coat, those with a tan seed coat can survive in the natural environment from year to year (Wang et al., 2004).

**Linkage group 2** (Fig. 1). QTLs found on this linkage group are all related to seed size and early stages of plant growth and affect many of the same traits as those of linkage group 1. QTLs for increased seed size (seed weight, seed length, seed width and seed thickness) and epicotyl length, stem and lower internode lengths were all detected in a 18-cM region on linkage group 2 (Fig. 1). The highest genetic contribution for all traits related to seed size occurred in this region.

**Linkage group 4.** On linkage group 4, the genes for epicotyl colour and black mottle on the seed coat are linked closely. The association between these two traits has been reported previously (Kaga et al., 1996). The only QTL detected for flowering date was located on this linkage group and is associated with the upper stem characteristics of upper internode length and stem twining.

**Linkage group 7.** Linkage group 7 appears to be the most important for changes in the pod during domestication as it is the only linkage group for which all three pod-related traits (pod length and width and pod twist number) were detected. In addition, several QTLs for growth habit such as stem length, upper internode length and stem twining were consistently detected in a narrow region of 6 cM.

**Linkage group 9.** QTLs for growth habit (stem length, internode length, stem thickness and determinancy) and leaf and seed size were detected mainly in a 10–15-cM region on linkage group 9 (Fig. 2). Cultivated parent alleles in this region shorten the stem and upper internode lengths and cause a non-twining plant type. This region has many QTLs in common with, and having the same influence as, cultivated azuki bean alleles on linkage group 7 discussed.
Comparison of QTLs and their location among warm-season legumes

Cross between azuki bean and V. angularis var. nipponensis. In Japan, cultivated and wild azuki bean exist as a crop complex as a result of gene flow (Wang et al., 2004). Many intermediate plant types can be found in many parts of Japan (Yamaguchi, 1992). To better understand the genetics of the azuki bean complex a parallel study of Japan (Yamaguchi, 1992). To better understand the genetics of the azuki bean complex a parallel study of Japan (Yamaguchi, 1992). To better understand the genetics of the azuki bean complex a parallel study between a Japanese cultivar of azuki bean, ‘Kyoto (T. Isemura, unpubl. res.) was conducted involving a cross between V. nepalensis (lower) from the cross between V. angularis var. nipponensis but QTLs for these traits were not found in the cross studied here. Only genes for black mottled seed coat and epicotyl colour on linkage group 4 were common on these linkage groups in the two crosses. If these differences in QTLs between these two crosses within the azuki bean complex reflect genetic differences, rather than genomic rearrangements, between the wild or cultivated parents azuki bean breeders may be able to use the different QTLs in their breeding programmes.

Cross between azuki bean and V. nakashimae (Ohwi) Ohwi & Ohashi. The genes controlling red colour in the epicotyl and black mottle on seed coat were detected on linkage group 4 in this study, and these two loci are closely linked. Kaga et al. (1996, 2000) mapped the gene for red colour in epicotyl on linkage groups 4 and 1 using interspecific populations derived from the crosses between azuki bean and V. nakashimae and azuki bean and rice bean (V. umbellata), respectively. These two linkage groups were identified as the same on the basis of the order of three common RFLP markers (Han et al., 2005). The position of the locus for red epicotyl also coincides in the three maps.

Common bean (Phaseolus vulgaris L.). One of the closest genera to Vigna is Phaseolus and these two genera have been taxonomically distinguished only since 1970 (Verdcourt, 1970). In order to compare the QTLs detected in this study with those in common bean (P. vulgaris) the relationships of the linkage groups were investigated on the basis of the distribution of common RFLP markers reported in the common bean maps (Vallejos et al., 1992; Koinange et al., 1996; Freyre et al., 1998; Yu et al., 2000; Blair et al., 2003) and azuki bean map (Han et al., 2005; this study). Although the number of common RFLP markers is low, the relationship between linkage groups in the two genera could be determined. Linkage groups 7 and 8 of azuki bean in this study corresponded to the linkage group B5 and B7 of common bean (Fig. 4). QTL for pod and growth habit traits were detected on linkage group 7 in this study, but no QTL for these traits were detected on linkage group B5 of common bean. However, Koinange et al. (1996) reported that linkage group B7 played an important role in pod length and seed size. QTLs for seed length and pod length were detected in almost the same region on linkage group 8 of azuki bean. It was not possible to clarify the relationship between linkage group 1 in this study and B1 of common bean where many QTL were detected in both crops. It will be necessary to determine if Phaseolus SSR markers can be placed on the genetic map of azuki bean and vice versa to clarify corresponding linkage group relationships between these two crops.

Cowpea [Vigna unguiculata (L.) Walp.] and mungbean [Vigna radiata (L.) Wilczek]. QTL analysis for seed weight was carried out in the Vigna domesticates cowpea and
mungbean since this is an important trait related to yield (Fatokun et al., 1992). Using populations derived from crosses between cowpea and wild cowpea and mungbean and wild mungbean, two and four QTLs for seed weight, respectively, were reported (Fatokun et al., 1992).

Linkage groups ii and vi in the cowpea map correspond to linkage groups 1 and 4, respectively, in the azuki bean map (Fig. 5). Linkage groups i, ii, iii and vi in the mungbean map correspond to linkage groups 9, 1, 8 and 10, respectively, in the azuki bean map (Fig. 5). In this study,
Fig. 4. Comparative QTL maps for domestication-related traits between azuki bean and common bean (*Phaseolus vulgaris*). Linkage groups 7 and 8 of azuki bean in this study corresponded to linkage groups B5 and B7 on the current map of common bean, respectively. It was not possible to clarify the relationships for remaining linkage groups between azuki bean and common bean. Linkage groups of common bean, left to right, are after the maps of Vallejos et al. (1992), Freyre et al. (1998) and Koinange et al. (1996). The QTL for SW (seed weight) and PL (pod length) on linkage group D7 in common bean are after Koinange et al. (1996).
FIG. 5. Comparative QTL maps for seed weight in three cultivated Vigna species, V. angularis (azuki bean), V. radiata (mungbean) and V. unguiculata (cowpea). Linkage groups of mungbean are after the maps of Fatokun et al. (1992) (Roman numerals) and Menancio-Hautea et al. (1993) (Arabic numerals). Linkage groups of cowpea are after the maps of Fatokun et al. (1992). The QTL regions for seed weight in mungbean and cowpea are after Fatokun et al. (1992).
a QTL for seed weight was detected on linkage group 1 at a location corresponding to that of a QTL for this trait on linkage group ii in cowpea and mungbean. This seed weight QTL appears to be conserved among these three species. QTLs for seed weight were also detected at similar locations on azuki bean linkage group 9 and mungbean linkage group i.

Although the QTL with largest effect for seed weight was detected on the linkage group 2 in azuki bean, no QTL was detected on the linkage groups corresponding to this linkage group in cowpea and mungbean. QTLs on linkage group vi of cowpea, iii and vi of mungbean and 8 of azuki bean appear to be specific to these crops. These results suggest that the main genome regions related to increased seed weight under domestication do not correspond among these related species despite high homology between the linkage groups. In azuki bean, seed weight in cultivated taxa is about eight times that of the wild parent. In contrast, seed weight in cultivated and wild parents of crosses analysed for both cowpea and mungbean only exhibited a 5-fold difference (Fatokun et al., 1992). Azuki bean has among the largest seed for the cultivated Asian Vigna (Tomooka et al., 2000). It seems that increase in seed size in azuki bean compared with cowpea and mungbean involves some different loci.

Other legumes. In soybean (tribe Phaseoleae), a QTL for seed weight was detected near RFLP marker sT153 on linkage group A (Maughan et al., 1996). This corresponds to linkage group 1 in azuki bean. However, this RFLP marker was well separated from the molecular markers associated with seed weight variation in azuki bean, mungbean and cowpea.

In pea (Pisum sativum L., tribe Vicieae) (Timmerman-Vaughan et al., 1996), a QTL for seed weight was also detected in the region that corresponds to the region with seed weight QTL on linkage group 1 of azuki bean and ii of cowpea and mungbean (tribe Phaseoleae) based on RFLP comparison. Therefore, it seems that this region has been conserved across the Leguminosae and plays an important role in increasing seed size.

CONCLUSIONS

A broad array of domestication-related traits in azuki bean have been analysed and their QTLs mapped on the azuki bean molecular linkage map. Most traits are controlled by two to nine QTLs that occur on different linkage groups. QTLs for domestication-related traits are not evenly distributed across the azuki bean linkage map and five out of the 11 linkage groups in azuki bean (linkage groups 1, 2, 4, 7 and 9) have 80% of the QTLs detected. In addition, within a linkage group, QTLs are clustered.

Comparison of the positions of QTLs for domestication-related traits studied here with reports for related legumes has revealed that some regions with seed size QTLs are conserved among azuki bean, mungbean, cowpea, soybean and pea. However, there are also many QTLs that are not common either within the azuki bean complex or across the warm-season legumes. This study provides a foundation for use of molecular-assisted selection of domestication-related QTLs in azuki bean breeding programmes. The results presented here can be used to further enhance understanding of domestication in the Phaseolus–Vigna group.

SUPPLEMENTARY INFORMATION

Four sets of supplementary information are provided online at http://aob.oxfordjournals.org/. Supplementary Information 1 provides temperature, rainfall and sunlight hours at Tsukuba, Japan, for the months azuki populations were growing in 2002–2004. Supplementary Information 2 shows the frequency distribution for all traits examined in all populations examined. Supplementary information 3 shows a comparison of QTLs found for BC1F1 and F2 molecular linkage maps. Supplementary Information 4 shows the correlation coefficients among each trait for all populations examined.

ACKNOWLEDGEMENTS

This research was conducted while the first author (T.I.) was a recipient of a fellowship from the Japan Society for the Promotion of Science. The authors thank T. Nobori, N. Karino, S. Hirashima, K. Sugimoto, H. Uchiyama, T. Taguchi and H. Tomiyama for technical support. Funding to pay the Open Access publication charges for this article was provided by the OECD.

LITERATURE CITED

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B 57: 289–300.

Blair MW, Pedraza F, Buendia HF, Geitan-Solis E, Beebe SE, Gepts P, et al. 2003. Development of a genome-wide anchored microsatellite map for common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics 107: 1362–1374.

Boutilier SR, Young ND, Olson TC, Yu ZH. 1995. Genome conservation among three legume genera detected with DNA markers. Genome 38: 928–937.

Cai HW, Morishima H. 2002. QTL clusters reflect character associations in wild and cultivated rice. Theoretical and Applied Genetics 104: 1217–1228.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963–971.

Doebley J, Stec A. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129: 285–295.

Doebley J, Stec A. 1993. Inheritance of morphological differences between maize and teosinte: comparison of results for two F2 populations. Genetics 134: 559–570.

Doebley J, Stec A, Hubbard L. 1997. The evolution of apical dominance in maize. Nature 386: 485–488.

Doganlar S, Frary A, Daunay MC, Lester RN, Tankersley SD. 2002. Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 161: 1713–1726.

Fatokun CA, Menancio-Haueta D, Danesh D, Young ND. 1992. Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. Genetics 132: 841–846.

Freyre R, Skroch PW, Jeffery V, Adam-Blondon AF, Shirimohamadali A, Johnson WC, et al. 1998. Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theoretical and Applied Genetics 97: 847–856.

Hammer K. 1984. The domestication syndrome. Die Kulturpflanze 32: 11–34 [in German with English summary].
Han OK, Kaga A, Isemura T, Tomooka N, Vaughan DA. 2005. A genetic linkage map for azuki bean (Vigna angularis). *Theoretical and Applied Genetics* **111**: 1278–1287.

Hawkes J. 1983. *The diversity of crop plants*. Cambridge, MA: Harvard University Press.

Jansen RC, Van Ooijen JM, Stam P, Lister C, Dean C. 1995. Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. *Theoretical and Applied Genetics* **91**: 33–37.

Kaga A, Ohnishi M, Ishii T, Kamijima O. 1996. A genetic linkage map of azuki bean constructed with molecular and morphological markers using an interspecific population (Vigna angularis × V. nakashimae). *Theoretical and Applied Genetics* **93**: 658–663.

Kaga A, Ishii T, Tsukimoto K, Tokoro E, Kamijima O. 2000. Comparative molecular mapping in *Ceratotropis* species using an interspecific cross between azuki bean (Vigna angularis) and rice bean (*V. umbellata*). *Theoretical and Applied Genetics* **100**: 207–213.

Koinange EMK, Singh SP, Gepts P. 1996. Genetic control of the domestication syndrome in common bean. *Crop Science* **36**: 1037–1045.

Korol A, Ronin Y, Hayes P, Nevo E. 1998. Multi-interval mapping of correlated trait complexes: simulation analysis and evidence from barley. *Heredity* **80**: 273–284.

Lebreton CM, Visscher PM. 1998. Empirical nonparametric bootstrap strategies in quantitative trait loci mapping: conditioning on the genetic model. *Genetics* **148**: 525–535.

Lumpkin TA, McClary DC. 1994. Azuki bean: botany, production and uses. Wallingford: CAB International.

Maughan PJ, Maroof MAS, Buss GR. 1996. Molecular-marker analysis of seed-weight: genomic locations, gene action, and evidence for orthologous evolution among three legume species. *Theoretical and Applied Genetics* **93**: 574–579.

Menacio-Hautea D, Fatokun CA, Kumar L, Danesh D, Young ND. 1993. Comparative genome analysis of mungbean (*Vigna radiata* (L.) Wilczek) and cowpea (*V. unguiculata* (L.) Walpers) using RFLP mapping data. *Theoretical and Applied Genetics* **86**: 797–810.

Papa R, Acosta J, Delgado-Salinas A, Gepts P. 2005. A genome-wide analysis of differentiation between wild and domesticated Phaseolus vulgaris from Mesoamerica. *Theoretical and Applied Genetics* **111**: 1147–1158.

Peng J, Ronin Y, Fahima T, Röder M, Li Y, Nevo E, Korol A. 2003. Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. *Proceedings of National Academy of Sciences of the USA* **100**: 2489–2495.

Pouzet V, Lamy F, Devos KM, Gale MD, Sarr A, Robert T. 2000. Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae). *Theoretical and Applied Genetics* **100**: 147–159.

Smartt J. 1976. *Tropical pulses*. London: Longman.

Somta P, Kaga A, Tomooka N, Kashiwaba K, Isemura T, Chaitieng B, et al. 2006. Development of an interspecific *Vigna* linkage map between *Vigna umbellata* (Thunb.) Ohwi & Ohashi and *V. nakashimae* (Ohwi) Ohwi & Ohashi and its use in analysis of bruchid resistance and comparative genomics. *Plant Breeding* **125**: 77–84.

Tateishi Y, Maxted N. 2002. New species combinations in *Vigna* subgenus *Ceratotropis* (Piper) Verdcourt (Leguminosae, Phaseoleae). *Kew Bulletin* **57**: 625–633.

Timmerman-Vaughan GM, McCallum JA, Frew TJ, Weeden NF, Russell AC. 1996. Linkage mapping of quantitative trait loci controlling seed weight in pea (*Pisum sativum* L.). *Theoretical and Applied Genetics* **93**: 431–439.

Tomooka N, Kashiwaba K, Vaughan DA, Ishimoto M, Egawa Y. 2000. The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetle in the genus *Vigna* subgenus *Ceratotropis*. *Euphytica* **115**: 27–41.

Tomooka N, Vaughan DA, Moss H, Maxted N. 2002. *The Asian Vigna: genus Vigna subgenus Ceratotropis genetic resources*. Dordrecht: Kluwer.

Tomooka N, Kaga A, Vaughan DA. 2006. The Asian *Vigna* (Vigna subgenus *Ceratotropis*) biodiversity and evolution. In: Sharma AK, Sharma A, eds. *Plant genome biodiversity and evolution*. Vol. 1. Part C. *Plumeraeae (Angiosperm-Dicotyledons)*. Enfield, NH: Science Publishers, 87–126.

Vaughan DA, Tomooka N, Kaga A. 2004. Azuki bean. In: Singh RJ, Jauhar PP, eds. *Genetic resources, chromosome engineering, and crop improvement: grain legumes*. Boca Raton, FL: CRC Press, 341–353.

Vallejos CE, Sakiyama NS, Chase CD. 1992. A molecular-marker-based linkage map of *Phaseolus vulgaris* L. *Genetics* **131**: 733–740.

Verdecourt B. 1970. Studies of the *Leguminosae-Papilionoideae* for the Flora of Tropical East Africa. IV. *Kew Bulletin* **24**: 507–569.

Wang XW, Kaga A, Tomooka N, Vaughan DA. 2004. The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). *Theoretical and Applied Genetics* **109**: 352–360.

Wojciechowski MF, Lavin M, Sanderson MJ. 2004. A phylogeny of legumes (*Leguminosae*) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *American Journal of Botany* **91**: 1846–1862.

Yamaguchi H. 1992. Wild and weedy azuki beans in Japan. *Economic Botany* **46**: 384–394.

Yu K, Park SJ, Pousya V, Gepts P. 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). *Journal of Heredity* **91**: 429–434.

Zong XX, Kaga A, Tomooka N, Wang XW, Han OK, Vaughan DA. 2003. The genetic diversity of the *Vigna angularis* complex in Asia. *Genome* **46**: 647–658.