นิพนธ์ต้นฉบับ

Characterization and genetic relatedness among 37 yardlong bean and cowpea accessions based on morphological characters and RAPD analysis

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Abstract

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Twenty four yardlong bean and 13 cowpea accessions were planted in the field to characterize their morphology and genetic relatedness. A randomized complete block design (RCBD) with two replications was used. Growth habit, days to flowering, pod color, pod length, number of pods/plant, yield/plant and consumption quality were recorded. The results showed that pod length, number of pods/plant and pod yield/plant among 37 accessions were highly significant differerence. Mean pod yield and pod length of 24 yardlong bean accessions were 212.1 g/plant and 48.7 cm, respectively, while mean pod yield and pod length of 13 cowpea accessions were 117.4 g/plant and 21.3 cm, respectively. Twenty two yardlong bean accessions exhibited indeterminate growth habit while 10 of 13 cowpea had determinate growth habit and the rests exhibited semi-determinate growth. Genetic variation and relationships among accessions were investigated based on RAPD technique. Total DNA was extracted from young leaf samples of all accessions using CTAB

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buffer. One hundred and twenty decamer oligonucleotide primers were screened and 5 primers (OPC-06, OPR-12, OPZ-03, OPZ-08, OPZ-13) were chosen for further evaluation. A dendrogram of genetic similarity was constructed based on 23 polymorphic bands obtained from 5 primers using UPGMA in SPSS program, which revealed separate groups between yardlong bean and cowpea. The similarity coefficient among yardlong bean and cowpea accessions ranged from 0.515 to 1.000 and 0.548 to 1.000, respectively.

Key words : yardlong bean, cowpea, genetic polymorphism, RAPD

บทคัดย่อ

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การศึกษาลักษณะและความสัมพันธ์ทางพันธุกรรมในถั่วฝักยาวและถั่วพุ่ม 37 สายพันธุ์ โดยอาศัยลักษณะทางสัณฐานและเครื่องหมายอาร์เอพีดี

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ปลูกถั่วฝักยาว 24 สายพันธุ์ และถั่วพุ่ม 13 สายพันธุ์ เพื่อเปรียบเทียบลักษณะสัณฐานและวิเคราะห์ความสัมพันธ์ทางพันธุกรรมโดยใช้เทคนิค RAPD วางแผนการทดลองแบบ RCBD จำนวน 2 ซ้ำ บันทึกลักษณะการเจริญเติบโต วันออกดอก สีฝัก ความยาวฝัก จำนวนฝัก/ต้น ผลผลิต/ต้น และคุณภาพการบริโภค พบว่า ระหว่างถั่วฝักยาวและถั่วพุ่ม 37 สายพันธุ์มีความแตกต่างทางสถิติในลักษณะความยาวฝัก จำนวนฝัก/ต้น และผลผลิต/ต้น กลุ่มถั่วฝักยาวมีผลผลิตและความยาวฝักเฉลี่ย 212.1 กรัม/ต้น และ 48.7 ซม. ตามลำดับ ส่วนผลผลิตและความยาวฝักเฉลี่ยในกลุ่มถั่วพุ่มมีค่า 117.4 กรัม/ต้น และ 21.3 ซม. ตามลำดับ ถั่วฝักยาว 22 สายพันธุ์มีการเจริญแบบเลื้อย (indeterminate) ส่วนถั่วพุ่ม 10 สายพันธุ์มีการเจริญแบบพุ่ม (determinate) ที่เหลือมีการเจริญแบบกึ่งเลื้อย (semi-indeterminate) ศึกษาความแปรปรวนและความใกล้ชิดทางพันธุกรรมระหว่างสายพันธุ์โดยใช้เทคนิคอาร์เอพีดี โดยสกัดดีเอ็นเอจากใบอ่อนใช้บัฟเฟอร์ CTAB และทดสอบกับไพรเมอร์จำนวน 120 ไพรเมอร์ คัดเลือกไพรเมอร์ที่ให้ผลชัดเจนที่สุด 5 ไพรเมอร์ (OPC-06, OPR-12, OPZ-03, OPZ-08 และ OPZ-13) เพื่อศึกษารูปแบบของแถบดีเอ็นเอของถั่วพุ่มและถั่วฝักยาว นำผลของรูปแบบแถบดีเอ็นเอที่มีความแตกต่างกันจากแต่ละสายพันธุ์รวม 23 แถบที่ได้มาสร้างเดนโดแกรม เพื่อดูความใกล้ชิดทางพันธุกรรมในกลุ่มพืชโดยวิธี UPGMA โปรแกรม SPSS พบว่าสามารถแยกกลุ่มระหว่างถั่วฝักยาวและถั่วพุ่มได้ชัดเจน โดยดัชนีความใกล้ชิดทางพันธุกรรม ในกลุ่มถั่วฝักยาวและถั่วพุ่มมีค่าระหว่าง 0.515-1.000 และ 0.548-1.000 ตามลำดับ

Yardlong bean (*Vigna unguiculata* ssp. sesquipedalis (L.) Verdc.) is known as vegetable cowpea, asparagus bean, string bean, snake bean, snake pea, snap pea, bodi, bora and sitao. Its origin is possibly in the Middle West Africa or in Southern China. Yardlong bean is widely grown in Southeast Asia, South China and West Africa for immature pods which are used as a vegatable. Yardlong bean is one of the economically important vegetable crops in Thailand. Production area of yardlong bean in Thailand is about 18,560-20,160 ha annually. Cowpea (*Vigna unguiculata* ssp. sinensis) is one of the important food grain legumes in all tropical areas, particularly in Africa. It is cultivated on at least 12.5 million hectares, with an annual production of over 3.0 million tons worldwide (Fana *et al*., 2004). It is usually grown intercropped with sorghum or millet and also in rotation cropping system. In Thailand, yardlong bean is considered have relatively low pod yield productivity and stability. It is quite sensitive to unfavorable environmental conditions such as

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high temperature and dry weather, too cloudy sky or heavy rain, and susceptible to various diseases and insect pests. Therefore, it is desirable to develop a new better adaptable and productive variety.

A clear characterization of accessions is a necessary first step to facilitate breeding efforts and will benefit plant breeder in choosing the proper parental materials. Germplasm characterizations are traditionally based on various morphological characters and on molecular markers. However, morphological characters are often highly influenced by environmental factors. Several moleculars markers are now in common use in studying plant genetic diversity or relationship. Molecular marker can provide information that can help in detecting the distinctiveness of species. They also have much to offer in the resolution of problems concerning hybridization (Karp and Edwards, 1997). Isozymes have been used for genetic diversity studies in cowpea (Pasquet, 1993; Vaillancourt *et al*., 1993). At the DNA level, RAPD is the effective markers used for diversity study in many crops including cowpea (Coulibaly *et al*., 2002; Fana *et al*., 2004; Laity *et al*., 2003; Nkongolo, 2003 and Yee *et al*., 1999) and yardlong bean (Pooprompan *et al*., 1996; Phansak *et al*., 2001). RAPD was also further applied together with other markers to construct genetic linkage map for this species (Menendez *et al*., 1997 and Ouedraogo *et al*., 2002).

The objectives of this study were to characterize agronomic traits and yielding ability and to determine the degree of genetic similarity among a total of 37 yardlong bean/cowpea accessions using RAPD markers.

Materials and Methods

Plant materials

A total of 24 accessions of yardlong bean and 13 accessions of common cowpea were collected from the Tropical Vegetable Research Center (TVRC) of Kasetsart University, Royal project at Chachoensao, Field crops research center at Ubon Ratchathani and some local markets. The accession numbers, varieties/lines and domestic data are shown in Table 1

Morphological character evaluation

Morphological characters and yield of total 37 yardlong bean/cowpea accessions were investigated, using RCBD with two replications. Agronomic data including growth habit, days to 50% flowering, pod color, pod length, number of pods/plant and pod yield were recorded from 20 plants per plot. Selected PSU#1 was used as a control variety. Data were analyzed by SAS program (SAS, 1985). Consumption quality was also recorded by trained taste panels with a consumption score of 1.0-5.0. A score of 1.0 is very poor and 5.0 is the best.

RAPD marker evaluation

1. DNA extraction and RAPD amplification procedures

DNA samples was extracted from approximately 200 mg of young clean fresh leaves of 36 accessions (SR00-1139 was not included because no seed was available). Procedures were modified from Doyle and Doyle (1990) as described by Nualsri and Konlasuk (2000). DNA prepared from all accessions was used for PCR reactions. One hundred and twenty 10-base oligonucleotide primers (Operon, Alameda, U.S.A.) were used for first step screening. The amplification reaction was performed in a reaction volumn of 25 µl, containing 2.5 µl of 10 X buffer, 3.0 µl of 25 mM $MgCl_2$, 200 µM of each dNTP, 0.3 µM of primer, 1.0 unit of Taq DNA polymerase and 1.0 µl of 100 ng template DNA. The thermal profile as described by Phansak *et al.* (2001) started from 35 cycles of 94°C for 30 sec, 37°C for 30 sec, 72°C for 1 min, and finally 72°C for 5 min. After amplification, 10 µl of PCR products were seperated by electrophoresis at 50 V for 2 h and 30 min on 1.75% LE agarose (Promega, Medison, U.S.A.) using TBE buffer, stained with 0.5 µg/ml of ethidium bromide for 30 min, and destained by soaking in deionized water for 20 min and photographed under UV light. Seventeen primers, which produced a clear polymorphic banding (OPA-09, OPB-04, OPB-07, OPB-08, OPB-17, OPC-06,

Table 1. Sources of yardlong bean and common cowpea germplasm used in this study

Accession	Source	Original source	
1 VU 012*	TVRC	Mukdahan	Thailand
2 VU 041-A*	TVRC	Narathiwat	Thailand
3 VU 051*	TVRC	Sing Buri	Thailand
4 VU 054*	TVRC	Chai Nat	Thailand
5 VU 063*	TVRC	-	
6 VU 136*	TVRC	Nonthaburi	Thailand
7 VU 144*	TVRC		Thailand
8 VU 163*	TVRC	UP Philippines	
9 VU 124*	TVRC	-	Thailand
10 VU 135*	TVRC	-	Thailand
11 VU 146*	TVRC	Ratchaburi	Thailand
12 VU 162*	TVRC	Songkhla	Thailand
13 VU 171*	TVRC	Chiangmai	Thailand
14 NR 001*	Royal project	Chachoengsao	Thailand
15 NR 002*	Royal project	Chachoengsao	Thailand
16 NR 003*	Local market	Songkhla	Thailand
17 KU-20*	KU	Nakhon Pathom	Thailand
18 NR 005*	Local market	Songkhla	Thailand
19 NR 006*	Local market	Songkhla	Thailand
20 NR 007*	Farmer	Chaiyaphum	Thailand
21 SR00-0274*	TVRC		
22Selected PSU#1*	PSU	Songkhla	Thailand
23 VU 189*	TVRC	-	China
24 VU 174	TVRC	-	Bangladesh
25 VU 176	TVRC	-	Bangladesh
26 VU 173	TVRC	-	Bangladesh
27 VU 178	TVRC	-	Bangladesh
28 VU 179	TVRC	-	Bangladesh
29 SR 00-379	TVRC	-	Sri Lanka
30 SR 00-379A	TVRC	-	Sri Lanka
31 SR 00-863	TVRC	-	Sri Lanka
32 SR 00-1139	TVRC	-	Sri Lanka
33 SR 00-0402	TVRC	-	Sri Lanka
34 SR 99-334*	TVRC	-	Sri Lanka
35 IT 82E-9	Field Crops Research center, Ubon Ratchatani	-	Thailand
36 IT 82E-16	Field Crops Research center, Ubon Ratchatani	-	Thailand
37 IT 84D-666	Field Crops, Research center, Ubon Ratchatani	-	Thailand

Remark * yardlong bean accession

OPC-07, OPC-10, OPC-14, OPR-02, OPR-08, OPR-12, OPZ-03, OPZ-07, OPZ-08, OPZ-12 and OPZ-13) were selected to re-screen for polymorphic fragments and five primers were chosen for further studies (Table 4). To ensure the quality of results, all samples were analyzed twice and a negative control was added.

2. Data analysis

Clear amplified fragments from the 5 selected primers were scored. Each band was treated as a separate putative locus and scored as present (1) or absent (0) in each accession. Hierarchical Cluster Analysis was performed using the SPSS programs (Vanichbancha, 2003) to identify genetic relatedness among accessions. Similarity coefficient was generated by Jaccard's method (Jaccard, 1908). These similarity coefficients were used to construct a dendrogram using unweighted pair-group method using arithmetic average (UPGMA).

Results and Discussion

Morphological character evaluation

Yardlong bean / cowpea accessions from field experiment showed various morphological characters. Twenty-two of 24 yardlong bean accessions were of indeterminate growth habit, except VU189 and NR001 were determinate. Ten of 13 cowpea were determinate while other three accessions (SR00-863, VU174, SR00-1139) had semi-determinate growth habit. Most yardlong bean had green to darkgreen pod color with the average score for consumption qualities of 3.96. Two yardlong bean accessions (VU144 and SR00-0402) were blue-green in pod color while KU20 had purple-red pod with the consuming quality ranging from 1.0 to 2.0. Pod color of most cowpea was light-green to dark green, except that of SR00-1139 which was gray-green. Most of yardlong bean accessions had usually had higher pod qualities than cowpea. Overall, mean consuming quality score of yardlong bean was 3.65 compared of 1.65 in cowpea. It was noted that among yardlong bean accessions, pods that were green to dark green in color had higher consuming quality compared to blue-green or purple-red pod. (Table 2)

Pod length, number of pods per plant and pod yield (g/plant) of all 37 accessions were highly significantly different. The top three highest pod yield were found in SR99-334, VU163 and VU171. They produced 360.6, 346.5 and 306.9 g fresh weight/plant, respectively. Mean pod yield of 24 yardlong bean was 212.1 g/plant while that of 13 cowpea was 117.4 g/plant, or only 45.4% compared to yardlong bean. Pod length of 37 accessions varied from 14.9 to 58.3 cm. Mean pod length of 13 cowpea was 21.3 cm while that of yardlong bean was 48.7 cm (Table 3)

VU189 and NR001, which were classified as yardlong bean, exhibited some morphological difference from other yardlong bean accessions. These two accessions had determinate growth habit with pod length of 34.0-34.9 cm. VU189 and NR001 might be improved varieties derived from yardlong bean and cowpea cross followed by selection or backcrossing.

RAPD marker evaluation

One hundred and twenty 10-base oligonucleotide primers were screened among 36 yardlong/cowpea accessions. Only five primers (OPC-06,OPR-12, OPZ-03,OPZ-08 and OPZ-13) were chosen to use for genetic diversity analysis. The total number of clear visible and polymorphic bands across 36 accessions varied among primers (Table 4). A total of 38 visible bands, 23 polymorphic bands was generated with the mean of 7.6 and 4.6 bands/primer, respectively. OPZ-03 gave the highest number of fragments (11 fragments) and 7 from these fragments were polymorphisms (Figure 1). The size of the amplified fragment ranged from approximately 225 bp to 1650 bp (Table 4). Pooprompan *et al.* (1996) identified various varieties of yardlong bean by RAPD and reported size of fragment varied from 500 to 2200 bp, while 940 to 1100 bp fragments were reported by Phansak *et al.* (2001). However, primers used by the two research groups were different from primers used in our experiment.

Genetic relatedness

A dendrogram constructed from 23 polymorphic bands revealed fairly good separation of genetic groups between yardlong bean and cowpea (Figure 2). However, VU189 and NR 001, two improved yardlong bean accessions derived from

Table 2. Morphological characters of 37 yardlong bean/cowpea accessions

accessions	growth habit	Day to 50% flowering	pod color	Consumed 2/ Àquality
1 SR99-334*	Indet.	41	green-dark green	4.0
2 VU 163*	Indet.	40	green	4.5
3 VU 171*	Indet.	37	green-dark green	4.5
4 VU 012*	Indet.	37	green	3.0
5 VU 162*	Indet.	40	green	4.0
6 VU 124*	Indet.	42	green	3.0
7 VU 041-A*	Indet.	40	green	4.0
8 Selected PSU #1*	Indet.	39	green	4.0
9 VU 146*	Indet.	40	green	4.0
10 VU 135*	Indet.	42	green	5.0
11 VU 144*	Indet.	39	blue-green	1.0
12 NR 003*	Indet.	38	green	4.5
13 VU 136*	Indet.	39	green	1.5
14 VU 051*	Indet.	38	dark green	3.0
15 VU 054*	Indet.	40	green	4.0
16 NR 005*	Indet.	40	green	4.5
17 NR 006*	Indet.	40	dark green	4.5
18 SR00-0402	Det.	42	blue-green	1.5
19 SR00-863	Semi-indet.	38	green	2.0
20 VU 063*	Indet.	39	green	2.0
21 KU 20*	Indet.	40	purple-red	2.0
22 NR 002*	Indet.	41	green	4.5
23 VU 176	Det	39	light green	2.0
24 VU 174	Semi-indet.	40	light green	1.0
25 VU 189*	Det.	35	light green	4.0
26 SR00-379A	Det.	39	dark green	2.0
27 NR 007*	Indet.	41	green	4.5
28 VU 173	Det.	39	light green	1.0
29 VU 178	Det.	37	light green	1.5
30 IT 82E-9	Det.	36	dark green	2.0
31 VU 179	Det.	39	light green	1.5
32 IT 82E-16	Det.	37	dark green	2.0
33 NR001*	Det.	39	green	4.0
34 SR00-1139	Semi-indet.	44	grey-green	1.5
35 IT 84D-666	Det.	37	dark green	2.0
36 SR00-0274*	Indet.	40	green	3.5
37 SR00-379	Det.	39	green	1.5

Remark * yardlong bean accession
2/ 1.0-5.0 score ; 5.0 = best, 1.0 = very poor

a cross between yardlong bean and cowpea which exhibited most characters resembling cowpea and were classified in the cowpea group. The result revealed some good relationship between growth habit and genetic relatedness. The relationship between growth habit and genetic relatedness was also realized among 38 Malawian cowpeas (Nkongolo *et al*., 2003) and 7 cultivated Senega-

Table 3. Yield and relative yield of 37 yardlong bean/cowpea accessions.

Accessions	Pod length (cm.)	No. of pod per plant	Pod yield (g./plant)	Relative yield (%)
1 SR99-334*	53.6	24.1	360.6	155.0
2 VU 163*	55.3	19.3	346.5	148.9
3 VU 171*	48.7	17.1	306.9	131.9
4 VU 012*	52.9	16.7	278.3	119.6
5 VU 162*	58.3	14.7	260.5	112.0
6 VU 124*	55.1	18.7	254.5	109.4
7 VU 041-A*	44.1	15.0	238.2	102.4
8 Selected PSU #1*	57.1	15.0	232.6	100.0
9 VU 146*	52.2	15.6	225.3	96.9
10 VU 135*	50.4	12.6	223.7	96.2
11 VU 144*	45.7	15.6	221.3	95.1
12 NR 003*	56.7	13.5	218.9	94.1
13 VU 136*	54.0	14.0	211.1	90.7
14 VU 051*	44.4	11.2	207.2	89.1
15 VU 054*	47.6	14.6	199.8	85.9
16 NR 005*	50.1	14.4	199.4	85.7
17 NR 006*	42.7	11.2	196.0	84.2
18 SR00-0402	39.8	14.8	195.3	83.9
19 SR00-863	23.6	24.5	176.9	76.0
20 VU 063*	46.3	12.0	164.2	70.6
21 KU 20*	44.1	12.2	154.8	66.5
22 NR 002*	50.3	10.7	152.5	65.5
23 VU 176	18.5	38.3	152.4	65.5
24 VU 174	20.8	33.1	150.8	64.8
25 VU 189*	34.9	13.6	145.8	62.7
26 SR00-379A	17.8	25.3	139.5	60.0
27 NR 007*	44.5	7.7	136.3	58.6
28 VU 173	19.4	26.9	123.6	53.1
29 VU 178	17.2	31.2	112.4	48.3
30 IT 82E-9	15.5	21.1	104.0	44.7
31 VU 179	19.0	25.5	101.2	43.5
32 IT 82E-16	15.9	19.7	98.5	42.3
33 NR001*	34.0	8.3	94.5	40.6
34 SR00-1139	37.7	6.3	76.5	32.9
35 IT 84D-666	16.3	13.5	66.3	28.5
36 SR00-0274*	45.2	5.5	60.3	25.9
37 SR00-379	14.9	6.1	29.3	12.6
F-test	**	**	**	
$LSD_{.01}$	3.26	3.67	50.98	
C.V. (%)	3.16	8.27	10.77	

Remark * yardlong bean accession ** significant at 0.01 level

lese cowpea (Laity *et al.*, 2003). Genetic diversity among yardlong bean was relatively higher than that of cowpea. Similarity coefficient among yardlong bean and cowpea accessions were 0.515

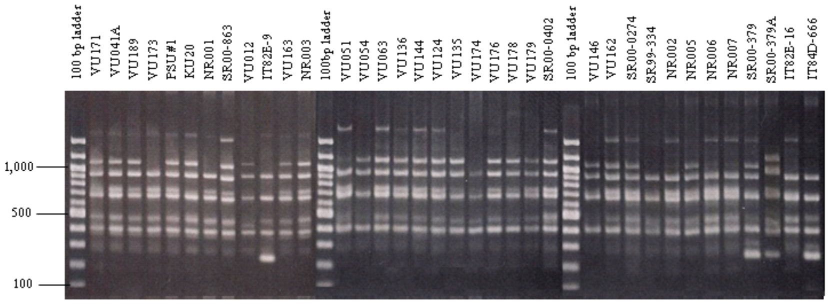

Figure 1. **RAPD amplification products of 36 yardlong bean/cowpea accessions generated from primer OPZ-13**

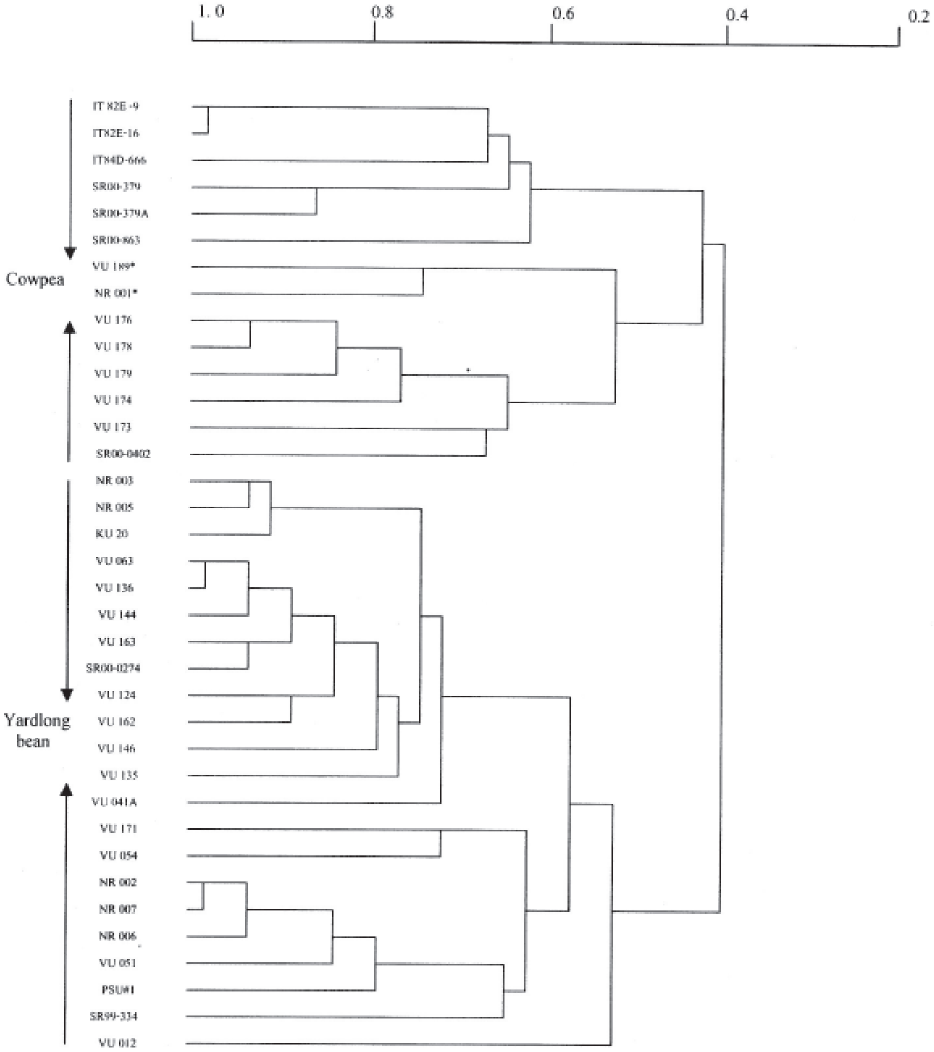

Figure 2. **Dendogram of genetic relationships among 36 yardlong bean/cowpea accessions using unweighted pair-group method of arithemetic average (UPGMA) based on the Jaccard similarity matrix (* accession that was classified as yardlong bean).**

Table 4. Total fragments, polymorphic fragments and size of RAPD bands produced from yardlong bean and cowpea accessions.

Primer ID	Primer sequence	Total fragment	Polymorphic fragment	Range of fragment size (bp)
OPC-06	GAACGGACTC	8	4	275-1,350
OPR-12	ACAGGTGCGT	5	3	675-1,200
OPZ-03	CAGCACCGCA	11	7	225-1,175
OPZ-08	GGGTGGGTAA	7	5	350-1,500
OPZ-13	GACTAAGCCC	7	4	250-1,650
Total	-	38	23	225-1,650

to 1.000 and 0.548 to 1.000, respectively. Relatedness among accessions was not influenced by geographical location, except all accessions from Bangladesh (VU173, 174, 176, 178 and 179) which originated from the same cross. They were grouped in the same cluster with similarity coefficient higher than 0.7. Phansak *et al.* (2005) studied relationship among 15 accessions of yardlong bean from Thailand, Bangladesh, China, Laos, Philippines and Taiwan using STMS (sequence tagged microsatellite site) and reported a similarity coefficient varying from 0.50 to 1.00. Among those accessions, 3 groups could be made with no relation to geographical origins.

Among 36 accessions, VU176 (cowpea) and SR99-334 (yardlong bean) exhibited the lowest genetic relatedness with similarity coefficient of 0.484. There were three pairs of very closely related accessions based on RAPD markers showing identical DNA patterns, there were IT82E-9/IT82E-16, NR002/NR007 and VU063/VU136. Based on STMS analysis studied by Phansak *et al.* (2005), they reported identical banding pattern of VU173 and VU 174 but we distinguished them with a similarity coefficient of 0.79.

Conclusion

A total of 37 yardlong bean/cowpea accessions were grown for morphological evaluation. Yardlong bean accessions had relatively higher yield and pod quality consumption compared to cowpea. A dendrogram based on 23 RAPD polymorphic fragments obtained from 5 primers (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13), revealed fairly good separation of groups between yardlong bean and cowpea.

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