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Varietal Evaluation and 30 kDa Protein Studies in Local Bitter Gourd (*Momordica charantia* L.)

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ABSTRACT

Twelve accessions of local bitter gourds were tested in comparison with a cultivated bitter gourd at Phichit Horticultural Research Center in summer 2000. Botanical and horticultural characteristics of leaf, pistillate flower, staminate flower, fruit and seeds were recorded. Ripe fruit yield of the accessions ranged from 3,227 to 6,912 kg/ha. Yield was not significantly different among the accessions but showed high variation in protein levels in extraction from endosperm. The partial protein powder ranged from 104.33 to 208.67 mg/5 g of endopsperm while the specific activity protein at 30 kDa evaluated by the polyacrylamide gel electrophoresis (PAGE) method ranged from 104.86 to 265.42 μg/5g of endosperm. One specific accession (No.16) gave the highest level of the specific activity protein at 30 kDa, however, it gave high level of the partial protein powder, 168.33 μg/5g. Contradictory, the other accession (No.11) which had more or less the same level of total protein as the mentioned accession, had rather low level of the specific activity protein at 30 kDa, 149.96 μg/5g of endosperm.

Key words: bitter gourd, 30 kDa protein, partial protein, specific activity protein

INTRODUCTION

Rural Thai people have used several indigenous vegetables and spices as medicine since the ancient times. The local medicine is called herbal medicine. Nowadays, there are various kinds of modern medicine, therefore the interest towards herbal medicine is quite limited. Some researchers are searching for new compounds to use for these disease such as HIV and cancer. It was found that bitter gourd reduced oxidation of methmyoglobin by hydrogenperoxide in laboratory. The reduction as such may be able to reduce the action of the virus.

Bitter gourd has long been used as local medicine in Asia. The plant has been investigated for the active ingredients and their effects. Ng *et al*.(1992 and 1997) at the Chinese Medical Material Research Centre of the Chinese University of Hong Kong found a series of protein in bitter gourd which has anti-HIV activity. Lee-Huang *et al*. (1994-1995a and 1995b) isolated MAP30 (*Momordica* anti-viral protein of 30 kDa) that has anti-HIV activity. There are several proteins involved in HIV replication, such as reverse transcriptase which converts the viral RNA genome to DNA, protease which modifies the protein products of the viral genome for new viral particles and integrase which inserts and removes the DNA from the host genome.

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The protein can block the infection of T lymphocytes and monocytes by HIV and inhibits the replication of HIV in already infected cells *in vitro*. (Lee-Huang *et al*., 1990) Although, MAP30 shows the inhibitory effect, but it appears to have limited toxicity to uninfected cells. MAP30 has a direct action against integrase activity and may inhibit the virus in other ways as well (Lee-Huang *et al*.,1995b). It has been quite sometimes that *M. charantia* protein was shown to have an action on HIV, but the protein has not been developed further for HIV treatment.

There are two types of bitter gourd in Thailand; local and cultivated types, the former scattered in Thailand as weeds. Variations of size and shape of fruits, plant type and leaf type are always observed. Because of its low economical values, little attention has been paid to the crop in terms of research. Collection of germplasm of local and cultivated bitter gourd and evaluation of horticultural characterization are the primary step for the breeding program. Subsequently, identification and separation of MAP30-like substance of the collected accessions would be operated. Then, breeding and development of varieties for high MAP30 variety should be the important step for the production of MAP30 in large scale.

MATERIALS AND METHODS

Twelve accessions of local bitter gourds were collected from various locations in Thailand in 1999. They were grown in the field at the horticultural research center, Department of Agricultural Extension in Phichit province, Thailand in 2000. Randomized complete block design with 4 replications was used. One cultivated bitter gourd called Deak bin brand or No.20 was used as a control variety. The experimental period was about 4 months. Fruits were harvested at ripe stage, they were weighted and seeds were taken out for seed weight.

General horticultural and botanical characteristics such as leaf shape and size, male and female flowers, fruit and seeds were recorded. They were grown for botanical and horticultural characterization.

Partial protein extraction, total protein analysis and specific activity protein at 30 kDa analysis were obtained by using frozen endosperm of bitter gourd seeds. The endosperm was ground at 4°C with normal saline solution at pH 3.6-4.0. The solution was filtered with cloth before it was centrifuged at 12,000 rpm, 4°C for 30 minutes for 3 times. The supernatant was shaked with 50%$(NH_4)_2SO_4$ and 50 mM Na_2PO_4 buffer, pH 6.3 before it was centrifuged again. The residue was dialysed with 20 mM Na_2PO_4 buffer, pH 6.3. Then active protein fraction was dried with a lyophilizer for 24 hrs for partial protein powder.

Total protein was analysed from the partial protein powder, using Bio-Rad protein assay reagent (Bradford) (Bio-Rad Laboratories, U.S.A.Cat.No. 500-0006). Five milligrams of the partial protein was mixed with 1 ml dei. H_2O and shaken by vortex. Ten µl of the solution was dropped into microliter plate wells. Various concentrations of Bovine Serum Albumin (BSA), 0 to 1000 µg/ml were dropped into adjacent wells. Then 200 µl/well of dyereagent was mixed in each well. They were measured at 620 nm absorbance. A regression line of standard protein (BSA) was calculated. Solution of each accession was compared with standard prtoein (BSA), to calculate total protein of each accession.

Specific activity of protein at 30 kDa was analysed from the partial protein powder. Deionized water was added into the powder. Buffer was added into the solution before it was heated at 95°C for 5 min. The sample was used for electrophoresis. Gels were stained with coomassie blue before washing and drying. Then specific activity of protein at 30 kDa was measured by an imaging densitometer, then calculated by comparison both amount of protein.

RESULTS

Characterization of botanical and horticultural traits of 12 local bitter gourds (Table 1-8) revealed that many botanical characteristics of leaf of all accessions were similar to each other. They had simple type of leaf, pubescent on surface covering and alternate arrangement, with palmate venation. Leaf shape of all accessions were the same, which showed circular shape of leaf, open shape of upper leaf sinus, concave shape of teeth, 7 lobes, acute apex of leaf, cordate base of leaf and parted margin of leaf. They were different on leaf size and color (Table 1). Leaves of all accessions were green in color but different in degree of greenness as measured by Hunter L, a, b color.

Pistillate flowers of all accessions were the same on many characteristics. (Figure 1). The flowers were regular (actinomorphic), solitary type of inflorescence, rotate corolla shape, polypetalous of corolla with 5 petals, yellow corolla, inferior ovary, epigynous of flower part, calyx adnates to ovary, 5 calyx lobe, 3 parietal placenta and reniform shape of sessile bract. Size of petal, peduncle length and size of sessile bract were different among the accessions (Table 2).

Staminate flowers of all accessions were the same on many characteristics (Figure 2). They had regular staminate flower (actinomorphic), axillary inflorescence, rotate corolla shape, polypetalous corolla with 5 petals, yellow corolla, 5 calyx lobe and reniform shape of sessile bract. Size of petal, peduncle length, size of sessile bract and distance of sessile bracts to peduncle base of the accessions were different (Table 3).

Fruit of bitter gourd was pepo type with 7 lobes. Size and weight of the fruits in various accessions were different (Table 4). The accessions showed differences in number of seeds per fruit and seed size (Table 5). Ripe fruit yield among the accessions was not significantly different (Table 6). The yield ranged from 3,227 to 6,912 kg/ha. Accession No.13 gave the highest fruit yield. Other high yield accessions were No.21, 11, 7 and 16. Fruits and seeds of accession 11, 13 and 16 were shown in Figures 3, 4 and 5. Fresh seed yield of the accessions was significantly different (Table 7). Seed yield ranged from 312.5 to 842.5 kg/ha. Accession No.13 gave the highest seed yield. The accession also gave the highest number of ripe fruit, 680,313 fruit/ha (Table 7). However, high fruit yielding accession was not always gave high seed yield.

Accession No.13 was among the accessions that had the longest harvesting life, 117-118 days after germination (Table 8). The last harvesting of ripe fruit after germination for Accession No.13 was 117 days. Number of times to harvest ripe fruits of this accessions was the highest, 50 times (Table 8).

Figure 1 Pistillate (female) flower of bitter gourd.

Figure 2 Staminate (male) flower of bitter gourd.

Table 1 Botanical characteristics of leaf of bitter gourd accessions.

Acc. No.	Size of leaf (cm)[1]		
	Width	Length	Petiole length
1	11.9	8.0	6.7
3	10.0	7.1	4.7
5	11.4	7.2	5.8
6	14.8	11.2	7.5
7	13.4	9.2	8.8
8	15.4	9.9	7.3
10	13.5	8.3	9.3
11	10.7	7.5	5.4
12	10.8	7.9	5.6
13	10.0	7.7	5.8
16	10.8	7.1	6.9
20	14.3	10.2	7.6
21	10.5	7.7	5.1

[1] Average of 10 mature leaves

Table 1 (continue) Botanical characteristics of leaf of bitter gourd accessions.

Acc. No.	General shape of petiole sinus	Colour of leaf[1]		
		L[2]	a[3]	b[4]
1	Wide open	35.19	-5.22	10.33
3	Open	53.05	-4.97	9.18
5	Wide open	87.19	-1.23	1.79
6	Wide open	87.44	-1.3	1.66
7	Wide open	78.85	0.94	10.05
8	Wide open	51.34	-4.89	7.67
10	Open	38.01	-6.83	14.83
11	Open	51.72	-4.89	9.67
12	Wide open	34.96	-6.35	13.03
13	Wide open	65.64	-2.16	7.52
16	Open	65.64	-2.16	7.52
20	Open	51.37	-4.8	8.41
21	Wide open	50.36	-4.03	6.74

[1] Color QUEST Hunter Lab
Evaluated date: 5 April 2000

[2] L - lightness

[3] a – redness or greenness, - a green, + a red

[4] b – yellowness or blueness, - b blue, + b yellow

Table 2 Botanical characteristics of pistillate flower of bitter gourd accessions.

Acc. No.	Pistillate flower			Sessile bract of pistillate flower[1]		
	Petal (mm)		Peduncle length	Width	Length	Distance to peduncle base
	Width	Length	(mm)	(mm)	(mm)	(mm)
1	8.4	10.9	29.0	7.7	5.5	3.5
3	11.2	14.5	47.8	8.4	6.2	9.3
5	10.3	13.5	29.4	8.9	5.8	7.3
6	9.3	11.6	37.4	7.4	5.1	2.9
7	8.4	11.1	33.8	10.6	8.3	3.9
8	7.5	11.3	52.7	11.0	6.7	5.2
10	9.8	12.6	41.7	6.7	4.6	2.8
11	6.7	10.0	44.6	9.5	5.9	8.5
12	11.3	18.9	87.7	8.9	5.6	8.8
13	10.1	16.1	63.7	7.2	4.7	5.0
16	7.1	9.5	45.3	6.3	4.6	3.9
20	8.1	10.7	55.1	3.7	3.2	5.8
21	6.8	10.2	51.1	7.4	4.9	6.6

[1] Average of 10 pistillate flowers

Table 3 Botanical characteristics of staminate flower of bitter gourd accessions.

Acc. No.	Staminate flower			Sessile bract of staminate flower[1]		
	Petal (mm)		Peduncle length	Width	Length	Distance to peduncle base
	Width	Length	(mm)	(mm)	(mm)	(mm)
1	9.4	15.1	66.2	8.5	6.9	10.1
3	13.6	20.3	69.6	7.6	5.4	20.4
5	10.7	15.8	50.9	8.8	6.7	15.2
6	9.5	13.9	52.2	10.0	7.4	8.6
7	10.4	15.1	29.9	6.6	4.3	4.6
8	8.4	16.3	61.8	10.5	7.5	10.5
10	10.1	16.5	48.6	6.0	4.2	5.2
11	10.1	14.8	51.8	10.3	6.6	14.1
12	12.9	21.3	68.3	11.2	6.2	12.6
13	11.3	17.1	75.5	6.2	4.5	7.6
16	9.0	12.3	54.7	5.7	3.5	5.6
20	10.9	14.8	77.2	7.7	5.7	28.0
21	7.8	12.7	45.2	7.8	5.6	12.4

[1] Average of 10 staminate flowers

Table 4 Size and shape of bitter gourd fruit in 13 accessions.

Acc. No.	Length[1] (cm)	Diameter[1] (cm)	Fruit base to sessile bract[1] (cm)	Weight[1] (g)
1	4.02	3.00	2.96	12.03
3	4.72	2.11	3.82	5.31
5	4.81	2.18	2.78	5.87
6	6.70	3.26	4.39	20.30
7	10.29	3.59	2.92	42.89
8	11.44	3.72	4.38	51.21
10	8.52	4.23	5.70	45.75
11	6.88	3.02	4.42	16.12
12	4.70	2.08	6.48	4.69
13	4.69	2.20	7.04	4.94
16	6.08	3.25	4.50	19.89
20	6.10	3.18	5.78	17.91
21	5.20	2.93	5.37	13.73

[1] Average of 10 fruits.

Table 5 Number of seed per fruit and size of bitter gourd accessions.

Acc. No.	Size (mm)[1]			Number of seeds per fruit[2]
	Width	Length	Thickness	
1	7.25	12.40	3.91	8
3	4.78	9.65	3.22	14
5	5.75	9.43	3.25	9
6	7.37	12.36	4.05	8
7	6.63	12.76	3.85	31
8	7.80	14.93	4.07	28
10	7.00	12.51	3.89	26
11	6.95	13.64	3.48	15
12	5.23	10.66	3.23	9
13	5.53	11.16	3.22	13
16	7.64	12.91	3.49	17
20	7.43	12.34	4.09	11
21	7.32	12.18	4.06	8

[1] Average of 10 seeds

[2] Average of 10 mature fruits

Table 6 Days to 50% female flowering, first harvest of ripe fruit and yield of ripe fruit of bitter gourd accessions.

Acc. No.	Days to 50% female flowering[2/]	Days to first harvest (days)[2/]	Weight of ripe fruit[1] (kg/ ha)[2/]
1	57 abcd	68 c	3,227
3	59 bcd	70 cd	3,853
5	55 ab	68 bc	4,403
6	61 cd	71 cd	4,956
7	53 e	59 a	5,188
8	62 de	69 c	4,516
10	56 abc	62 ab	4,878
11	66 a[1]	75 d	5,376
12	57 abcd	66 bc	4,827
13	55 ab	66 bc	6,912
16	56 abc	68 bc	5,168
20	57 abcd	70 c	4,640
21	55 ab	67 bc	5,701
F-test	**	**	NS
CV (%)	5.96	5.36	27.11

1 Harvested ripe fruits per area.

2 Same letters indicate no differences in means (DMRT).

Table 7 Number of ripe fruit and seed yield of bitter gourd accessions.

Acc. No.	No. of ripe fruit (fruit/ha)	Fresh seed yield (kg/ha)
1	170,938 cde	360 cd
3	448,750 b	390 cd
5	575,625 a[1]	533.75 bc
6	176,563 cde	420 bcd
7	89,063 de	382.5 cd
8	63,125 e	380 cd
10	72,813 e	312.5 d
11	190,625 cd	485 bcd
12	655,938 a	610 b
13	680,313 a	842.5 a
16	150,000 cde	477.5 bcd
20	138,438 de	428.75 bcd
21	255,313 c	501.25 bcd
F-test	**	**
CV (%)	25.08	25.33

1 Same letters indicate no differences in means (DMRT)

Table 8 Days to last harvest and number of time to harvest ripe fruit of bitter gourd accessions.

Acc. No.	Last harvesting of ripe fruit after germination (days)	No. of times to harvest ripe fruits
1	107 cd	33 de
3	117 a[1]	45 ab
5	117 a	48 a
6	118 a	39 bcd
7	98 f	23 f
8	109 bc	24 f
10	101 e	24 f
11	117 a	36 cde
12	117 a	49 a
13	117 a	50 a
16	106 d	34 de
20	107 cd	32 e
21	110 b	41 bc
P	< 0.01	< 0.01
CV (%)	1.81	11.56

[1] Same letters indicate no differences in means (DMRT)

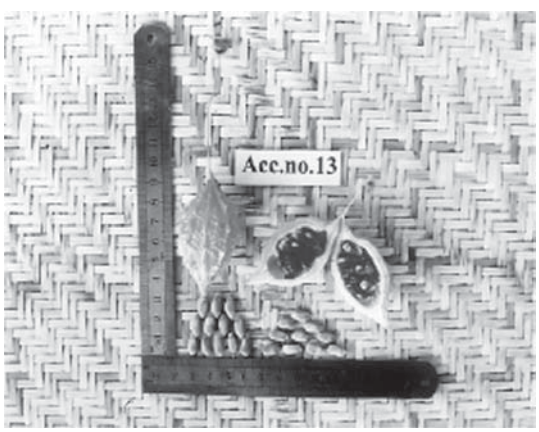

Figure 3 Fruit and seeds of bitter gourd, accession No. 13.

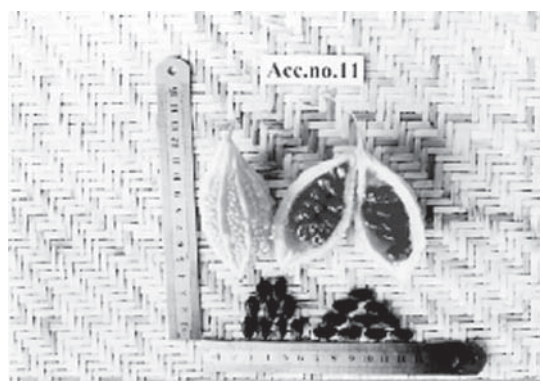

Figure 4 Fruit and seeds of bitter gourd, accession No. 11.

Total protein, and specific activity protein at 30 kDa in 5 grams of endosperm are showed in Table 9. Levels of these proteins showed variation among accessions of bitter gourd. The levels of total protein did not have any correlation correlated with the levels of the specific activity protein at 30 kDa. The levels showed the range of 104.86 to 265.42 μg/5g of endosperm.

When total protein and specific activity at 30 kDa were extracted from the endosperm of each accessions, it was found that high fruit and seed yielding accession such as No.13 gave low level of total protein 106.67 mg/ml H_2O and low level partial protein and specific activity protein. It gave

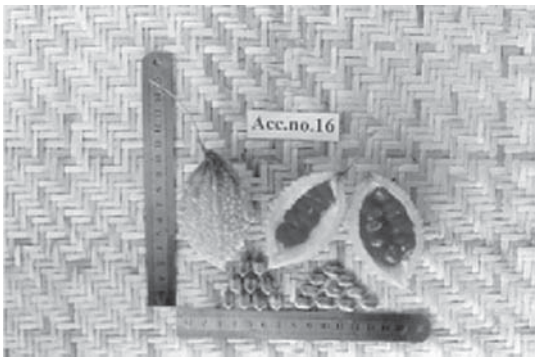

Figure 5 Fruit and seeds of bitter gourd, accession No. 16.

104.33 μg/5 g of endosperm of partial protein powder while the highest level was 208.67 mg/5g and gave 104.86 mg/5g of endosperm of specific activity protein at 30 kDa while the highest level was 265.42 μg/5g of endosperm. Accession No.16 gave the highest level of specific activity protein at 30 kDa. The accession gave lower yield of ripe fruit than accession No.13. However, the difference was not statistically significant.

DISCUSSION

Accession No.13 gave the highest fruit yield. It gave also the highest number of harvesting times and number of fruits. Number of female flowers and number of fruit per plant were found to be yield components of bitter gourd (Ramachandran and Gopalakrishnan, 1979, Paranjape and Rajput, 1995 and Rajput *et al*., 1995). Fruit yield was not as important as seed yield because only endosperm was used for extraction for specific protein at 30 kDa. Accession No.13, again, gave the highest seed weight.

When specific protein at 30 kDa or MAP30 was extracted from the bitter gourd accessions, wide range of protein levels was observed. Lee Huang *et al*. (1995a) reported the identification and purification of MAP30 in bitter melon *Momordica charantia* were different cultivar from our local bitter gourd.

High yielding variety such as accession No.13 did not give high level of MAP30. It gave the lowest level of MAP30, 104.80 μg/5g endosperm.

Table 9 Total protein , partial protein and specific activity protein at 30 kDa in 5g of endosperm of bitter gourd accessions.

Acc. No.	Total protein (μg/ml dei.H_2O)	Partial protein (mg)	Specific activity protein at 30 kDa (μg)[1]
1	148.17	155	158.40
3	142.5	163.33	206.39
5	126	115	121.96
6	93.17	142	162.30
7	162	142	197.51
8	172	179	179.86
10	138.3	208.67	215.91
11	160.8	207.33	149.96
12	87	110	120.22
13	106.67	104.33	104.86
16	136.50	168.33	265.42
20	150.67	117.67	139.28
21	175.33	162.67	147.81

[1] Specific activity protein calculated from imaging densitometer (model Bio-Rad GS-700).

While accession No.16 gave lower fruit yield than accession No.13 but it gave the highest level of MAP30, 265.42 μg/5g of endosperm. Since the difference in fruit yield was not statistically significant, therefore accession No.16 would better than accession No.13 for MAP30 extraction, eventhough seed yield of this accession was about half of accession No.13.

CONCLUSION

General botanical characteristics of local bitter gourd and cultivated bitter gourd were the same on type of leaf, pistillate flower, staminate flower fruit and seed. The differences were found only size fruit yield and seed yield. When total protein, and specific activity protein at 30 kDa were extracted from endosperm of the seeds. It was found that high yielding accessions did not always give high level of protein content in the seed. Some low yielding accessions gave high specific protein at 30 kDa levels.

Variation of specific activity protein at 30 kDa of bitter gourd accessions as shown in Table 9 illustrated differences in genetic control of MAP30 biosynthesis in the plants. It is very interesting that the range of production is wide, from 104.86 to 265.42 μg/5 g of endosperm. The substance which was extracted from bitter gourd fruit so called MAP 30 kDa is under investigation for its characteristics such as amino acid sequences. Plant breeding may play an important role on improvement for high production of the protein. Many methods of breeding such as pedigree method, mass selection, recurrent selection and F_1 hybridization can be used for varietal improvement.

LITERATURE CITED

Lee-Huang, S., H. Kung, P. Huang, J. Morell, P. Huang, A. Bourinbaiar, F. Brown, W. Tsai, A. Chen, H. Huang, and H. Chen. 1994. HIV-1 inhibition, DNA binding, RHA binding and ribosome inactivation activities in the N-terminal segments of the plant anti HIV protein GAP31. Proc. Natl. Acad. Sci. USA. 91 : 12208-12212.

Lee-Huang, S., H. Kung, P. Huang, P.Huang, A. Bourinbaiar, H. Huang, and H. Chen. 1995a. Anti-HIV and anti-tumor activities of recombiant MAP30 of bitter melon. Gene. 161 : 151-156.

Lee-Huang, S., H. Kung, P. Huang, P. Huang, A. Bourinbaiar, and H. Chen. 1995b. Inhibition of HIV-1 intergrase by plant antiviral proteins MAP30 and GAP31. Proc. Natl. Acad. Sci. 92 : 8818-8822.

Lee-Huang, S., P. Nara, P. Huang, P. Huang, H. Huang, and H. Chen. 1990. MAP30 : a new inhibitor of HIV-1 infection and replication. FEBS letters 272 : 12-18.

Ng, T., W. Chan, and H. Yeung. 1992. Proteins with abortifacient, ribosome-inactivation, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plant. General Pharmacology 23 : f575-590.

Ng, T., B. Huang, W. Fong, and H. Yeung. 1997. Anti-human imunodeficiency virus (anti HIV) natural products with special emphasis on reverse trancriptase inhibitors. Life Science 61 : 933-949.

Paranjape, S.P. and J.C. Rajput. 1995. Association of various characters in bitter gourd and their direct and indirect effects on yield. Journal of Maharashtra Agricultural Universities 20 : 193 – 195.

Ramachandran, C. and P.K. Gopalakrishnan. 1979. Correlation and regression studies in bitter gourd. Indian Journal of Agricultural Science 49 : 850 – 854.

Rajput, J.C., S.P. Paranjape, and B.M. Jamadagni. 1995. Correlation and path analysis studies for fruit yield in bitter gourd. Journal of Maharashtra Agricultural Universities 20 : 377-379.

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