アジア原産ササゲ属マメ科植物のネオ・ドメスティケーションとケツルアズキの多器官大型化突然変異
Neo-Domestication of *Vigna* (Leguminosae) Species and Identification of a *Multiple Organ Gigantism (mog)* Mutant of Cultivated Black Gram (*Vigna mungo*)

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**Introduction**

*Vigna* is a pan-tropical genus in the Leguminosae family. Nine wild species have been domesticated from 98 species in the genus *Vigna* (MAXTED et al., 2004). The genus *Vigna* is subdivided into 6 subgenera: subgenera *Vigna* and *Haydonia* in Africa, *Plectotropis* in Eurasia, *Ceratotropis* in Asia, and *Sigmoidotropis* and *Lasiospron* in America (Fig. 1). Among the 9 domesticated *Vigna* species, 2 (cowpea [*V. unguiculata*] and bambara groundnut [*V. subterranea*]) belong to the subgenus *Vigna*, 1 (tuber cowpea [*V. vexillata*]) belongs to the subgenus *Plectotropis*, and 6 (mungbean [*V. radiata*], black gram [*V. mungo*], moth bean [*V. aconitifolia*], rice bean [*V. umbellata*], azuki bean [*V. angularis*], and creole bean [*V. reflexa-pilosai*]) belong to the subgenus *Ceratotropis*.

The subgenus *Ceratotropis* is a morphologically homogeneous taxonomic group that consists of 21 species (TOMOOKA et al., 2002). This subgenus is also referred to as the Asian *Vigna* based on its natural distribution. Multiple and independent domestication events make the Asian *Vigna* species unique and interesting materials for studies of domestication genetics (TOMOOKA et al., 2006). In the present paper, we compare the genetic changes accumulated during the process of domestication using 4 economically important Asian *Vigna* crops: mungbean, black gram, rice bean, and azuki bean.

**Comparison of quantitative trait loci (QTLs) for domestication among 4 Asian *Vigna* crops**

Numerous common differences in morphological and physiological traits associated with domestication are observed between the domesticated and wild forms of different species. These differences, collectively called the domestication syndrome, result from selection over several thousands of years of adaptation to cultivated environments, human nutritional requirements, and human preferences (HAWKES, 1983). The 4 cultivated species mungbean, black gram, rice bean and azuki bean are ideal materials for improving our understanding of the gene evolution related to domestication among *Vigna* species and for characterizing useful traits as QTLs for use in breeding.

![Fig. 1. Relationships among Phaseolus, African Vigna (subgenus Vigna), Eurasian Vigna (subgenus Plectotropis), and Asian Vigna (subgenus Ceratotropis) (after TOMOOKA et al., 2010).](image-url)
also reported several QTLs for domestication-related traits of azuki bean (ISEMURA et al., 2007, KAGA et al., 2008), rice bean (ISEMURA et al., 2010a, 2010b) and mungbean (ISEMURA et al., 2012) in populations derived from crosses between cultivated and wild forms. The order of common markers in the linkage groups (LGs) was highly conserved between the 4 species.

Among the domestication syndrome traits, we compared the QTLs for 3 important traits in the present study: seed size (100-seed weight), pod dehiscence, and water absorption by seeds (Fig. 2). We found several differences in the genetics of domestication of these closely related Asian Vigna crops.

First, the number of QTLs detected differed among traits and among species. A total of 5 to 7 QTLs per species were detected for seed size increase, but only 1 to 3 QTLs were detected for pod dehiscence (Fig. 2). For water absorption by seeds, 2 to 5 QTLs were detected.

Second, the percentages of common QTLs (red ovals) and species-specific QTLs (green ovals) differed among the 3 domestication traits (Fig. 2). For seed size increase, a total of 16 loci were detected in the 4 species; among these 16 loci, 6 (37.5%) were common among 2 or more species. In particular, the QTL detected in linkage group 2 (LG2) was associated with seed size increase in all 4 species. For pod dehiscence, a total of 4 loci were detected among the 4 species. Among these 4 loci, one QTL in LG7 was found in azuki bean, rice bean, and mungbean but not in black gram. In contrast, a pod dehiscence QTL located in LG5 was detected only in black gram. Only 1 pod dehiscence QTL was detected in azuki bean, rice bean and black gram, but 3 were found in mungbean. Interestingly and unexpectedly, no common QTLs were detected for water absorp-
tion by seeds among the 13 loci detected.

Third, several of the QTLs showed effects opposite those that would be expected based on the parental phenotypes (blue ovals, Fig. 2). For example, the cultivated-parent allele in LG6 of mungbean was associated with pod dehiscence in spite of the fact that the pods of the cultivated parent showed phenotypically lower pod dehiscence than those of the wild parent. Likewise, the wild-parent alleles in LG6 of azuki bean and in LG2 and LG10 of rice bean facilitated water absorption by seeds, contrary to the expectation based on the phenotypic evaluations of the parents.

**MOG (Multiple Organ Gigantism) phenotype of cultivated black gram**

Black gram (*V. mungo*) is an ancient food legume that was domesticated in India (Fuller, 2007). Seeds of black gram are exported to Japan from Southeast Asia to produce bean sprouts. Thailand is one of the exporting countries, and has been conducting a black gram breeding program at the Chai Nat Field Crops Research Center. To develop hairless and large-seeded cultivars, mutation breeding using gamma rays was applied to the promising breeding line ‘BC48’ (later released as a registered cultivar in Thailand, ‘Phitsanulok 2’). As a result, a mutant line which has larger seeds, pods, and vegetative organs was generated (Fig. 3; Chinchest and Nakeeraks, 1991). Based on its prominent gigantism effects on multiple organs, the line was named ‘MOG’ (Multiple Organ Gigantism).

MOG showed higher biomass production than BC48 under a 12-hour day length (Table 1). The total leaf area and total leaf dry weight of MOG were significantly higher than those of BC48 at 20, 40, and 80 DAP. The total stem dry weight and total dry weight of MOG were significantly higher than those of BC48 at 20 and 40 DAP, but not significantly different at 80 DAP. The maturity of MOG was slightly later than that of BC48. At 90 DAP, MOG plants had a mean of 36 immature pods and 47 mature pods (43% immature pods), whereas BC48 plants had a mean of 12 immature pods and 62 mature pods (16% immature pods). The mean number of mature seeds per plant at 90 DAP was 226.5 for MOG and 397.5 for BC48. The mean number of mature seeds per pod was 4.8 for MOG and 6.4 for BC48. Since the 100-seed weight of MOG (7.9 g) was higher than that of BC48 (4.7 g), total seed weight per plant at 90 DAP was not significantly different between MOG (17.9 g) and BC48 (18.6 g). Since a higher percentage of immature pods remained on MOG than on BC48 at 90 DAP, the potential seed yield per plant will be higher for MOG if the growth duration is longer than 90 days.

**Domestication, super-domestication and neo-domestication**

A locus (*mog*) linked to the MOG phenotype, which behaves as a single-gene recessive trait, was mapped to the upper part of LG8 (Fig. 2). The 100-seed
Table 1. Comparison of growth traits between BC48 and MOG plants under 12 hrs day length

<table>
<thead>
<tr>
<th>DAP^1</th>
<th>Traits</th>
<th>Short day (12 hrs)</th>
<th>BC48 (%)</th>
<th>MOG (%)</th>
<th>P^2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Stem length (cm)</td>
<td></td>
<td>18.6</td>
<td>100</td>
<td>24.2</td>
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<tr>
<td>20 days</td>
<td>No. of nodes</td>
<td></td>
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<td>100</td>
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</tr>
<tr>
<td></td>
<td>Stem DW (g)</td>
<td></td>
<td>0.1</td>
<td>100</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Leaf DW (g)</td>
<td></td>
<td>0.3</td>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Total DW (g)</td>
<td></td>
<td>0.4</td>
<td>100</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Stem length (cm)</td>
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<td>97.1</td>
<td>100</td>
<td>131.0</td>
</tr>
<tr>
<td>40 days</td>
<td>No. of nodes</td>
<td></td>
<td>10.8</td>
<td>100</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>Leaf area (cm^2)</td>
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<td>1368.2</td>
<td>100</td>
<td>2115.9</td>
</tr>
<tr>
<td></td>
<td>Stem DW (g)</td>
<td></td>
<td>2.1</td>
<td>100</td>
<td>2.7</td>
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<tr>
<td></td>
<td>Leaf DW (g)</td>
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<td>3.0</td>
<td>100</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Flower + immature pod</td>
<td></td>
<td>0.4</td>
<td>100</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Total DW (g)</td>
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<td>125.5</td>
<td>100</td>
<td>134.8</td>
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<td></td>
<td>Stem length (cm)</td>
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<td>127.9</td>
<td>100</td>
<td>2205.9</td>
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<td>80 days</td>
<td>No. of nodes</td>
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<td></td>
<td>No. of branches</td>
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<td>100</td>
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<td>Leaf area (cm^2)</td>
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<td>100</td>
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<td>0.4</td>
<td>100</td>
<td>0.1</td>
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<td>Total DW (g)</td>
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<tr>
<td></td>
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<td>3.6</td>
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<td>DW (g)</td>
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<td>15.9</td>
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<td>Total DW (g)</td>
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<td>30.5</td>
<td>100</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>No. of mature pods</td>
<td></td>
<td>62.0</td>
<td>100</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>No. of seeds/pod</td>
<td></td>
<td>6.4</td>
<td>100</td>
<td>4.8</td>
</tr>
<tr>
<td>90 days</td>
<td>No. of mature seeds</td>
<td></td>
<td>397.5</td>
<td>100</td>
<td>226.5</td>
</tr>
<tr>
<td></td>
<td>Total seed weight (g)</td>
<td></td>
<td>18.6</td>
<td>100</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td>100 seed wt (g)</td>
<td></td>
<td>4.7</td>
<td>100</td>
<td>7.9</td>
</tr>
</tbody>
</table>

BC48: A black gram breeding line released as recommended cultivar "Phitsanulok 2" in Thailand
MOG: A mutant line of BC48 produced by irradiation of y ray and shows Multiple Organ Gigantism
1. DAP: Days After Planting
2. DW: Dry Weight
3. Significance level:
   **: at 1% level; *: at 5% level; n.s.: not significant, based on the analysis of variance

weight of black gram cultivar BC48 was 4.7 g, which is 5.2 times that of the wild parent (0.9 g). In azuki bean, the 100-seed weight of the domesticated parent 'Kyoto Dainagon' was 24.0 g, 9.6 times that of the wild parent (2.5 g). In rice bean, the 100-seed weight of the domesticated parent was 27.4 g, 15.2 times that of the wild parent (1.8 g). In mungbean, the 100-seed weight of the domesticated parent was 6.9 g, 5.8 times that of the wild parent (1.2 g). In spite of the fact that the domesticated azuki bean, rice bean, and mungbean have larger seeds than domesticated black gram, a mutant mog gene gene does not appear to be responsible for the larger seed size in these three Vigna crops because no QTL at the location of mog was detected in the map-
NEO-DOMESTICATION OF VIGNA AND IDENTIFICATION OF MOG MUTANT

ping populations. Therefore, if the recessive mog allele can be introduced into these crops, or derived by mutation, further seed size increase could be expected.

Among the 4 Asian Vigna crops compared here, fertile F1 plants can be obtained between azuki bean and rice bean, and between mungbean and black gram. If the rice bean cultivar QTL allele for 100-seed weight detected in LG4 could be introduced into azuki bean, development of a novel azuki bean cultivar having larger seeds than Kyoto Dainagon might be possible. Kyoto Dainagon is a very popular cultivar because of its large seed size and therefore commands a high market price. Low water absorption by seeds sometimes decreases the quality of azuki bean paste; thus, there is demand for an azuki bean cultivar having higher seed water permeability. For this purpose, the rice bean cultivar allele for water absorption by seeds in LG4 could be used for azuki bean breeding.

For mungbean, demand for cultivars suitable for machine harvesting is high. The black gram cultivar-specific QTL allele for decreasing pod dehiscence in LG5 could be incorporated into mungbean for this purpose. These approaches to detect species-specific domestication QTL alleles by comparative genomic studies of closely related crop species and use them to develop new cultivars of related species could be called “super-domestication”, as proposed by VAUGHAN et al. (2007).

We are planning to isolate several of the domestication QTLs (including the mog gene) described in the present study by map-based cloning. Recent studies have revealed that most of the useful phenotypic changes accumulated during domestication are based on loss-of-function mutations (DOEBLEY et al., 2006). Such mutations have already proved to be useful for agriculture and can be induced by gamma radiation or chemical mutagen treatment. Therefore, once the sequence of the target gene becomes available, screening for mutations in that gene could be conducted by reverse-genetics methods such as TILLING (TILL et al., 2003). Hence, these useful mutations can be applied to related species even though there is no cross-compatibility and, more importantly, without using genetic transformation.

Using this strategy, it should be possible to incorporate basic phenotypic changes that occurred during domestication such as seed size increase, low pod dehiscence, and high water absorption by seeds into non-domesticated wild species. In other words, “neo-domestication” should be possible within a relatively short time span. In the genus Vigna, there are many wild species that are adapted to marginal land with stressful environments. In some of these marginal land areas, wild Vigna species are used as an occasional food for humans. For example, Vigna marina, which grows on tropical seaside beaches, can grow in soils with NaCl concentrations of 400 mM, while Vigna trilobata, which grows in sandy soils under drought conditions, develops a long tap root; both species are reported to be used as human food (TOMOOKA et al., 2011).

Over many years, attempts to develop stress-resistant cultivars by using stress-resistance genes from wild species have met with little success. This failure is mainly because the mechanisms of physiological adaptation to stressful environments are complex and therefore cannot be achieved by incorporating only a few genes. Compared with genetic adaptations to stressful environments, the genetic changes that have occurred during the domestication process are relatively simple. We anticipate that by changing a few domestication genes, wild Vigna species could be domesticated to the primitive-cultivar level. To develop crops for sustainable use under marginal conditions, “neo-domestication” of wild species using domestication genes might be a better choice than attempting to transfer stress-tolerance genes into cultivated species.

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アジア原産ササゲ属マメ科植物のネオ・ドメスティケーションと
ケツルアズキの多器官大型化突然変異

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我々は、マメ科作物とその近縁野生種を対象に、進化と栽培化の過程で生み出された遺伝的多様性を解明し、それを利用することを目的に研究を進めている。

本稿では、アジアにおいて独立に栽培化が進行した4種のササゲ（Vigna）属マメ科作物であるアズキ、ツルアズキ、リョクトウおよびケツルアズキの栽培化に伴う遺伝変異について考察を行う。栽培化の過程で、これらの作物は、多くの器官とくに種子の大型化、裂果性と種子休眠性の消失という共通の形質変異を獲得し、人間による栽培に適した作物となった。そこで、これら共通の形質変異をもたらした遺伝的背景を、QTL解析を行うことによって明らかにし、検出したQTLの種間比較を行った。その結果、共通形質獲得の遺伝的背景には、種を超えて共通して利用された遺伝変異と、ある種の栽培化においてのみ利用された遺伝変異が存在し、その数と割合は形質によって大きく異なることが明らかになった。検出したQTLのうち、形質変化への寄与率が高いものに関して、現在遺伝子の単離に向けた解析を進めている。

ケツルアズキにおいては、栽培化で大型化した栽培品種へのガンマー線照射によって、さらに多器官が顕著に大型化した突然変異体（MOG: Multiple Organs Gigantism）が得られている。この突然変異遺伝子のマッピングを行った結果、本突然変異は、近縁種の栽培化過程における器官大型化には用いられなかった突然変異であることが明らかになった。本突然変異は、種子やその他の器官を約2倍に大型化する極めて作用力が大なる突然変異であり、しかも種子稔性の低下がほとんど見られないことから、産業利用上有望な遺伝子であると考えられ、単離に向けた解析を行っている。

これらの研究で明らかになってきた、近縁作物では利用されなかった栽培化関連有用変異をもたらす遺伝子が単離できれば、交雑親和性がない種においても、化学物質や放射線照射によって誘起された変異体を用いたTILLINGによるスクリーニングによって効率的に有用変異体を獲得し、作物のさらなる改良を行ったり（Super-domestication）、限界環境に適応した野生種を新規に作物化（Neo-domestication）したりできるようになると期待している。