การฟื้นฟูเชื้อพันธุกรรมถั่วในสกุล *Vigna* ในประเทศไทย

Regeneration of *Vigna* subgenus *Ceratotropis* collections in Thailand

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บทคัดย่อ: โครงการวิจัยนี้มีวัตถุประสงค์เพื่อฟื้นฟูและเก็บรักษาเชื้อพันธุกรรมถั่วในสกุล *Vigna* จำนวน 750 พันธุ์/สายพันธุ์ ดำเนินการระหว่างปี 2552-2553 โดยปลูกฟื้นฟูและผลิตเชื้อพันธุ์รุ่นใหม่ของพืชสกุล *Vigna* ซึ่งได้แก่ ถั่วเขียว (*Vigna radiata*) ถั่วเขียวผิวดำ (*V. mungo*) ถั่วป่าในสกุลใกล้เคียง (*Vigna spp.*) ดำเนินงานที่ศูนย์วิจัยพืชไร่ชัยนาท และถั่วพุ่ม (*V. unguiculata*) ดำเนินงานที่ศูนย์วิจัยพืชไร่อุบลราชธานี บันทึกข้อมูลลักษณะทางสัณฐานวิทยา ลักษณะทางการเกษตร รวมทั้งถ่ายภาพของพืชในขั้นตอนการเจริญเติบโตต่างๆ เพื่อจัดทำเป็นฐานข้อมูล ผลการดำเนินงานในปี 2552-2553 ทำการปลูกฟื้นฟู จำนวน 774 พันธุ์/สายพันธุ์ และสามารถเก็บเมล็ดพันธุ์ได้จำนวน 734 พันธุ์/สายพันธุ์ เมล็ดพันธุ์ที่ฟื้นฟูเรียบร้อยแล้วนำเข้าเก็บรักษาที่ธนาคารเชื้อพันธุ์พืชของกรมวิชาการเกษตรเพื่อการอนุรักษ์ และเก็บไว้ที่ศูนย์วิจัยฯ สำหรับใช้ประโยชน์ในการปรับปรุงพันธุ์ ส่วนหนึ่งส่งไปเก็บไว้แบบถาวรที่ Svalbord Seed Vault ซึ่งเป็นศูนย์เก็บรักษาเมล็ดพันธุ์พืชแห่งใหม่ของโลก และอีกส่วนหนึ่งเก็บไว้ที่ธนาคารเชื้อพันธุ์ในระดับนานาชาติ (AVRDC) โดยคาดว่าจะเสร็จเรียบร้อยภายในเดือนพฤศจิกายน 2554

คำสำคัญ: ถั่วเขียว, ถั่วเขียวผิวดำ, ถั่วพุ่ม, ถั่วป่าในสกุล *Vigna*, เชื้อพันธุกรรม

ABSTRACT: The implementation of this project was carried to regenerate and safety duplicate 750 accessions of *Vigna* species in 2009-2010. Mungbean (*Vigna radiata*) blackgram (*V. mungo*) and wild *Vigna* spp. were regenerated at Chai Nat Field Crops Research Centre and cowpea (*V. unguiculata*) was examined at Ubon Field Crops Research Centre Thailand. Morphological characteristics, preliminary evaluation data, agronomic evaluation data and photographs were recorded. A total target of *Vigna* species to be regenerated was 750 accessions. In summary for two years, 774 accessions were grown but only 734 accessions could obtain the seed for further research and safety duplication. All accessions were already deposited at the Thai-Department of Agriculture Genebank. The process of safety duplication at AVRDC and Svalbord Seed Vault expected to be finished and shipped sometime around November 2011.

Keywords: mungbean, blackgram, cowpea, wild *Vigna*, genetic resources

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Introduction

Plant genetic resources for food and agriculture in Thailand are divided into four crop categories (field crops, horticultural crops, para rubber and rice). Field crops consisting of cassava, sugarcane, maize, soybean, mungbean, related *Vigna* species, sesame, sunflower and cotton are under the responsibility of the Field Crop Research Institute. Horticultural crops including fruits, vegetables, ornamentals, and herbal and medicinal crops are under the responsibility of the Horticultural Research Institute. Para rubber is under the responsibility of the Para Rubber Research Institute and Rice is under the responsibility of the Rice Department.

The research on the regeneration of *Vigna* species is the ongoing project in Thailand. The Department of Agriculture (DOA) has recently cooperated with FAO in the implementation of the Global Plan of Action (GPA) and *in situ* conservation and characterization of *Vigna* species subgenus *Ceratotropis*. The success of the initial phases of the project led to the next project of regeneration and safe duplication of priority crop collections supported by the Global Crop Diversity Trust.

In this project, *Vigna* species were chosen because the species are at the risk for genetic erosion and extinction due mainly to human disturbance such as slash-and-burn cultivation, highway expansion and deforestation. Pests and natural disasters (mud slide) are also the causes. Moreover, the genebank is under genetic erosion caused by low seed viability and the number of accessions is greatly reduced because of no regeneration for more than 10 years.

During 2005-2006, the mission for collection of *Vigna* germplasm conducted 65 surveys for *in situ* conservation sites of wild *Vigna* and collected 66 samples. The *Vigna* species were available in only 24 sites of 65 sites (7 were new sites and 17 were previously reported) (Veerana et al., 2007).

In view of *in situ* conservation of field crops in Thailand, *Vigna* has been recorded and publicized more than other species, but the information is still not complete. Several surveys had been carried out accordingly. For example, Lairungreang et al. (1991) conducted a survey in 1989 in the central and northern part of Thailand. A survey along the border lines of Thailand and Myanmar, Laos and China was conducted in collaboration with a Japanese Research Organization (TARC) (Pichitporn et al., 1993). Some more surveys were carried out in the central and northern part of Thailand, covering nine provinces namely Chai Nat, Pichit, Pitsanulok, Sukhothai, Tak, Lampang, Chiang Mai, Mae Hong Son and Chiang Rai (Tomooka et al., 1997). Ngampongsai et al. (1999) also conducted a survey and collected *V. umbellata* var. *gracilis* in Tak, Kanchanaburi and *V. unguiculata* in Chiang Rai. Another survey was also carried out to search for wild *Vigna* species that may contain a gene of high temperature tolerance in order to transfer it to adzuki bean (Tomooka et al., 2000).

This research is part of the project for conservation and utilization of field crop genetic resources of the Thai Department of Agriculture and the budget is partly supported by the Global Crop Diversity Trust of FAO. We concern for regional and international cooperation in conservation, regeneration, safe duplication and utilization of field crops genetic resources.

National policy emphasises on plant breeding programme, supports from international organization (like Crop Trust) might lead a light to policy makers to realize the equally importance of pre-breeding research in plant genetic resources. The objectives of the project were to regenerate, characterize and evaluate genetic resources of *Vigna* accessions under threats, accessions from poor viability or under long term conservation in genebank and enhance cooperation in safety duplication of *Vigna* accessions at regional and international levels.

Materials and Methods

The implementation of the project was commenced in March 2009, and the target was to regenerate and safely duplicate 750 accessions of *Vigna* species. Mungbean (*Vigna radiata*), blackgram (*V. mungo*) and wild *Vigna* spp. were regenerated at the Chai Nat Field Crops Research Centre, and cowpea (*V. unguiculata*) was regenerated at the Ubon Ratchathani Field Crops Research Centre in Thailand. Morphological characteristics, preliminary data, agronomic data and photographs were recorded using mungbean descriptors described by IPGRI and the Asian *Vigna*: Genus *Vigna* subgenus *Ceratotropis* genetic resources (Tomooka et al., 2002).

In May 2009, 150 accessions of mungbean were regenerated in a planting area of 2x5 m^2 for each accession. The seeds of 135 accessions were collected. One hundred accessions of blackgram (*V. mungo*) were regenerated in the late rainy season 2009 and the seeds of all accessions were obtained.

One hundred and two accessions of wild *Vigna* species were grown in pots in late September 2009. Some accessions were obtained from the surveys during 2005-2006 in Thailand, and some accessions were introduced from overseas. Wild *Vigna* species are photosensitive and could produce seeds only once a year between late October and late March. They are also an indeterminate type and only 94 accessions could be harvested.

Eighty eight accessions of cowpea (*V. unguiculata*) were planted in April 2009 and only 78 accessions could be regenerated and harvested in June 2009. Sixty-eight accessions were for pulses and ten accessions were for fresh consumption. These accessions were kindly provided by the International Institute of Tropical Agriculture (IITA), the International Rice Research Institute (IRRI), and Khon Kaen University. The total number of 435 accessions of *Vigna* species was regenerated, 407 accessions were harvested and 28 accessions yield no seed.

In 2010, 239 accessions of *V. radiata* were grown in the early rainy season, the late rainy season and the dry season (with irrigation). All accessions could be regenerated successfully. One-hundred accessions of wild species were also regenerated in August 2010.

Results and Discussion

Evaluation of mungbean germplasm between 2009 and 2010

Variations among the accessions of mungbean were observed for morphological characteristics and agronomic traits. For example, shapes of terminal leaflets varied from ovate to

ovate-lanceolate. Petal colour, the typical characteristic of the subgenus *Ceratotropis*, varied from light to dark yellow. The numbers of days to 50% harvest varied between 36 to 58 days (average 42±4 days). For agronomic traits, the numbers of pods per plant were from 3 to 36 pods (average 12±5 pods). The numbers of seeds per pod varied between 7-19 seeds (average 11±1 seeds/pod). Seed sizes varied between 25.0-89.5 g (average 62.7±12 g/1000 seeds). Seed weights per plant varied between 2.1-41.3 g/plant (average 10.9±7.0 g/plant), whereas total seed yields were from 0.01-2.93 kg (average 0.86±0.69 kg). The morphological characteristics and agronomic traits were then recorded in a database for a sustainable and efficient use in breeding programs **(Table 1, Figure 1 and 2)**.

Evaluation of blackgram (*Vigna mungo*) germplasm for beansprout breeding programs in 2009

Differences among accessions of blackgram were observed for seed yield, seed size, plant height, number of pods per plant, number of seeds per pod, days to flowering, days to maturity and morphological characters. Seed yield per plant was 4.7-37.9 g (average 14.6±6.97 g), whereas seed yield per plot (5 m^2) varied between 139.7-1135.6 g (average 439.3±209.1 g). Seed size varied from 30-57 g (average 45.7±5.5 g/1000 seeds). Plant height ranged from 63-162 cm. (average 118±20 cm.) and the number of pod/plant was 17-103 pods (average 47±20.4 pods). Number of seed/pod was 6-8 seeds (average 7±0.5 seed). Days to 50% flowering was 32-87 days (average 63±13 days), whereas the maturity ranged from 63-118 days (average 94±13 days). Shape of terminal leaflets of most accessions (98 accessions) was deltate, and petal colour of most accessions (82 accessions) was yellow, whereas remaining accessions had greenish yellow petals. Dark brown colour of mature pods was found in 49 accessions, black colour of mature pods was found in 31 accessions and the remaining accessions had brown pods, whereas the seed coat colour of all accessions was black. From the database, 20 accessions were selected for beansprout breeding programs **(Table 2, Figure 3 and 4)**.

Evaluation of cowpea germplasm in 2009

The results showed that, given the differences in their own genetics, the morphological characteristics and agronomic traits of cowpea varied dramatically. Sixty-eight accessions of pulse cowpea showed indeterminate growth, 29 accessions showed semi erect growth habit, 32 accessions showed canopy type and 7 showed viny growth habit. Ten accessions of vegetable cowpea showed indeterminate growth, 7 accessions showed viny growth habit and 3 accessions showed erect growth habit. Leaf colours were green and dark green, petal colours varied from purple, dark purple and white. Young pod colours were light green and dark green, whereas mature pod colours were straw-like, light brown and dark brown. Seed colours varied from black (15 accessions), light brown (7 accessions), dark brown or red (26 accessions), white (14 accessions) and varies colour (6 accessions) and seed shapes were kidney and rhomboid.

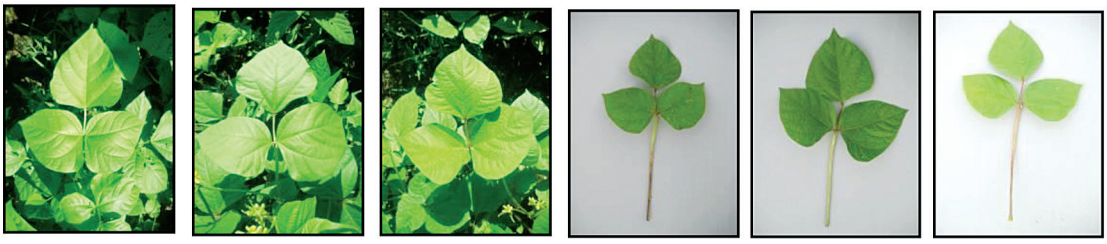
Evaluation of wild *Vigna* spp. germplasm in 2009 and 2010

All accessions of wild *Vigna* species evaluated in 2009 showed viny and indeterminate growth habit, there were differences among accessions for leaf shape, petal colour, days to harvest, and other agronomic traits. The results showed that, given the differences in their own genetics, *in situ* conservation sites, soil type, microclimate and imposed threats, the morphological characteristics and agronomic traits of wild *Vigna* species varied dramatically. Petal colours, the typical characteristic of the subgenus *Ceratotropis*, varied from light to dark yellow. Days to first harvest varied between 49 to 122 days (average 77±18 days). For agronomic traits, number of pods per plant was from 8 to 175 pods (average 62±42 pods). Number of seeds per pod varied between 5-14 seeds (average 9±2 seeds/pod). Seed sizes varied between 5-82 g (average 24±14 g/1000 seeds). Seed weights per plant varied between 0.5-23 g/plant (average 8.7±6.2 g/plant), whereas total seed yield was from 0.5-67.8 g (average 26±18.6 g). The morphological characteristics and agronomic traits were then recorded in a database for a sustainable and efficient use in breeding programs (Table 3, Figure 5 and 6).

Eighty eight accessions of wild *Vigna* species evaluated in 2010 had viny and indeterminate growth habit. Similar to those in 2009, the accessions were different for morphological characteristics and agronomic traits. Shapes of terminal leaflet shapes could be cuneate, ovate-lanceolate, ovate or deltoid. Petal colours varied from yellow to greenish yellow. Days to first maturity varied between 65 to 238 days (average 132±21 days). The late maturing genotypes (238-day maturity) were harvested on 28 March 2011. Unexpected rain storm in March 2010 caused severe losses to the experiment. Twelve accessions were completely damaged and yielded no seed. The accessions had 21 pods per plant to 691 pods per plant (average 290±173 pods), showing high variation for this trait. High variation was also found for number of seeds per pods, ranging from 3 to 10 seeds (average 8±1 seeds/pod). The smallest seed size was 8.8 g/1000 seeds, whereas the largest seed size was 108 g/1,000 seeds (average 29.6±20.8 g/1,000 seeds). Seed weights ranged between 4.9-96.1 g/plant (average 42.3±22.4 g/plant). The morphological characteristics and agronomic traits were then recorded in a database for a sustainable and efficient use in breeding programs.

Table 1 Range (min-max) and average±SD of morphological characteristics and agronomic traits of 374 accessions of mungbean evaluated at Chai Nat Field Crops Research Centre in 2009-2010.

No	Morphological characteristics and agronomic traits	Min-Max (average±SD)
1	Terminal leaflet length (cm)	6.8-16.8(10.1±1.5)
2	Days to 50% first flowering (DAE)	36 - 58 (42±4)
3	Days to harvest (DAE)	53 - 81 (61±7)
4	Plant height (cm)	20.8-96.4(54.4±13.7)
5	Number of pod/plant (pods)	3 - 36 (12±5)
6	Pod length (cm)	6.4-17.3(9.7±1.3)
7	Number of seed/pod (seeds)	7-19(11±1)
8	Seed size ; 1000 Seed weight (g)	25.0 - 89.5 (62.7±12)
9	Seed yieldl/plant (g)	2.1 - 41.3 (10.9±7.0)
10	Germination (%)	55.5-99.0(92.7±5.1)



a) Terminal leaflet shape: deltoid and ovate lanceolate

b) Petal colour : light yellow and dark yellow

Figure 1 Morphological characteristics of mungbean grown in 2009 and 2010 at Chai Nat FCRC; a) terminal leaflet shape and b) petal colour.

a) Mature pod color : black, brown and straw

b) Seed coat color : green group 137A, green group 138B, green group N137A, yellow green 146A, yellow green 151A

c) Seed coat luster: shiny and dull

Figure 2 Morphological characteristics of wild Vigna grown in 2009 at Chai Nat FCRC; a) immature pod color, b) mature pod color and c) seed coat color.

Table 2 Range (min-max) and average±SD of morphological characteristics and agronomic traits of 100 accessions of blackgram (*Vigna mungo*) regenerated at Chai Nat Field Crops Research Centre in 2009.

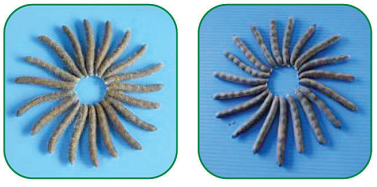
No.	Morphological characteristics and agronomic traits	Min-Max (average±SD)
1	Seed yield/plant (g)	4.7-37.9 (14.6± 6.97)
2	Seed yield/plot (g/5 sq.m)	139.7-1,135 (439.3±209.1)
3	Seed size; 1,000-seed weigth (g)	30-57 (45.7±5.5)
4	Plant height (cm.)	63-162 (118±20)
5	Number of pods/plant	17-103 (47±20.4)
6	Number of seeds/pod	6-8 (7±0.5)
7	Days to 50% flowering (DAE)	32-87 (63±13)
8	Days to harvest (DAE)	63-118 (94±13)

a) Growth type : erect, semi-erect and viny

b) Terminal leaflet shape : deltate and rhombic

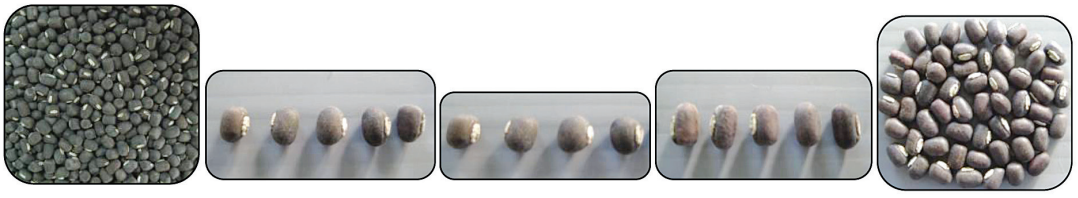
c) Petal colour: yellow, greenish yellow

d) Immature pod colour: light green



e) Mature pod color: dark brown, black

Figure 3 Morphological characteristics of blackgram accessions regenerated in 2009 at Chai Nat FCRC; a) growth type, b) terminal leaflet shape, c) petal colour, d) immature pod color, and e) mature pod colour : dark brown and black



a) Seed coat colour: black b) Seed shape: ovate, rounded and cylindrical c) Hilum shape: non concave

Figure 4 Morphological characteristics of blackgram accessions regenerated in 2009 at Chai Nat FCRC; a) Seed coat colour, b) Seed shape and c) Hilum shape.

Table 3 Range (min-max) and average±SD of morphological characteristics and agronomic traits of 94 accessions in 19 species of wild *Vigna* regenerated at CNFCRC 2009.

No	Morphological characteristics and agronomic traits	Min-Max (average±SD)
1	Terminal leaflet length (cm)	1.5-12.7(6.8±2.4)
2	Days to 50% first flowering (DAE)	24 - 95 (53±15)
3	Days to first harvest (DAE)	49 - 119 (72±18)
4	Plant height (cm)	16-200(133±53)
5	Number of pod/plant (pods)	8 - 175 (62±42)
6	Pod length (cm)	2.5-9.3(5±1.5)
7	Number of seed/pod (seeds)	5 - 14 (9±2)
8	Seed size ; 1000-Seed weight (g)	5 - 82 (24±14)
9	Seed yieldl/plant (g)	1 - 23 (8.7±6.2)
10	Total seed yield (g)	0.5-67.8 (26±18.6)

a) Growth type : erect, semi-erect and viny b) Hypocotyl colour: green, purple and greenish purple

c) Terminal leaflet shape: deltoid, ovate, acute, ovate lanceolate, cuneate, lobed

d) Petal colour : light yellow, dark yellow and greenish yellow

Figure 5 Morphological characteristics of wild Vigna grown in 2009 at Chai Nat FCRC; a) growth type, b) hypocotyl colour, c) terminal leaflet shape and d) petal colour.

a) Immature pod color: light green, green and dark green b) Mature pod color: black, brown and straw

c) Seed coat color : red 185 B group, straw 160 B group, dark brown 200 B group, dotted green 143 A group, brown 165 A group and dark red 183 B group.

Figure 6 Morphological characteristics of wild Vigna grown in 2009 at Chai Nat FCRC; a) immature pod color, b) mature pod color and c) seed coat color.

Conclusion

In summary for two years, 774 accessions were grown but only 734 accessions including 374 accessions of mungbean, 100 accessions of blackgram, 78 accessions of cowpea and 182 accessions of wild *Vigna* spp. yielded viable seeds for further research and safe duplication. All accessions were already deposited at the Thai-Department of Agriculture genebank. The process of safe duplication at AVRDC and Svalbord could be finished, and transfer of the genetic materials will be carried out in November 2011.

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