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Introduction of Yam Bean (*Pachyrhizus* spp.) in Korea

Sang-Kuk Kim[1], Hong-Jib Choi[1], Jae-Hee Won[2], Jun-Hong Park[3], In-Jung Lee[4] and Shin-Young Park[5]*

[1]Division of Crop Sciences, Gyeongbuk Provincial Agricultural Technology Administration, Daegu 702-708, Republic of Korea

[2]Daegu Gyeongbuk Institute for Oriental Medicine Herb Quality Certification Center, Daegu 700-230, Korea

[3]Bonghwa Alpine Agricultural Experiment Station, Gyeongbuk Provincial Agricultural Technology Administration, Bonghwa 755-843, Republic of Korea

[4]Division of Plant Biosciences, College of Agriculture and Life Science, Kyungpook National University, Daegu 702-701, Korea

[5]Department of Clinical Pathology, Cheju Halla College, Cheju 690-708, Republic of Korea

Abstract - We examined the growth characteristics, fresh tuber yield, rotenone content of two yam beans (*Pachyrhizus erosus* and *P. ahipa*) introduced from Mexico. *P. erosus* species showed better adaptable ability than *P. ahipa* species in seed productivity and tuber yield. Rotenone content in the *P. erosus* species extracted with chloroform was 3.6 folds much more extracted than ethanol extraction. The order of rotenone content found in crude extract obtained by different solvent extraction from the highest to the lowest was mature seed (484.7 ㎍) and leaves (17.2 ㎍) of the *P. erosus* species, respectively.

Key words - Yambean, *Pachyrhizus erosus*, *Pachyrhizus ahipa*, Rotenone, Seed, Tuber

Introduction

The genus *Pachyrhizus* (yam bean) is a close relative to the soybean and phaseolus bean (Ingham, 1990). The genus comprises three cultivated species with different ecogeographical origins: *P. ahipa*, from the highland tropics of Bolivia and northern Argentina; *P. erosus*, from the semiarid tropics of Central America; and *P. tuberous*, from the tropical lowlands of both sides of the Andean mountain range. *Pachyrhizus erosus* was introduced to the Philippines during the 16th century, and it is nowadays a popular crop in many countries of Asia as well as West Africa. The yam beans are exclusively used as a tuber crop and the crisp and fruity tubers are consumed raw as refreshment (Sørensen, 1996).

Unlike its close relatives the soybean and the *Phaseolus* beans, the yam bean is exclusively used for its tuberous roots (Sørensen *et al*., 1997). The name yam bean is used to designate the species within the genus *Pachyrhizus*, in particular the three cultivated species; *P. erosus*, from the semiarid tropics of Central America; *P. tuberosus* from the tropical lowlands of both slopes of the Andean mountain range and *P. ahipa* from Andean highland (Sørensen *et al*., 1996). Moreover, *P. erosus* is also cultivated in many South East Asian countries.

Studies on yam bean seed composition are scarce and concentrated exclusively on *P. erosus*. For this species, It was reported an approximate composition of 6.7% moisture, 26.2% protein, 27.3% oil, 20.0% carbohydrates, 7.0% fiber, and 3.6% ash (Duke, 1981). Additionally, potential uses of yam bean seed have mainly focused on the extraction of rotenone as a source of a natural insecticide (Duke, 1981). Additionally, it was pointed out that the potential value of yam bean meal for human could be consumed after the elimination of rotenone (Santos *et al*., 1996). The seeds of the yam bean are not used due to the high rotenone content (Santos *et al*., 1996, Grüneberg *et al*., 1999). It was reported that rotenone would be reduced from 0.06 to 1.0% by heating and solvent extraction (Santos *et al*., 1996). Recently, yam bean in our country has been first attempted to establish cultural practices like planting time, fertilizer application and propagation. Therefore, The objective of the present study was to evaluate the growth habit, rotenone content, root and

*Corresponding author. E-mail : shiny@hc.ac.kr

seed yield on two yam bean species.

Materials and Methods

General procedures

Seeds of the two yam bean species, *Pachyrhizus erosus* and *Pachyrhizus ahipa* which were introduced from Mexico were used in this study. Two yam bean (*Pachyrhizus* spp.) species were hill-seeded with the planting distance 60 cm between plants within rows and 40 cm between rows on ridges on 1 April. Prior to planting, fertilizer was supplied with nitrogen, phosphorus, and potassium at the rate of 23, 14, and 16 kg ha^{-1}, incorporating as basal and top dressing (7:3) to the soil. Experimental design was a randomized complete block with three replicates.

Plant samples

The leaves of *P. erosus and ahipa* were sampled 20 days after appearance of the five or nodes from each yam bean plant. The seeds were only harvested for *P. erosus*. The pods were not set in spite of flowering in *P. ahipa*. The tuber of *P. erosus and P. ahipa* were also sampled 30 days after flowering. Each sample including leaves, seeds and tubers for rotenone analysis was lyophilized for 48 h. The extraction of endogenous rotenone was followed as described by Sae-Yun *et al*. (2006).

Extraction of rotenone from plant organ

The lyophilized plant sample (1 g) was placed in a stopped conical flask and macerated with 10 ml of five different solvents, methanol (MeOH), ethanol (EtOH), *n*-hexane, chloroform, and acetonitrile at room temperature (28 to 30℃) for 2 days with occasional stirring under dark condition. The solvent was then filtered and finally massed up to 10 ml. The extraction was carried out on three separate occasions ($n = 3$).

HPLC analysis for rotenone

The chromatographic system (Waters Alliance, USA) consisted of an automated gradient controller fitted with separations module 2695, a model 2996 photodiode array detector and Empower 2 software. The column was a SunFire C_{18} 150mm×4.6 mm, 5μm, Waters, USA). The gradient profile for the separation of rotenone was as follows: initial mobile phase 20% methanol/100% methanol (70:30, %, v/v for 4 min, 40:60, %, v/v for 8 min, 0:100, %, v/v for 10 min) reaching 70:30 (%, v/v) in 40 min. Prior to injection, the LC system had to be stabilized for 20 min with an 20% methanol/100% methanol phase (70:30, %, v/v).

Standard solutions of rotenone (0 to 20 μg/ml) were prepared in methanol, and 20 μl of each was injected onto the HPLC column. The mean peak areas for each concentration were calculated, and standard calibration curves were constructed by plotting concentrations against peak areas. The injection volume was 20 μl, and the flow rate was 1 ml min^{-1}. The analysis was performed at a wavelength of 290 nm.

Results

Growth characteristics of two yam beans

Growth characteristics of vines, stem diameter, number of internodes, flowers on two yam bean species are presented in Table 1 and Fig. 1. Vine length in both species ranged 1.8 to 5.4m. Vine length in *Pachyrhizus ahipa* species was three times longer than that in *P. erosus* species. Stem diameter in *P. ahipa* species about two folds thicker than that in *P. erosus* species and internode number per plant was also much in *P.*

Table 1. Characteristics of vine length, stem diameter, number of internode, flower in two yam bean species

Species	Vine length (m)	Stem diameter (mm)	No. of internode	Flower color	No. of flower ($plant^{-1}$)	Fertilization rate (%)	Plant type
P. ahipa	5.4±0.2 a	5.1±1.9 a	18.5±1.5 a	violet	25.7±3.9 a	NF	9
P. erosus	1.8±0.3 b	2.7±1.6 b	7.8±2.8 b	white/violet	11.4±1.6 b	78.8±7.3	5

Plant type measured on a score 3: erect, 5:semi-erect, 7: spreaded, and 9: extremely spreaded.
NF: not fertilized. Data are standard deviations.
In each column, means followed by a common letters are not significantly different at 5% level by DMRT.

ahipa species than in *P. erosus* species. Flowers showed two different colors with violet and white in two yam bean species, and white color in flower was only observed in *P. erosus* species. Flower number per plant in both yam beans was 11.4 to 25.7, and *P. erosus* species were higher than *P. erosus* species. Fertilization rate accounted for 78.8% in the only *P. erosus* species. Growing pattern of main stem which is commonly regarded as the represented plant type has differently two plant types which are divided into the semi-erect and the extremely spread in these yam bean plants.

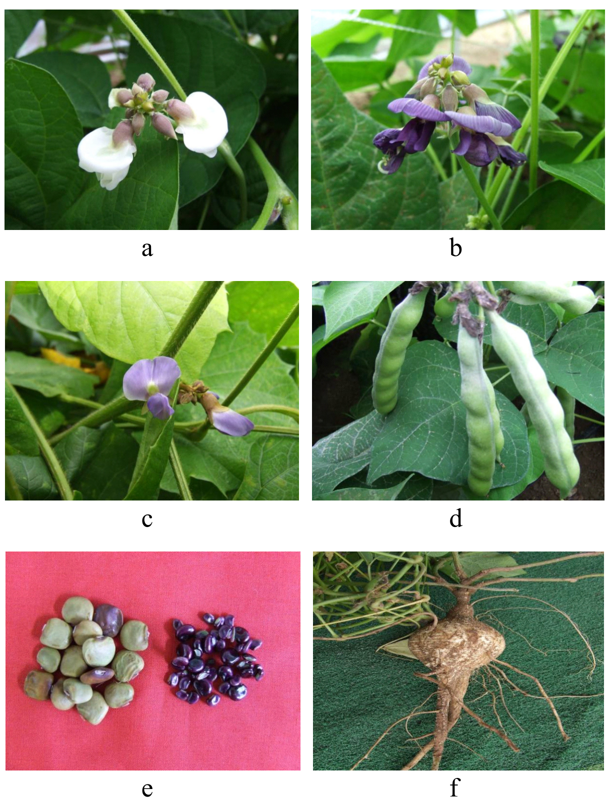

Fig. 1. a. b. Flowers of *P. erosus*, c. Flowers of *P. ahipa*, d. Pods of *P. erosus*, e. Seeds of *P. erosus*, left: mature seed, right: immature seed, and f. Tuber of *P. erosus*.

Changes of seeds and tuber yields

The number of pods, seeds and tuber yield was also evaluated from the two yam beans (Table 2). The Seeds were only obtained in *P. erosus* species. Number of pods and seeds per plant in the *P. erosus* species was 17.4 and 8.5 respectively. The one hundred-seed weight was 23.7 g with determining at 12% water content. The fresh tuber weight of individual plant in both yam beans ranged 188 to 259 g, respectively. The fresh tuber weight was heavier in the *P. erosus* species than in the *P. ahipa* species. Fresh tuber yield per 10a was more increased in the *P. erosus* species than that of the *P. ahipa* species accompanying by increased individual tubers.

Rotenone content of plant organs

The rotenone content was measured from various plant organs with different five solvents on two yam beans (Table 3). The standard rotenone and rotenone separated from the sample (*P. erosus* leaves) was shown in Fig. 2. The each retention time at the peak of standard rotenone and sample rotenone appeared 20.809 and 20.944 min, respectively.

Samples were analyzed and divided into three parts, leaves, immature and matured seeds. In the leaves of the *P. ahipa* species, highest rotenone content was obtained when extracted with non-polar solvent, chloroform to 78.7 μg per g^{-1} dry wt. Rotenone content recovered from chloroform was 3.6 folds much more extracted than that of ethanol. This tendency was similar to results determined from the leaves of the *P. ahipa* species. However, rotenone content was higher in the *P. erosus* species than in the *P. ahipa* species. Rotenone content was also analyzed from the mature and immature seeds for the only *P. erosus* species (Table 3). In the mature seeds, rotenone content extracted to chloroform was 484.7 μg showing a highest concentration among the used five

Table 2. Characteristics of seed and tuber in two yam bean species

Species	No. of pods (plant^{-1})	No. of seeds (pod^{-1})	100-seeds weight (g)	Fresh tuber wt. (g plant^{-1})	Fresh tuber yield (kg 10a^{-1})
P. ahipa	NF	NF	NF	188.6 b	784.6 b
P. erosus	17.5±2.4	8.5±1.0	23.7±0.7	259.2 a	1,078 a

In each column, means followed by a common letters are not significantly different at 5% level by DMRT.
NF: Not formed. Data are standard deviations.

Table 3. Change of rotenone contents as affected by different solvents in leaves and seeds of two yam bean species

Organs	Species	Rotenone content ($\mu g g^{-1}$ dry wt)				
		MeOH	EtOH	n-hexane	Chloroform	Acetonitrile
Leaves	*P. ahipa*	42.4±2.8	21.6±1.8	48.8±3.8	78.7±6.2	49.7±4.1
	P. erosus	47.9±1.5	17.2±1.3	45.5±4.3	75.1±5.6	44.3±3.8
Seeds mature	*P. erosus*	236.0±3.8	288.1±5.9	255.7±9.3	484.7±9.6	464.1±8.7
Seeds immature	*P. erosus*	148.1±5.1	211.3±6.9	257.6±4.8	478.4±8.8	387.7±6.7

Data are standard deviations.

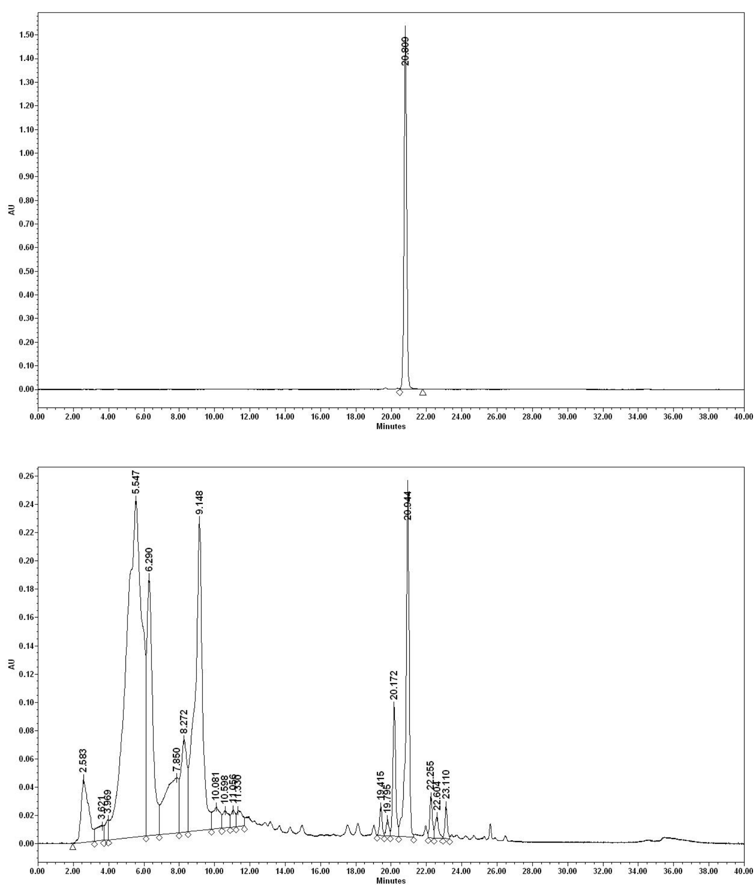

Fig. 2. Chromatogram of rotenone from the standard and leaves of *P. erosus* species.

solvents, whereas the lowest rotenone content was 236.0 μg when extracted with methanol. In the immature seeds, highest rotenone content was observed when extracted with chloroform showing a same tendency like that of the mature seeds. Rotenone content in both mature seeds and immature seeds was always highest as it was extracted with chloroform.

Discussion

For the first time, we examined the growth characteristics, fresh tuber yield, rotenone content of two yam beans, which were introduced from Mexico, grown in Andong, Korea. Vine growth *in Pachyrhizus erosus* was very different from that of *Pachyrhizus ahipa*. Vine length in *Pachyrhizus ahipa* species was three folds longer than that in *Pachyrhizus erosus* species. The *P. ahipa* species were distinguished morphologically from the species of *P. erosus* by the entire leaflets possessing dentate leaflets with short racemes and the general absence of lateral axes. The flowers of *P. erosus* were white or violet in color otherwise violet in flower color showed in the *P. ahipa* species. The number of flowers per lateral raceme of *P. ahipa* species was as high as 12.0 to 16.6 compared to the *P. erosus* species. The wings curl outwards following anthesis, a feature seen only in *P. ahipa* species. The seeds in the *P. erosus* species were purple or thin yellow/grey in color, in some part, kidney shaped, and it measured 9 to 10 mm. The 100-seed weight was 23.7 g (ranged 23.0 - 24.4 g). Meanwhile, the seeds in the *P. ahipa* species were not s*et al*though it flowered. The *P. ahipa* species are predominantly self-pollinating species (Ørting *et al*., 1996). It suggested that the pollen fertility between these yam bean varieties had different mode mediated by insect or wind pollination, and possibly unfavorable cultural conditions such as late plating time, higher temperature with lower humidity during flowering time. Flower colors were apparently distinguished by violet and white in two yam bean species, and white flower in color was only observed in *P. erosus* species. Main stem has differently two plant types which were divided into the semi-erect and the extremely spread. The fresh tuber weight of individual plant in these yam beans ranged 188 to 259 g, and it was heavier in the *P. erosus* species than in the *P. ahipa* species.

Rotenone content in the *P. erosus* species extracted with chloroform was 3.6 folds much more extracted than ethanol extraction. The result was similar to the leaves of the *P. ahipa* species. Rotenone is a natural toxin extracted from the roots and stems of several tropical and subtropical plants belonging to the genus *Lonchocarpus*, *Tephrosia*, or *Derris* (Fukami and Nakajima, 1971). In addition to rotenone, natural extracts of the *Pachyrhizus* spp. contain other active rotenoids such as deguelin, tephrosin, and rotenolone (Fang and Casida, 1998). Rotenone content from leaves and seeds was revealed that mature seeds had a highest concentration extracted with chloroform (Table 3). Rotenone content varied with different solvents. The order of rotenone content found in crude extract obtained by different solvent extraction from the highest to the lowest was mature seed (484.7 ㎍) and leaves (17.2 ㎍) of the *P. erosus* species, respectively. Moreover, the results from this study indicated that chloroform was considerably effective solvent. These results demonstrated that chloroform shows better solvent properties for rotenone extraction since the crude extract using chloroform contains significantly higher amount of rotenone compared to the crude extract using ethanol (Sae-Yun *et al*., 2006). The primary purpose of this study was to estimate the degree of adaption with growth characteristics, and variation of rotenone content for each organ of two yam beans. In fact, tropical tuber crop as a use new cash crop has been overlooked because of potential hazard such as foreign insect, an ecological disturbance in introduction of several foreign crops. In order to establish the introduced crop, further study is needed for better cultural practices of yam bean in Korea.

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