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# Genetic diversity of the *Vigna* germplasm from Thailand and neighboring regions revealed by AFLP analysis

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## Abstract

Thailand is a center of diversity for section *Angulares* of the Asian *Vigna* (genus *Vigna* subgenus *Ceratotropis*) and 4 *Vigna* species are cultivated in Thailand. Using newly collected wild and cultivated germplasm of *Vigna* from Thailand and outgroup accessions AFLP analysis was conducted to clarify genetic diversity and relationships. The results suggest that cultivated *V. umbellata* and *V. mungo* evolved from wild relatives in a single domestication event. *Vigna umbellata* is poorly differentiated from its wild and weedy relatives compared to *V. mungo*. Results suggest northern Thailand and the neighboring Shan state, Myanmar, is the probable center of domestication for *V. umbellata* as wild accessions from this area and cultivated rice bean from a wide area in Asia are not greatly diverged. *Vigna minima*, *V. tenuicaulis* and *V. exilis* accessions in Thailand are well differentiated with considerable intra-specific variation. *Vigna hirtella* consists of two well differentiated subgroups, suggesting taxonomic revision may be necessary. Close genetic relationships between *V. radiata* and *V. grandiflora*, and between *V. mungo* and *V. trinervia* are confirmed. Naturally growing *V. mungo* populations in northern Thailand appear to be true wild species as they are well differentiated from Indian wild and Thai cultivated populations. The origin of naturally growing cowpea in Thailand needs to be further studied using a more comprehensive set of materials. This study clarifies inter and intra-specific genetic diversity and inter species relationships of Thai *Vigna* species.

## Introduction

Four *Vigna* cultigens are cultivated in Thailand. Among them mungbean [*Vigna radiata* (L.) Wilczek] is the most important crop (Srinives et al. 1988). Rice bean [*V. umbellata* (Thunb.) Ohwi et Ohashi] is a traditional food crop cultivated mainly in the mountainous area in northern Thailand, whilst black gram [*V. mungo* (L.) Hepper] has been recently introduced to the central part of Thailand as an export crop (Chavalvut et al. 1988). These three crops belong to the genus *Vigna* subgenus *Ceratotropis* that is called the Asian *Vigna* (Tomooka et al. 2002a). Cowpea [*V. unguiculata* (L.) Walp.] is a traditional food legume grown throughout Southeast Asia including Thailand, although its origin is considered to be in Africa (Ba et al. 2004). This crop belongs to the genus *Vigna* subgenus *Vigna*. Wild relatives of these cultigens distributed in Thailand

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are considered to be useful gene sources for breeding programs.

Until recently the wild *Vigna* in Thailand were poorly documented. This is mainly because of the poor representation of *Vigna* specimens conserved in major European herbaria (Maréchal et al. 1978). Based on recent systematic collections of the wild *Vigna* in Thailand, many new germplasm accessions and herbarium specimens have been collected and conserved (Tomooka 1991; Tomooka et al. 1997, 2000). Information regarding the distribution, ecology and taxonomic treatments of the *Vigna* in Thailand are much improved (Tomooka et al. 2002a).

Based on new germplasm and herbarium specimens, 9 wild species of *Vigna* subgenus *Ceratotropis* are recognized in Thailand (Tomooka et al. 2002a). These are *V. radiata* var. *sublobata* (Roxb.) Verdc., *V. mungo* var. *silvestris* Lukoki, Maréchal et Otoul, *V. grandiflora* (Prain) Tateishi et Maxted, *V. umbellata*, *V. exilis* Tateishi et Maxted, *V. hirtella* Ridley, *V. minima* (Roxb.) Ohwi et Ohashi, *V. trinervia* (Heyne ex Wall.) Tateishi et Maxted and *V. tenuicaulis* Tomooka et Maxted. Among them, *V. exilis* and *V. tenuicaulis* are recently described (Tateishi and Maxted 2002, Tomooka et al. 2002b). While mungbean is the most important cultigen in Thailand the wild form in Thailand is only known from herbarium specimens. In contrast, wild and weedy forms of rice bean (*V. umbellata*) have frequently been found mainly in northern Thailand. Wild forms of black gram (*V. mungo* var. *silvestris*) were recorded for the first time in 1996 (Tomooka et al. 1997). However, it is not clear whether this is a native wild species or an escape from cultivation. In addition, the origin of naturally growing populations of *V. unguiculata* is unknown (Tomooka et al. 1997) since the natural distribution of wild cowpea (*V. unguiculata* var. *spontanea*) has been considered to be limited to Africa.

The Asian *Vigna* have been studied using a variety of biochemical and molecular techniques, however, they used a limited number of accessions of each species (e.g. Konarev et al. 2002; Tomooka et al. 2002c). The only studies of the Asian *Vigna* in East Asia assessed genetic diversity using large numbers of accessions of different species (Xu et al. 2000a, b; Yoon et al. 2000; Zong et al. 2003). There are no reports of detailed studies of the Asian *Vigna* in Thailand using molecular techniques. Thailand has representatives of 40% of the species in the subgenus *Ceratotropis* and is considered at the center of diversity of section *Angulares* of this subgenus (Tomooka et al. 2002a). The objectives of this study were to:

(1) Analyze a comprehensive collection of the *V. umbellata* crop complex from Thailand to determine the relationships among wild, weedy and cultivated forms using AFLP analysis supplemented with passport and morphological data;

(2) Analyze a representative collection of other Asian *Vigna* native to Thailand to determine inter and intra specific relationships using AFLP analysis. Selected accessions of the species that occur in other countries were included for comparative purposes.

## Materials and methods

### *Plant materials*

Materials analyzed are listed in the Appendix 1. All materials analyzed are diploid species with $2n = 2x = 22$ chromosomes. The accessions were analyzed in two sets since the number of accessions included in this study could not be fitted onto one gel to enable reliable comparisons. The first set includes accessions of closely related species in section *Angulares* except *Vigna trinervia.* Most of the accessions analyzed in this set were of the *V. umbellata* (rice bean) crop complex that is widely distributed across Thailand. *V. umbellata* accessions were divided into 4 types based on status when collected (wild or cultivated) and seed size and color. These types were wild accessions with typical seed size and color (wild A), wild accessions with seeds of similar size to typical wild accessions but with atypical seedcoat color (wild B), wild accessions with seeds of larger size than typical wild seeds and atypical seed color (wild C) and cultivated (Appendix 1, Figure 1a).

The second set includes *Vigna* species that occur in Thailand that are not so closely related. *Vigna radiata*, *V. mungo* and *V. grandiflora* are in section *Ceratotropis*, *V. trinervia* in section *Angulares*, and *V. unguiculata* in subgenus *Vigna*. For both *V. mungo* and *V. unguiculata*, cultivated and wild forms (Figure 1b and c) were included. To enable the diversity of Thai *Vigna* to be compared with that of neighboring countries, accessions from Myanmar were included for *V. hirtella, V. minima, V. tenuicaulis* and *V. umbellata* and from Malaysia

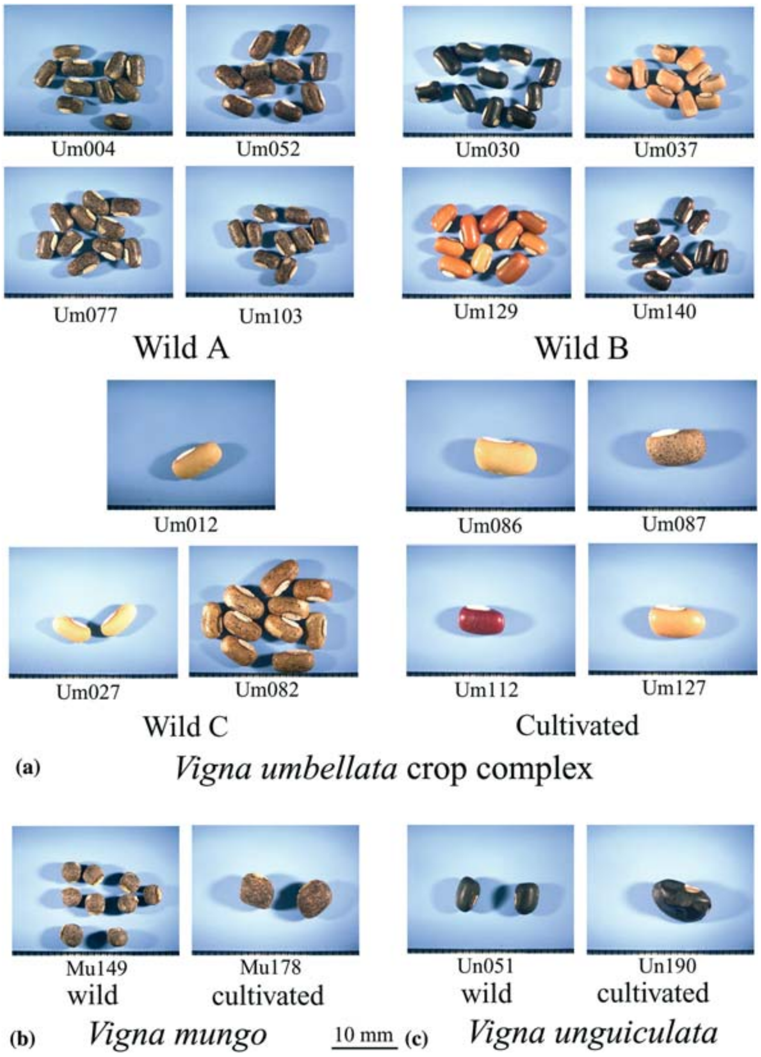

*Figure 1*. Variation in seed shape and color of different components of (a) the *V. umbellata* crop complex, (b) V. mungo, (c) V. unguiculata.

for *V. trinervia*. Cultivated accessions of *V. mungo* from Australia and India were included to estimate the geographical differentiation among cultivars. For the same reason, one accession each from Nepal and Japan of cultivated *V. umbellata* were included. The distribution status of accessions analyzed from Thailand of each species used in this study is shown (Figure 2a–g).

### *DNA extraction*

Five seeds were planted in pots in the greenhouse. Total genomic DNA was extracted from a bulk sample per population of young primary leaves by a modification of a previously published procedure (Dellaport et al. 1983). DNA concentrations were estimated and standardized against known concentrations of $\lambda$ DNA on 1% agarose gels.

### *AFLP analysis*

AFLP analysis followed the procedure described in Zong et al. (2003). Briefly, the restriction reaction was carried out with 0.5 $\mu$g genomic DNA digested with *EcoRI* and *MseI*. *EcoRI* and *MseI* adapters were ligated to the fragments. PCR preamplifica-

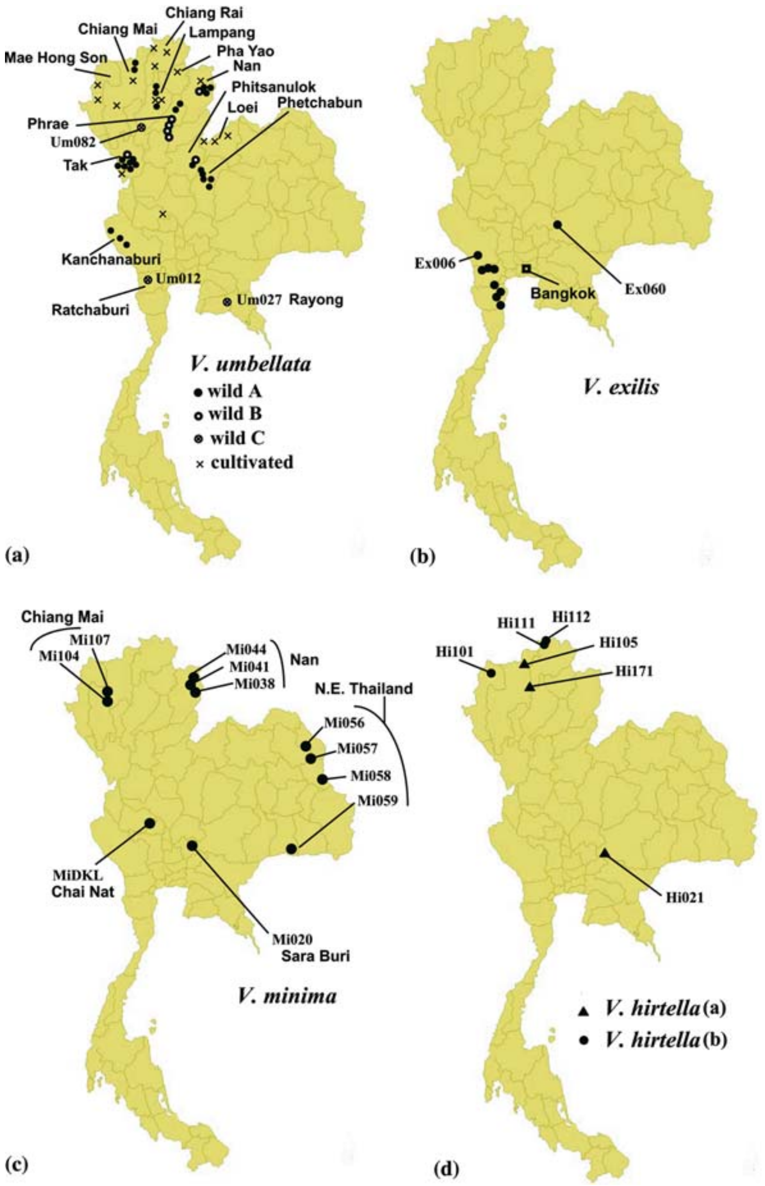

*Figure 2.* Distribution of Thai accessions analyzed: (a) *V. umbellata* (cultivated,×; wild (A) ●; wild (B)○; wild (C)⊗); (b) *V. exilis*; (c) *V. minima*; (d) *V. hirtella* (a) ▲, *V. hirtella* (b) ●; (e) *V. tenuicaulis*; (f) *V. grandiflora* (○), *V. radiata* (▲), *V. unguiculata*, cultivated (); *V. unguiculata*, wild (●). (g) *V. trinervia* (▲), *V. mungo* var. *mungo* (×); *V. mungo* var. *silvestris* (●). See text and figure 1 for wild A, B and C.

tion was carried out with E00 and M00 primers without addition of selective nucleotide using diluted restriction ligation mixture as template DNA.

Selective amplification was carried out with *Eco*RI and *Mse*I primers with 3 selective nucleotides added using diluted preamplification products as template DNA. The selective AFLP primers used were as follows: *Eco*RI, 5′-GAC TGCGTACCAATTCNNN-3′ (where NNN was one of the following AAC, AAG, AGA, CAA, CAT, GAA); *Mse*I, 5′-GATGAGTCCTGAG TAANNN-3′ (where NNN was one of the following AAC, AAG, AGA, CAA, CAT, GAA). For set 1, all accessions of species in section *Angulares* except *Vigna trinervia*, the following 6

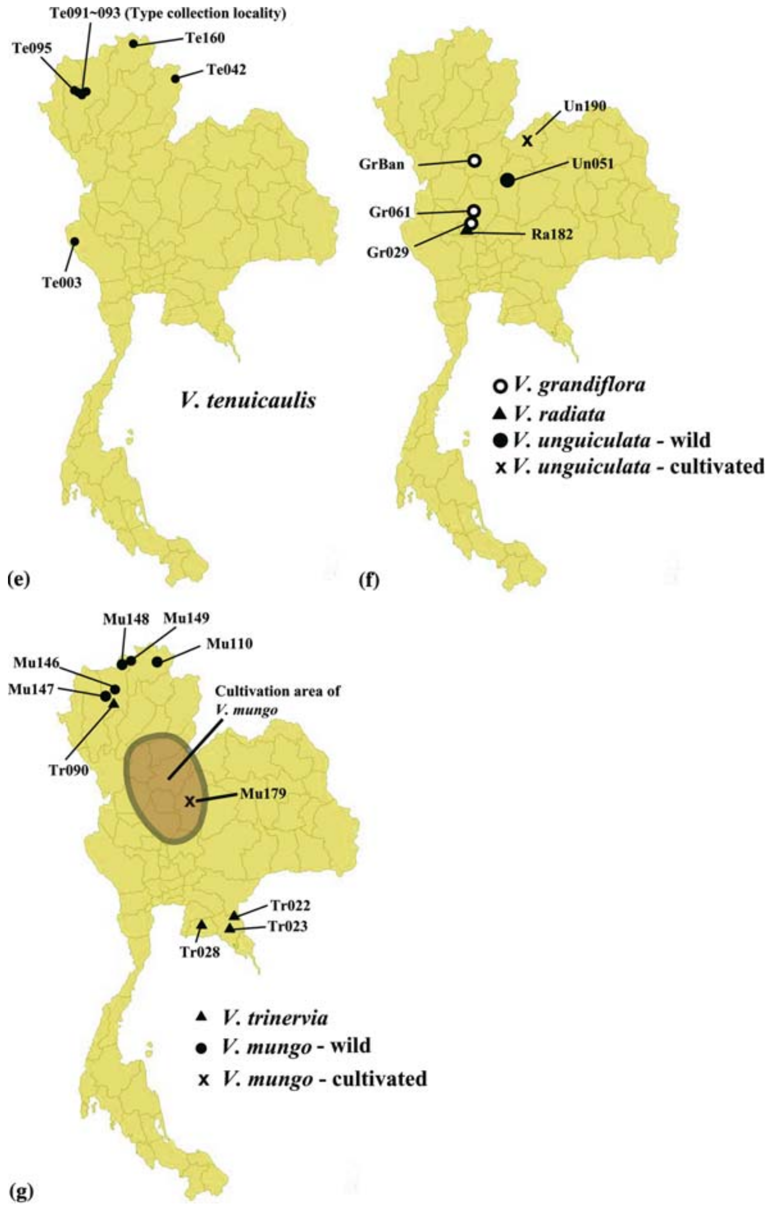

Figure 2. Continued.

primer pairs were used AAC/AAC, AAG/AAG, AGA/AGA, CAA/CAA, CAT/CAT, GAA/GAA. For set 2, all accessions of *V. trinervia*, *V. unguiculata* and species in section *Ceratotropis* of subgenus *Ceratotropis*, the following three primer pairs were used AAC/AAC, AAG/AAG, AGA/AGA. A different number of primers was used for each set because set 1 consists of more closely related materials than set 2. Hence a larger number of primers was considered necessary to resolve differences among and within species of set 1 than set 2. Amplified products were run in 6% acrylamide gel and the banding patterns were visualized according to the silver staining method described by Panaud et al. (1996).

### *Data analysis*

AFLP bands were scored as present (1) and absent (0), and only bands showing unambiguous polymorphism were entered into a data matrix.

Two methods were used to estimate diversity. The first method estimates genetic distance at the species level based on inferred nucleotide diversity ($\pi \times 1000$) designed especially for AFLP data (Innan et al. 1999). The average value of all the pairwise distances was regarded as a measure of nucleotide polymorphism (nucleotide diversity). A neighbor-joining analysis was conducted by PHYLIP ver. 3.57 on the basis of the pairwise distance (Felsenstein 1992) and an unrooted tree was constructed using Treeview ver. 3.2. For wild and cultivated accessions of *Vigna umbellata* and *V. mungo*, the population genetics statistics total genetic diversity (Ht), within-population gene diversity (Hs), relative magnitude of gene differentiation among populations (Gst), Nei's gene diversity (h) and Shannon's gene diversity index (I) were calculated using POPGENE software ver. 1.32.

## Results

### *(a) Set 1*

For the first set of accessions (see Appendix 1) AFLP analysis revealed a total of 1074 bands of which 1021 (95.1%) were polymorphic (Table 1a). Neighbor joining cluster analysis of this set of accessions revealed 6 groups of accessions consisting of five species with 2 subgroups within *Vigna hirtella* (Figure 3). The average number of amplified bands per accession, number of polymorphic bands and estimated nucleotide diversity ($\pi \times 1000$) are summarized for these six groups (Table 1a). Average number of amplified bands ranged from 302.7 [*V. hirtella* (a)] to 337.8 (*V. umbellata*). Percentage of polymorphic bands ranged from 37.9% [*V. hirtella* (b)] to 76.0% (*V. minima*). Nucleotide diversity for each species/subgroup ranged from 6.928 (*V. umbellata*) to 18.25 [*V. hirtella* (a)].

### *Vigna umbellata (Rice bean)*

A total of 57 accessions consisting of different components of this crop complex were analyzed (Figure 1, Table 2a). Cultivated accessions consisted of 16 Thai landraces, 1 Nepalese and 1 Japanese landrace. Wild accessions consisted of 36 from Thailand and 3 from Myanmar. Although the number of accessions analyzed was more than other species and came from a wider geographic range, *V. umbellata* showed the lowest level of nucleotide diversity (6.928) (Table 1a).

Genetic variation parameters for the 4 population types of *V. umbellata*, typical wild [wild (A)], wild with small seeds and atypical seedcoat color [wild (B)], wild with larger seeds and atypical seedcoat color [wild (C)] and cultivated are summarized (Table 2a). Average number of amplified

*Table 1.* Summary of AFLP fragments and diversity statistics calculated for each species.

| Species | No. of accessions | Average no. of amplified bands/accession | No. of polymorphic bands/Total no. of amplified bands/ species (%) | Estimated nucleotide diversity $\pi \times 1000^{a}$ |
|---|---|---|---|---|
| (a) *Angulares* subgenus *Ceratotropis* species (AFLP bands generated by 6 primer pairs) | | | | |
| *V. exilis* | 9 | 316.9 | 320/505 (63.4) | 12.681 |
| *V. hirtella* (a) | 3 | 302.7 | 217/418 (51.9) | 18.250 |
| *V. hirtella* (b) | 5 | 320.4 | 152/401 (37.9) | 7.834 |
| *V. minima* | 13 | 306.5 | 453/596 (76.0) | 15.458 |
| *V. tenuicaulis* | 9 | 307.2 | 305/475 (64.2) | 12.866 |
| *V. umbellata* | 57 | 337.8 | 281/497 (56.5) | 6.928 |
| Total | 96 | | 1021/1074 (95.1) | |
| (b) other *Vigna* species (AFLP bands generated by 3 primer pairs) | | | | |
| *V. trinervia* | 5 | 147.8 | 68/182 (37.4) | 7.698 |
| *V. grandiflora* | 3 | 184.3 | 106/240 (44.2) | 22.300 |
| *V. mungo* | 13 | 166.5 | 115/226 (50.9) | 8.095 |
| *V. radiata* | 1 | 158 | – | – |
| *V. unguiculata* | 2 | 156.5 | 48/180 (26.7) | 10.290 |
| Total | 24 | | 513/526 (97.5) | |

[a] $\pi$ = Innan's nucleotide diversity (Innan et al. 1999).

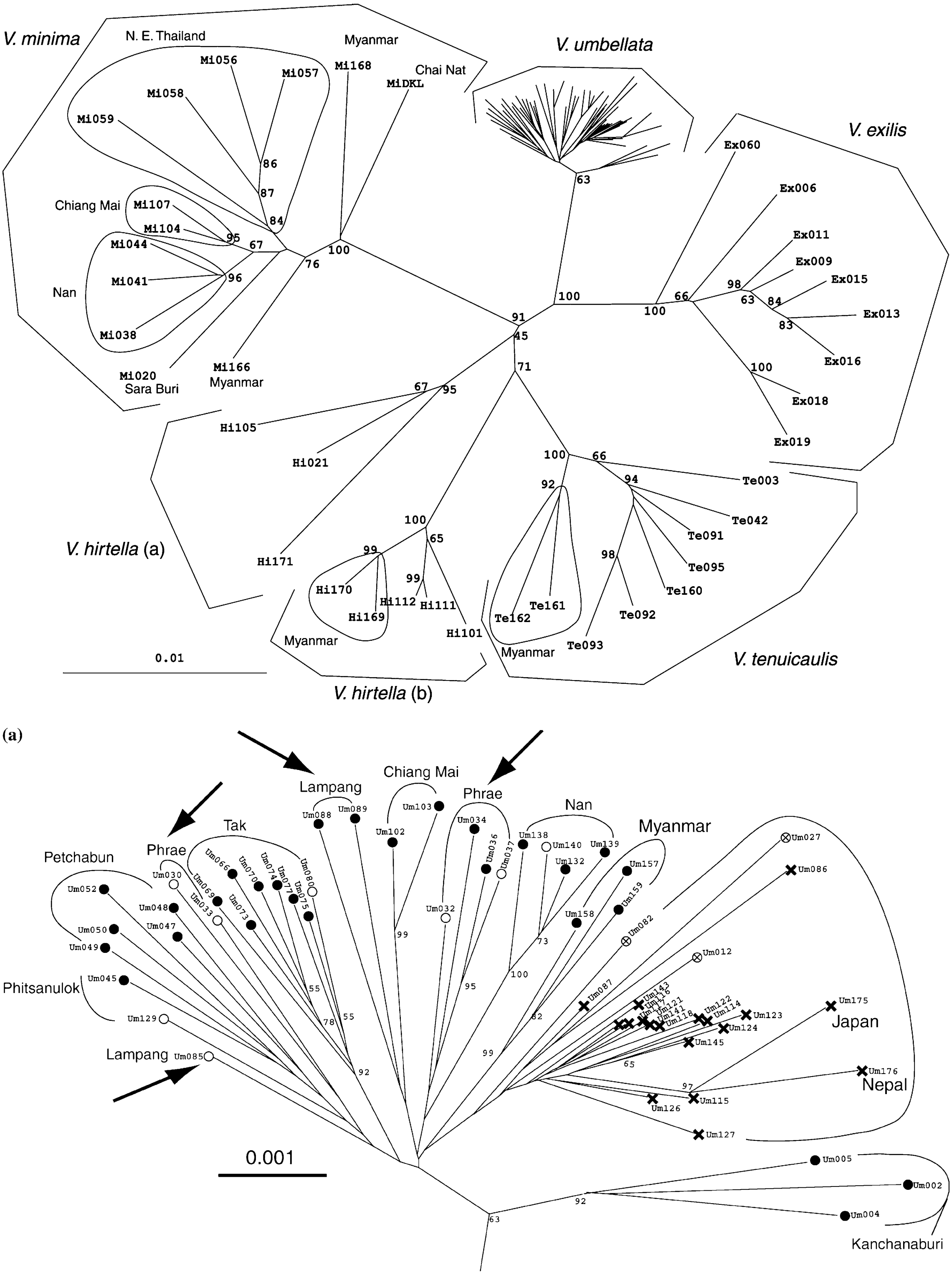

*Figure 3*. Unrooted neighbor-joining tree of *Vigna* species analyzed in section *Angulares* (except *V. trinervia*) based on a matrix of inferred nucleotide diversity using AFLP variation to prepare the matrix. % bootstrap values based on 1000 replications over 50% are shown. (a) Enlargement of *V. umbellata* (cultivated ×; wild (A) ●; wild (B) ○; wild (C) ⊗). See text and Figure 1 for wild A, B and C explanation.

bands was less in cultivated (330.6) and large, colored seeded wild (C) (331.8) populations compared with typical wild (A) accessions (339.0) and highest in small, colored seeded wild (B) accessions (348.6). Percentage of polymorphic bands was smallest in large colored seeded wild (C) (18.7%) and highest in typical wild (A) accessions (48.1%). Within-population gene diversity estimates (Hs) were low in cultivated (0.084) and large, colored seeded wild (C) (0.074), compared with high in typical wild (B) (0.132) and wild, small colored seeded (B) accessions (0.101). The tendency of within-population gene diversity is consistent with the results based on Nei's gene diversity (h) and Shannon's gene diversity index (I).

All cultivated accessions clustered together except two accessions found in a farmer's seed lot (Um086, 087) (Figure 1a, Figure 3a). These two accessions (Um086, 087) positioned outside of the main Thai cultivated accessions. These two accessions have an intermediate position, as do wild (C) accessions (Um012, 027, 082), between the cultivated cluster and wild accessions. Japanese and Nepalese cultivated accessions were included in the same cluster as Thai cultivated accessions but were significantly differentiated from each other and from other Thai cultivated accessions (Figure 3a).

Cultivated accessions analyzed were very weakly differentiated from wild accessions (Figure 3a). Within wild populations, three accessions from Kanchanaburi province (Um002, 004, 005) were highly differentiated from other Thai wild accessions. Although other wild accessions were closely related to each other, geographic differentiation was observed (Figure 3a). Generally, accessions from the same province clustered together, and most of the exceptional cases were observed for wild (B) accessions with seed color not found in typical wild (A) accessions from the same location (indicated with arrows in Figure 3a). Three wild (A) accessions from Myanmar (Um157, 158, 159) were not significantly differentiated from Thai cultivar and Thai wild populations.

### *Vigna exilis*

*Vigna exilis* was genetically well differentiated from other species and is most closely related to *V. umbellata* of the species analyzed (Figure 3, Table 3a). Among the nine accessions of *V. exilis* analyzed, geographic differentiation was detected. Eight were collected from western and southwestern region of Bangkok and one from northeast of Bangkok (Ex060) (Figure 2b). The accession from northeast of Bangkok showed the highest level of differentiation.

*Table 2.* Gene diversity statistics for population groups of *V. umbellata* and *V. mungo*.

| Population groups | No. of accessions | Average no. of amplified bands/accession | No. of polymorphic bands/Total no. of amplified bands (%) | Ht[a] | Hs[b] | Gst[c] | h[d] | I[e] |
|---|---|---|---|---|---|---|---|---|
| (a) *V. umbellata* | | | | | | | | |
| wild A[f] | 29 | 339 | 239/497 (48.1) | | 0.132 | | 0.135 | 0.208 |
| wild B | 7 | 348.6 | 130/497 (26.2) | | 0.101 | | 0.101 | 0.148 |
| wild C | 3 | 331.8 | 93/497 (18.7) | | 0.074 | | 0.074 | 0.109 |
| cultivated | 18 | 330.6 | 144/497 (29.0) | | 0.084 | | 0.084 | 0.129 |
| All accessions | 57 | 337.8 | 281/497 (56.5) | 0.131 | 0.104 | 0.205 | 0.134 | 0.211 |
| (b) *V. mungo* | | | | | | | | |
| Thai wild | 5 | 166.6 | 38/226 (16.8) | | 0.061 | | 0.061 | 0.09 |
| Indian wild | 2 | 161 | 14/226 (6.2) | | 0.026 | | 0.026 | 0.034 |
| cultivated | 6 | 168.3 | 24/226 (10.6) | | 0.045 | | 0.045 | 0.062 |
| All accessions | 13 | 166.5 | 115/226 (50.9) | 0.171 | 0.044 | 0.745 | 0.161 | 0.246 |

[a] Ht = total genetic diversity.

[b] Hs = within-populations gene diversity.

[c] Gst = relative magnitude of gene differentiation among populations.

[d] h = Nei's (1973) gene diversity.

[e] I = Shannon's gene diversity index (Shannon and Weaver 1949).

[f] see text for description of wild A, B and C.

*Table 3*. Genetic distances within and between species/subgroup based on Innan's nucleotide diversity ($\pi \times 1000$).

| | *V. minima* | *V. hirtella* (a) | *V. hirtella* (b) | *V. tenuicaulis* | *V. exilis* | *V. umbellata* |
|---|---|---|---|---|---|---|
| (a) Section *Angulares* species | | | | | | |
| *V. minima* | 15.458[a]<br>3.371[b]<br>78[c] | | | | | |
| *V. hirtella* (a) | 35.321<br>3.254<br>39 | 18.250<br>3.424<br>3 | | | | |
| *V. hirtella* (b) | 36.041<br>1.724<br>65 | 27.869<br>1.896<br>15 | 7.834<br>2.766<br>10 | | | |
| *V. tenuicaulis* | 35.723<br>2.144<br>117 | 32.141<br>2.288<br>27 | 27.284<br>1.884<br>45 | 12.866<br>2.834<br>36 | | |
| *V. exilis* | 32.684<br>2.087<br>117 | 32.146<br>2.537<br>27 | 31.772<br>2.013<br>45 | 32.831<br>2.375<br>81 | 12.681<br>3.931<br>36 | |
| *V. umbellata* | 34.072<br>2.458<br>741 | 27.697<br>2.233<br>171 | 29.665<br>1.286<br>285 | 29.819<br>1.770<br>513 | 25.723<br>1.569<br>513 | 6.928<br>1.635<br>1596 |
| (b) Other *Vigna* species | | | | | | |
| | *V. trinervia* | *V. mungo* | *V. grandiflora* | *V. radiata* | *V. unguiculata* | |
| *V. trinervia* | 7.698[a]<br>4.73669[b]<br>10[c] | | | | | |
| *V. mungo* | 63.271<br>2.883<br>65 | 6.095<br>4.261<br>78 | | | | |
| *V. grandiflora* | 82.659<br>5.597<br>15 | 87.327<br>8.831<br>39 | 22.300<br>15.854<br>3 | | | |
| *V. radiata* | 10.161<br>11.854<br>5 | 79.508<br>4.024<br>13 | 61.760<br>10.144<br>3 | | | |
| *V. unguiculata* | 10.501<br>6.656<br>10 | 116.468<br>7.575<br>26 | 82.659<br>5.597<br>15 | 84.675<br>–<br>2 | 10.290<br>–<br>1 | |

(a) – Species in section *Angulares* excluding *V. trinervia* (set 1).
(b) – Species in section *Ceratotropis*, *V. trinervia* and *V. unguiculata* (set 2).
[a] Average genetic distance.
[b] Standard Error.
[c] Number of comparisons.

### *Vigna minima*

Among species examined in this set of accessions, *V. minima* (13 accessions) showed the highest nucleotide diversity (15.458) and geographic differentiation was observed. Four accessions from northeast Thailand (Mi056, 057, 058, 059), 2 accessions from Chiang Mai province (Mi104, 107) and 3 accessions from Nan province (Mi038, 041, 044) formed distinct sub-clusters (Figure 2c, Figure 3). Accessions from Myanmar (Mi166, 168) and central provinces of Thailand (MiDKL, Mi020) were the most diverged of the *V. minima* accessions.

### *Vigna hirtella*

Neighbor joining clustering of *V. hirtella* accessions based on morphological characteristics formed two genetically distinct subgroups [*V. hirtella* (a): 3 accessions and *V. hirtella* (b): 5 accessions] (Figure 2d, Figure 3). Genetic distance based on nucleotide diversity between the two subgroups was 27.869 (Table 3). *Vigna hirtella* (a) subgroup showed higher nucleotide diversity (18.25) than *V. hirtella* (b) subgroup (7.834) (Table 1a).

*Vigna tenuicaulis*
Nine *V. tenuicaulis* accessions showed the third highest nucleotide diversity (12.866). A high level of genetic differentiation was observed between Myanmar (Te161, 162) and Thai accessions (Figure 3). Among Thai accessions, the accession (Te003) that was collected in Kanchanaburi, western Thailand was the most diverged. Accessions collected from locations in close proximity in northern Thailand each showed high inter accession genetic diversity. Three accessions (Te091, 092, 093) that had morphological differences in pod shape and bracteole color were collected at the same site (Figure 2e). However, they showed a high level of genetic diversity.

### *(b) Set 2*

AFLP analysis of the second set of accessions, consisting of *Vigna trinervia* of section *Angulares*, *V. grandiflora*, *V. mungo* and *V. radiata* of section *Ceratotropis*, and *V. unguiculata* in subgenus *Vigna*, was based on 3 primer pairs (*E*-AAC/*M*-AAC, *E*-AAG/*M*-AAG, *E*-AGA/*M*-AGA). A total of 526 bands were amplified and 513 (97.5%) showed polymorphism (Table 1b). Highest number of bands was amplified in *V. grandiflora* (184.3) and lowest for *V. trinervia* (147.8). Percentage of polymorphic bands ranged from 26.7% (*V. unguiculata)* to 50.9% (*V. mungo*). Nucleotide diversity ($\pi \times 1000$) was lowest in *V. trinervia* (7.698) and highest in *V. grandiflora* (22.3) (Table 1b).

*Vigna grandiflora*
*Vigna grandiflora* was the most closely related to *V. radiata* of the species analyzed (Table 3b, Figure 4). Although the three accessions analyzed were collected from a limited area in central Thailand (Figure 2f), they showed the highest nucleotide diversity (Table 1b).

*Vigna trinervia*
*Vigna trinervia* was the most closely related to *V. mungo* of the species analyzed in this set (Table 3b, Figure 2g). In contrast to *V. grandiflora*, accessions of *V. trinervia* showed a lower level of nucleotide diversity although accessions were from widely separated locations in northern Thailand (Tr090), southeastern Thailand (Tr022, 028, 023) and Malaysia (Tr163) (Table 3b, Figure 4).

*Vigna mungo (Black gram)*
Accessions of *V. mungo* include cultivated accessions from Thailand (Figure 2g), India and Australia, and wild accessions from India and Thailand. However, nucleotide diversity was low (8.095) compared with *V. grandiflora* (22.3). Cultivated accessions, wild accessions from India and wild accessions from Thailand formed 3 distinct sub-clusters (Figure 4a). Among 3 sub-clusters, wild populations from Thailand showed the highest gene diversity (Table 2b).

*Vigna unguiculata (Cowpea)*
One accession each of cultivated and wild growing *V. unguiculata* were analyzed. Relatively high nucleotide diversity (10.29) was recorded (Table 1b) and relatively high genetic differentiation was observed between these two accessions (Figure 4).

## Discussion

### *(a) Set 1*

Based on the distribution of the Asian *Vigna*, Thailand appears to be the center of diversity of section *Angulares* of the subgenus *Ceratotropis* (Tomooka et al. 2002a). Northern Thailand is at the center of distribution for many section *Angulares* species. Consequently analysis of the Asian *Vigna* germplasm from this area is expected to reveal insights into the evolution of section *Angulares*.

*Vigna umbellata (rice bean)*
*Vigna umbellata* is a minor cultigen but its prolific vegetative growth and high seed production suggest it may be a more useful cover crop, forage or food legume than realized hither-to-fore. This crop has been used as a staple food mainly in mountainous regions of mainland Southeast Asia and southern China where it is cultivated under shifting cultivation agricultural systems. These areas are considered to be the center of origin and diversity.

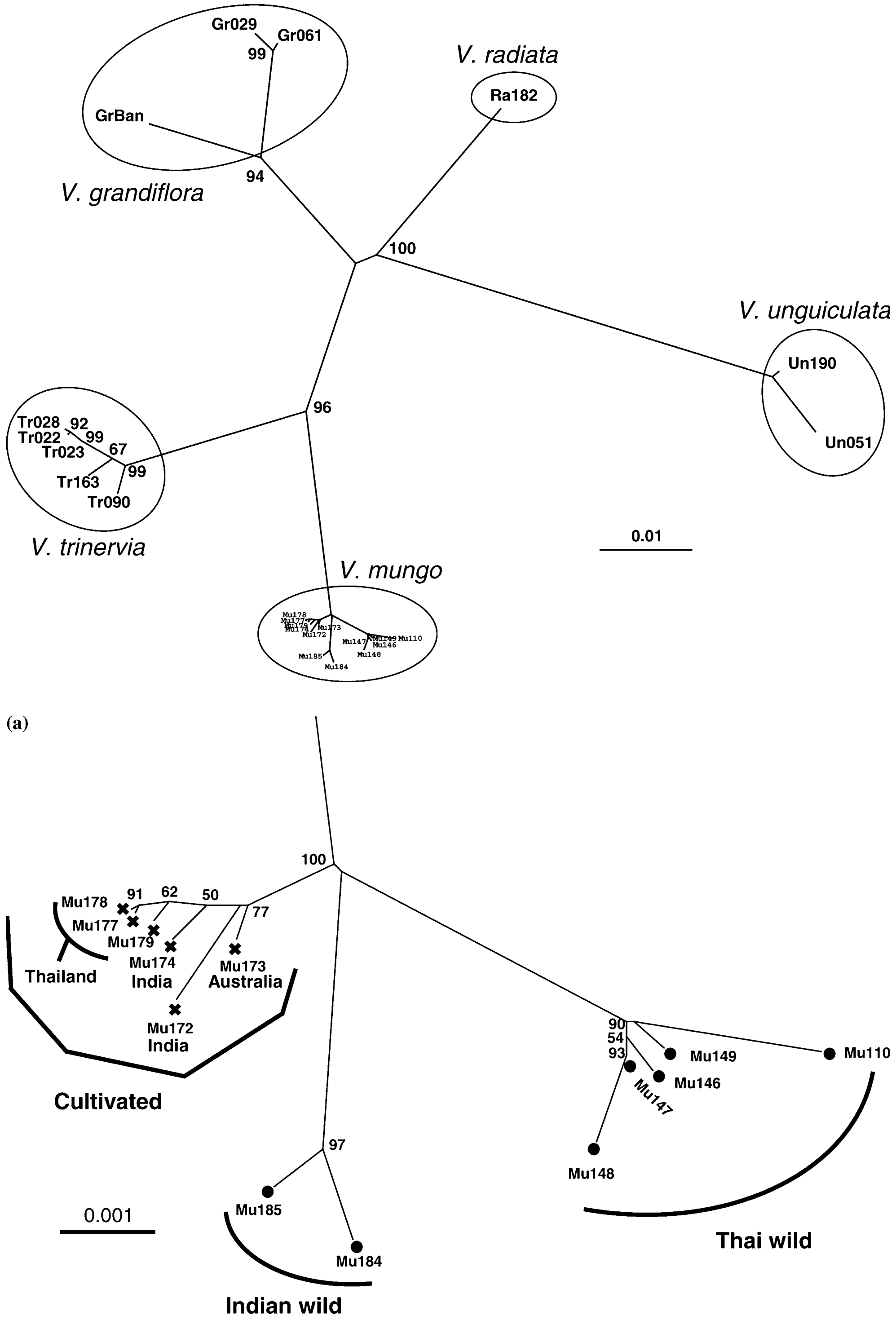

*Figure 4*. Unrooted neighbor-joining tree of species in section *Ceratotropis*, *V. trinervia* and *V. unguiculata* based on a matrix of inferred nucleotide diversity using AFLP variation to prepare the matrix. % bootstrap values based on 1000 replications over 50% are shown. (a) Inset enlargement of *V. mungo*. *V. mungo* var. *mungo* (×) and *V. mungo* var. *silvestris* (●).

This is the first detailed molecular analysis of germplasm of the *V. umbellata* crop complex. The lack of variation based on inferred nucleotide diversity in *V. umbellata* accessions from northern Thailand, a region in the center of distribution of this crop complex, might imply that this is a recently evolved species. Based on morphological studies of the Asian *Vigna* species, Tateishi (1985) suggested *V. umbellata* to be a recently evolved species and closely related to *V. exilis*. This is partly supported from our results that show *V. umbellata* and *V. exilis* are most closely related (genetic distance = 25.723, (Table 3a).

The reproductive system of *V. umbellata* and its ecological habitat may also help explain the low intraspecific diversity. Isozyme and SSR (microsatellite) studies of plant species with different breeding systems have revealed that annual, inbreeding species have higher inter population variation than perennial, outcrossing species (Hamrick and Godt 1990; Kuroda et al. 2003).

Field observations in Thailand of *V. umbellata* suggest it has a perennial habit based on its very thick stem (Tomooka et al. 1997). It has a conspicuous inflorescence consisting of several large, bright yellow flowers raised above the leaf canopy. These characteristics suggest it is adapted to outbreeding. The prominent inflorescence with large, bright yellow flowers of *V. umbellata* is in contrast to the other species of section *Angulares* analyzed here that have smaller flowers and less prominent inflorescence. Thus outcrossing maybe more common in *V. umbellata* than other *Vigna* species from Thailand. In addition, during germplasm collection many insects including bees were observed visiting flowers of *V. umbellata*. The frequent existence of populations having atypical seed color [wild (B) and wild (C)] may be the result of gene flow between cultivated and wild populations. However, inter- and intra-population genetic structures, rate of outcrossing and annual/perennial habit of *Vigna* species require clarification. It is necessary to analyze breeding system and population structure to clarify why the genetic diversity is much lower in *V. umbellata* than other *Vigna* in Thailand.

All the cultivated accessions of *Vigna umbellata* analyzed, including those from Japan and Nepal, form a single cluster within the wild accessions. This suggests a single domestication event for *Vigna umbellata*. The lack of differentiation between the wild and cultivated accessions suggests that northern Thailand is the area where domestication occurred. In both Thailand and Myanmar farmers collect wild *V. umbellata* for consumption (Tomooka et al. 2003). However, frequent post-domestication gene flow between wild and cultivated forms may also explain the lack of differentiation as has been suggested for African cowpea, *V. unguiculata* (Coulibaly et al. 2002). To better understand the domestication of rice bean an analysis of materials from southeast Asia and southern Chinese materials is required.

Three accessions [wild (C)] from roadside habitats (Um012, Um027, Um082) have considerably larger seeds and seed color not found in typical wild *V. umbellata* [wild (A)] (Figures 1 and 2a). If these accessions have escaped from cultivation, they should be included in the cluster with cultivated *V. umbellata* accessions. However, AFLP results show this is not the case. Their position is intermediate between cultivated and wild populations (Figure 3a). Intermediate seed size and AFLP results as well as seed color similar to some cultivated accessions suggests these populations originate from natural hybridization between wild and cultivated rice bean.

Natural introgression from wild to cultivated rice bean is the possible origin of cultivated accessions Um086 and Um087 that were found in a farmers seed bulk lot in Lampang province (Figure 2a). Um086 has tan color seed and Um087 has tan with black mottled seed. The genetic position based on AFLP analysis of these two accessions suggests they have originated from wild gene introgression into the cultigen. Due to preference or market demand, some Thai farmers report that they select only red seeded *V. umbellata*. Farmers reported that during cultivation mottled color seeds were frequently found and discarded. Hence these off-types may form escaped populations.

### *Vigna exilis*

This is the first detailed study of *V. exilis* which has a very restricted habitat on limestone outcrops scattered across parts of west and central Thailand (Tateishi and Maxted 2002; Tomooka et al. 2002b). The disjunct distribution of populations of this species might help explain the high level of inter-population diversity of this species (Table 1a).

### *Vigna minima*

*Vigna minima* is the only species in section *Angulares* that is found as scattered plants on the forest floor. It is widely distributed in southeast and east Asia (Tomooka et al. 2002a). The high level of genetic differentiation reflects the wide distribution of the analyzed accessions (Figure 2b). The isolation of this species in forests on different mountain ranges across northern Thailand and in remaining sporadic patches of forest in northeast Thailand probably accounts for the high level of population divergence.

### *Vigna hirtella*

Previous studies have suggested that materials identified as *V. hirtella* may consist of two taxa (Tomooka et al. 2002c). This study also suggests this, since the genetic distance between *V. umbellata* and *V. exilis* (25.723) is less than between the two groups (27.869) of *V. hirtella* (a and b) (Table 3a). *V. hirtella* was first described in peninsular Malaysia. An accession collected in peninsular Malaysia close to the collection site of the type specimen and therefore considered to be true *V. hirtella* was included in a previous analysis (Tomooka et al. 2002c). Judging from DNA similarity with Malaysian accession, the *V. hirtella* (a) in this study is probably a true *V. hirtella*. The average altitude of the 3 *V. hirtella* (a) accessions is 628 m, while that of the 5 *V. hirtella* (b) accessions is 1243 m. Although *V. hirtella* (a) consist of 3 Thai accessions each accession is well differentiated (Figures 2d and 3, Table 3). However, for *V. hirtella* (b) the level of genetic differentiation among accessions is relatively small (Figures 2d and 3). It remains to be determined whether clear morphological differences exist between these two groups of *V. hirtella* accessions.

### *Vigna tenuicaulis*

*Vigna tenuicaulis* is a recently described species (Tomooka et al. 2002b). Myanmar accessions of this species analyzed are genetically distinct from Thai accessions (Figure 3). Accessions from northern Thailand show considerable genetic differentiation. Even 3 accessions (Te091, Te092: Type plant, Te093) collected at the same site (the collection site of Type specimens) show a high level of genetic difference (Figure 2e).

### *(a) Set 2*

Section *Ceratotropis* consists of 4 species; of them three were analyzed here. *Vigna subramaniana* is only known from south Asia and was not included. The center of diversity for this section is south Asia (Tomooka et al. 2002a) and in Thailand only a few wild populations of species in this section have been found. While Thailand is an important producer of mungbean (*V. radiata*) the presumed wild ancestor of mungbean (*V. radiata* var. *sublobata*) is not now found in Thailand. *Vigna unguiculata* belonging to the subgenus *Vigna* was also included to investigate genetic relationship between cultivated and naturally growing populations. Each species analyzed is highly differentiated compared with those analyzed in section *Angulares* (Table 3).

### *Vigna grandiflora and Vigna trinervia*

Results of analyzing Thai representatives of section *Ceratotropis* species and *V. trinervia* confirm earlier studies that *V. grandiflora* is more closely related to *V. radiata* than *V. mungo* (Tomooka et al. 2002c). Although Niyomdham (1992) proposed a treatment of *V. grandiflora* as a variety of *V. radiata*, AFLP distance suggests this to be a distinct species. In addition, *V. trinervia* of section *Angulares* is more closely related to *V. mungo* than the other section *Ceratotropis* species analyzed here.

### *Vigna mungo (Black gram)*

This study is the first to analyze both wild and cultivated *V. mungo* by molecular techniques in detail. The results show that cultivated *V. mungo* is most probably monophyletic since cultivated accessions from Thailand, India and Australia are all clustered together and separately from accessions of its presumed wild ancestor *V. mungo* var. *silvestris* (Figure 4a). In addition, all cultivated accessions are differentiated from both Thai and Indian wild accessions. This genetic relationship is in contrast to the *V. umbellata* crop complex discussed above. India has been proposed as the most likely country where *V. mungo* was domesticated (Chandel et al. 1984). However, based on genetic distance between analyzed wild and cultivated germplasm of *V. mungo*, this cannot be confirmed. A larger number of wild *V. mungo* accessions covering its distribution range especially from

Myanmar should be included to clarify the center of diversity of this crop complex.

The wild accessions analyzed show Thai accessions of *V. mungo* var. *silvestris* are well differentiated from Indian wild accessions and cultivated accessions from India, Thailand and Australia. Only recently have populations of wild *V. mungo* var. *silvestris* been found in Thailand. In addition, *V. mungo* was introduced into Thailand within the last 50 years (P. Srinives, Kasesart University, 2004, pers. comm.). Consequently it is unclear whether naturally growing wild *V. mungo* populations in Thailand are true wild populations of *V. mungo* or recently introduced wild contaminants of cultivated *V. mungo* that have become naturalized. Judging from the genetic distance between wild populations of *V. mungo* in Thailand and cultivated and Indian wild populations, and the geographic distance between the main area of black gram cultivation in Thailand and where wild populations have been collected (Figure 2g), they appear to be truly native to Thailand.

### *Vigna unguiculata (Cowpea)*

Cowpea was domesticated in Africa but is considered an ancient introduced crop in Asia (Ng and Maréchal 1985). The wild progenitor of cowpea (*V. unguiculata* var. *spontanea)* is reported only from Africa. However in Thailand, there are natural growing black seeded cowpea populations. Plants of these populations have easy shattering pods and seed size is much smaller than present cowpea landraces cultivated in Thailand (Figure 1c). In the present study, a wild population and cultivar from Thailand were compared. A high degree of genetic differentiation between these two populations suggests the wild population is not a recent escape from landrace cowpea. A larger number of wild populations and landraces should be analyzed to clarify the origin of wild small seeded cowpea in Thailand.

In conclusion, this study is the first to shed light on the process and possible place of domestication for *Vigna umbellata*. The results support the view that both *V. umbellata* and *V. mungo* evolved from wild relatives in a single domestication event. *V. umbellata* probably was domesticated in northern Thailand or nearby regions. While *V. exilis*, *V. minima* and *V. tenuicaulis* are well-differentiated species; *V. hirtella* has characteristics of a complex species with at least two genetically distinct types. Species relationships of section *Ceratotropis* confirm earlier studies. The Asian *Vigna* in Thailand, particularly the north of Thailand, can furnish germplasm that is useful for understanding both speciation and domestication in this agriculturally important group.

## Acknowledgements

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**Appendix 1.** *Vigna* accessions used in this study.

| Code | JP no.[a] | Species name (status) | Origin | Province | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|---|---|---|
| Set 1. subgenus *Ceratotropis* section *Angulares* (except *V. trinervia*) | | | | | | | |
| Um002 | 210639 | *V. umbellata* (wild A) | Thailand | Kanchanaburi | N14–44 | E98–34 | 200 |
| Um004 | 210640 | *V. umbellata* (wild A) | Thailand | Kanchanaburi | N14–40 | E98–23 | 867 |
| Um005 | 210642 | *V. umbellata* (wild A) | Thailand | Kanchanaburi | N14–44 | E98–38 | 195 |
| Um045 | 210672 | *V. umbellata* (wild A) | Thailand | Phitsanulok | N16–52 | E100–38 | 165 |
| Um047 | 210674 | *V. umbellata* (wild A) | Thailand | Phetchabun | N16–40 | E101–05 | 860 |
| Um048 | 210675 | *V. umbellata* (wild A) | Thailand | Phetchabun | N16–40 | E101–06 | 970 |
| Um049 | 210676 | *V. umbellata* (wild A) | Thailand | Phetchabun | N16–33 | E101–02 | 715 |
| Um050 | 210677 | *V. umbellata* (wild A) | Thailand | Phetchabun | N16–25 | E101–11 | 165 |
| Um052 | 210679 | *V. umbellata* (wild A) | Thailand | Phetchabun | N16–32 | E101–18 | 235 |
| Um070 | 108525 | *V. umbellata* (wild A) | Thailand | Tak | N16–48 | E98–57 | 510 |
| Um074 | 108529 | *V. umbellata* (wild A) | Thailand | Tak | N16–46 | E98–51 | 380 |
| Um088 | 108547 | *V. umbellata* (wild A) | Thailand | Lampang | N18–30 | E99–20 | 390 |
| Um089 | 108548 | *V. umbellata* (wild A) | Thailand | Lampang | N18–31 | E99–31 | 400 |
| Um102 | 108563 | *V. umbellata* (wild A) | Thailand | Chiang Mai | N19–27 | E99–00 | 510 |

*Appendix 1*. Continued.

| Code | JP no.[a] | Species name (status) | Origin | Province | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|---|---|---|
| Um103 | 108564 | *V. umbellata* (wild A) | Thailand | Chiang Mai | N19–28 | E99–00 | 510 |
| Um157 | 210797 | *V. umbellata* (wild A) | Myanmar | Mandalay | N21–52 | E96–10 | 100 |
| Um158 | 210802 | *V. umbellata* (wild A) | Myanmar | Mandalay | N21–59 | E96–23 | 855 |
| Um159 | 210803 | *V. umbellata* (wild A) | Myanmar | Mandalay | N21–52 | E96–21 | 860 |
| Um012 | 210647 | *V. umbellata* (wild C) | Thailand | Ratchaburi | N13–19 | E99–34 | 260 |
| Um027 | 210662 | *V. umbellata* (wild C) | Thailand | Rayong | N12–50 | E101–44 | 100 |
| Um030 | 210665 | *V. umbellata* (wild B) | Thailand | Phrae | N17–51 | E100–02 | 350 |
| Um032 | 210666 | *V. umbellata* (wild B) | Thailand | Phrae | N17–51 | E100–02 | 350 |
| Um033 | 210667 | *V. umbellata* (wild B) | Thailand | Phrae | N17–56 | E100–04 | 240 |
| Um034 | 207985 | *V. umbellata* (wild A) | Thailand | Phrae | N18–14 | E100–17 | 260 |
| Um036 | 210668 | *V. umbellata* (wild A) | Thailand | Phrae | N18–22 | E100–24 | 300 |
| Um037 | 211786 | *V. umbellata* (wild B) | Thailand | Phrae | N18–22 | E100–24 | 300 |
| Um066 | 108521 | *V. umbellata* (wild A) | Thailand | Tak | N16–48 | E99–03 | 109 |
| Um069 | 108524 | *V. umbellata* (wild A) | Thailand | Tak | N16–48 | E99–03 | 109 |
| Um073 | 108528 | *V. umbellata* (wild A) | Thailand | Tak | N16–47 | E98–53 | 405 |
| Um075 | 108530 | *V. umbellata* (wild A) | Thailand | Tak | N16–44 | E98–34 | 220 |
| Um077 | 108532 | *V. umbellata* (wild A) | Thailand | Tak | N16–47 | E98–37 | 235 |
| Um080 | 108536 | *V. umbellata* (wild B) | Thailand | Tak | N16–58 | E98–41 | 340 |
| Um082 | 108539 | *V. umbellata* (wild C) | Thailand | Lampang | N17–36 | E99–13 | 210 |
| Um085 | 108543 | *V. umbellata* (wild B) | Thailand | Lampang | N18–29 | E99–30 | 370 |
| Um129 | 109670 | *V. umbellata* (wild B) | Thailand | Phitsanulok | N16–47 | E100–51 | 150 |
| Um132 | 109677 | *V. umbellata* (wild A) | Thailand | Nan | N18–47 | E100–44 | 420 |
| Um138 | 109679 | *V. umbellata* (wild A) | Thailand | Nan | N18–47 | E100–44 | 420 |
| Um139 | 109676 | *V. umbellata* (wild A) | Thailand | Nan | N18–47 | E100–44 | 420 |
| Um140 | 109678 | *V. umbellata* (wild B) | Thailand | Nan | N18–47 | E100–44 | 420 |
| Um086 | 108544 | *V. umbellata* (cult.) | Thailand | Lampang | N18–29 | E99–30 | 370 |
| Um087 | 108545 | *V. umbellata* (cult.) | Thailand | Lampang | N18–29 | E99–31 | 371 |
| Um114 | 108550 | *V. umbellata* (cult.) | Thailand | Chiang Rai | N19–11 | E99–30 | 620 |
| Um115 | 105863 | *V. umbellata* (cult.) | Thailand | Mae Hong Son | N18–18 | E97–56 | 750 |
| Um116 | 105883 | *V. umbellata* (cult.) | Thailand | Loei (seed dealer) | N17–22 | E101–16 | 410 |
| Um117 | 105885 | *V. umbellata* (cult.) | Thailand | Loei | N17–22 | E101–16 | 650 |
| Um118 | 105870 | *V. umbellata* (cult.) | Thailand | Chiang Mai | N19–05 | E98–51 | 420 |
| Um121 | 105880 | *V. umbellata* (cult.) | Thailand | Loei | N17–34 | E100–54 | 710 |
| Um122 | 105855 | *V. umbellata* (cult.) | Thailand | Uthaitani (Uthaitani market) | N15–22 | E100–01 | 80 |
| Um123 | 105859 | *V. umbellata* (cult.) | Thailand | Tak (Mae Sot market) | N16–42 | E98–34 | 400 |
| Um124 | 105875 | *V. umbellata* (cult.) | Thailand | Chiang Rai (Chiang Rai market) | N19–54 | E99–49 | 500 |
| Um126 | 105879 | *V. umbellata* (cult.) | Thailand | Nan (Nan market) | N18–46 | E100–46 | 450 |
| Um127 | 105871 | *V. umbellata* (cult.) | Thailand | Chiang Mai (Chom Thong market) | N18–24 | E98–40 | 360 |
| Um141 | 110837 | *V. umbellata* (cult.) | Thailand | Pha Yao | N19–18 | E100–09 | 240 |
| Um143 | 105825 | *V. umbellata* (cult.) | Thailand | Mae Hong Son (market) | N19–17 | E97–58 | 450 |
| Um145 | 105822 | *V. umbellata* (cult.) | Thailand | Chiang Rai | N20–11 | E99–34 | 420 |
| Um175 | 100311 | *V. umbellata* (cult.) | Nepal | Phabgdwam Pakhribas V.P. | N27–20 | E87–42 | 1580 |
| Um176 | 99485 | *V. umbellata* (cult.) | Japan | Nagasaki | N34–28 | E129–20 | 20 |
| Ex006 | 207983 | *V. exilis* (wild) | Thailand | Kanchanaburi | N14–01 | E99–14 | 110 |
| Ex009 | 205884 | *V. exilis* (wild) | Thailand | Ratchaburi | N13–34 | E99–46 | 150 |
| Ex011 | 210646 | *V. exilis* (wild) | Thailand | Ratchaburi | N13–35 | E99–40 | 130 |
| Ex013 | 210648 | *V. exilis* (wild) | Thailand | Ratchaburi | N13–22 | E99–47 | 105 |
| Ex015 | 210650 | *V. exilis* (wild) | Thailand | Petchaburi | N13–06 | E99–55 | 80 |
| Ex016 | 210651 | *V. exilis* (wild) | Thailand | Petchaburi | N10–33 | E104–42 | 130 |
| Ex018 | 210653 | *V. exilis* (wild) | Thailand | Petchaburi | N12–57 | E99–54 | 110 |
| Ex019 | 210654 | *V. exilis* (wild) | Thailand | Petchaburi | N12–51 | E99–56 | 60 |
| Ex060 | 210684 | *V. exilis* (wild) | Thailand | Sara Buri | N14–38 | E101–09 | 315 |
| Te003 | 205883 | *V. tenuicaulis* (wild) | Thailand | Kanchanaburi | N14–42 | E98–28 | 662 |
| Hi021 | 205885 | *V. hirtella* (wild) | Thailand | Nakhon Ratchasima | N14–25 | E101–25 | 750 |
| Te042 | 210671 | *V. tenuicaulis* (wild) | Thailand | Nan | N19–05 | E101–09 | 625 |
| Te091 | 108551 | *V. tenuicaulis* (wild) | Thailand | Chiang Mai | N19–07 | E98–41 | 760 |
| Te092 | 108552 | *V. tenuicaulis* (wild) | Thailand | Chiang Mai | N19–07 | E98–41 | 760 |

*Appendix 1*. Continued.

| Code | JP no.[a] | Species name (status) | Origin | Province | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|---|---|---|
| Te093 | 108553 | *V. tenuicaulis* (wild) | Thailand | Chiang Mai | N19–07 | E98–41 | 760 |
| Te095 | 108555 | *V. tenuicaulis* (wild) | Thailand | Chiang Mai | N19–15 | E98–36 | 1365 |
| Hi101 | 108562 | *V. hirtella* (wild) | Thailand | Mae Hong Son | N19–26 | E98–24 | 1085 |
| Hi105 | 108566 | *V. hirtella* (wild) | Thailand | Chiang Mai | N19–33 | E100–06 | 535 |
| Hi111 | 108515 | *V. hirtella* (wild) | Thailand | Chiang Rai | N20–18 | E99–50 | 1075 |
| Hi112 | 108542 | *V. hirtella* (wild) | Thailand | Chiang Rai | N20–19 | E99–50 | 1130 |
| Te160 | 109682 | *V. tenuicaulis* (wild) | Thailand | Chiang Rai | N19–52 | E99–49 | 460 |
| Te161 | 217444 | *V. tenuicaulis* (wild) | Myanmar | Shan | N22–60 | E97–46 | 735 |
| Te162 | 217486 | *V. tenuicaulis* (wild) | Myanmar | Chin | N22–55 | E93–40 | 1590 |
| Hi169 | 217435 | *V. hirtella* (wild) | Myanmar | Shan | N20–43 | E97–03 | 1360 |
| Hi170 | 217491 | *V. hirtella* (wild) | Myanmar | Chin | N23–23 | E93–39 | 1567 |
| Hi171 | 109681 | *V. hirtella* (wild) | Thailand | Chiang Mai | N19–04 | E99–23 | 530 |
| Mi020 | 210655 | *V. minima* (wild) | Thailand | Sara Buri | N14–26 | E100–54 | 90 |
| Mi038 | 210669 | *V. minima* (wild) | Thailand | Nan | N18–03 | E100–57 | 340 |
| Mi041 | 205886 | *V. minima* (wild) | Thailand | Nan | N19–05 | E101–09 | 625 |
| Mi044 | 205888 | *V. minima* (wild) | Thailand | Nan | N19–07 | E101–09 | 850 |
| Mi056 | 205890 | *V. minima* (wild) | Thailand | Sakhon Nakhon | N16–58 | E103–59 | 310 |
| Mi057 | 210682 | *V. minima* (wild) | Thailand | Mukdahan | N16–50 | E104–08 | 305 |
| Mi058 | 210683 | *V. minima* (wild) | Thailand | Mukdahan | N16–19 | E104–31 | 235 |
| Mi059 | 205891 | *V. minima* (wild) | Thailand | Surin | N14–27 | E103–42 | 305 |
| Mi104 | 101829 | *V. minima* (wild) | Thailand | Chiang Mai | N19–33 | E99–06 | 535 |
| Mi107 | 108568 | *V. minima* (wild) | Thailand | Chiang Mai | N19–37 | E99–09 | 665 |
| Mi166 | 210806 | *V. minima* (wild) | Myanmar | Kalaw | N20–39 | E96–33 | 1230 |
| Mi168 | 210824 | *V. minima* (wild) | Myanmar | Pa-an | N16–51 | E97–41 | 15 |
| MiDKL | 107869 | *V. minima* (wild) | Thailand | Chai Nat | N15–15 | E99–59 | 150 |
| Set 2. genus *Ceratotropis* section *Ceratotropis, V. trinervia* and *V. unguiculata* | | | | | | | |
| Tr022 | 210657 | *V. trinervia* (wild) | Thailand | Chantaburi | N12–52 | E102–16 | 250 |
| Tr023 | 210658 | *V. trinervia* (wild) | Thailand | Chantaburi | N12–52 | E102–16 | 250 |
| Tr028 | 210663 | *V. trinervia* (wild) | Thailand | Rayong | N12–48 | E101–28 | 150 |
| Tr090 | 108130 | *V. trinervia* (wild) | Thailand | Chiang Mai | N19–02 | E99–18 | 545 |
| Tr163 | 108840 | *V. trinervia* (wild) | Malaysia | Pahang | N04–03 | E102–18 | 50 |
| Mu110 | 1085123 | *V. mungo* (wild) | Thailand | Chiang Rai | N20–17 | E100–01 | 410 |
| Mu146 | 219130 | *V. mungo* (wild) | Thailand | Chiang Mai | N19–23 | E98–59 | 450 |
| Mu147 | 107878 | *V. mungo* (wild) | Thailand | Chiang Mai | N19–07 | E98–46 | 520 |
| Mu148 | 218939 | *V. mungo* (wild) | Thailand | Chiang Mai | N19–50 | E99–11 | 640 |
| Mu149 | 219131 | *V. mungo* (wild) | Thailand | Chiang Mai | N20–01 | E99–17 | 460 |
| Mu172 | 212357 | *V. mungo* (cult.) | India |  | – | – | – |
| Mu173 | 212358 | *V. mungo* (cult.) | Australia |  | – | – | – |
| Mu174 | 212362 | *V. mungo* (cult.) | India |  | – | – | – |
| Mu177 | 219132 | *V. mungo* (cult.) | Thailand | Breeding line (mutant) | – | – | – |
| Mu178 | 106710 | *V. mungo* (cult.) | Thailand | Breeding line | – | – | – |
| Mu179 | 109668 | *V. mungo* (cult.) | Thailand | Phetchabun | N16–17 | E101–04 | 160 |
| Mu184 | 107874 | *V. mungo* (wild) | India |  | – | – | – |
| Mu185 | 107873 | *V. mungo* (wild) | India |  | – | – | – |
| Gr029 | 207984 | *V. grandiflora* (wild) | Thailand | Chai Nat | N15–11 | E100–06 | 46 |
| Gr061 | 108509 | *V. grandiflora* (wild) | Thailand | Nakhon Sawan | N15–34 | E100–03 | 50 |
| GrBan | 107862 | *V. grandiflora* (wild) | Thailand | Phitsanulok | N16–45 | E100–07 | 260 |
| Ra182 | 110830 | *V. radiata* (cult.) | Thailand | Released variety | – | – | – |
| Un051 | 210678 | *V. unguiculata* (wild) | Thailand | Phetchabun | N16–25 | E101–11 | 165 |
| Un190 | 105881 | *V. unguiculata* (cult.) | Thailand | Loei | N17–35 | E100–53 | 450 |

[a] JP no. is the number of the accession in the Genebank of the Ministry of Agriculture, Forestry and Fisheries, Japan.

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