Agung Karuniawan

Cultivation status and genetic diversity of yam bean (*Pachyrhizus erosus* (L.) Urban) in Indonesia

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Cultivation status and genetic diversity of yam bean (*Pachyrhizus erosus* (L.) Urban) in Indonesia

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Agung Karuniawan
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List of Abbreviations

ARTC	Andean Root and Tuber Crops
CATIE	Centro Agronomico de Investigaticon y Ensenanza
PC-i	Principal component-i
DNA	Deoxyribo Nucleic Acid
EC	Erosus cultivated (*P. erosus*) accession
ENT	East Nusatenggara
G	Genotype
ha	Hectare
L	Location
mM	Millimolar
mm	Millimeter
ng	Nanogram
PC	Principal component
PCA	Principal Component Analysis
PCoA	Principal Co-ordinate Analysis
QTL	Quantitative trait loci
R	Replication
RAPD	Random amplified polymorphic DNA
t	Ton
TAE buffer	TRIS acetate buffer
TE buffer	TRIS EDTA buffer
UPGMA	Unweighted pair group method average
UV	Ultra violet
µl	Microliter
µM	Micromolar

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I. INTRODUCTION

1.1. Distribution, cultivation, and uses

The genus *Pachyrhizus* consists of five different species. The Mexican yam bean (*P. erosus*), the Andean yam bean (*P. ahipa*) and the Amazonian yam bean (*P. tuberosus*) are cultivated, whereas *P. panamensis* and *P. ferrugineus* are only found wild (NRC, 1979; Sørensen, 1996). Yam beans *P. ahipa* and *P. tuberosus* are only of local importance in South America, whereas *P. erosus* is grown in many tropical and sub-tropical regions in South America, Asia, and Africa. Yam bean is exclusively used for its tuberous roots (Sørensen, 1996; Sørensen et al., 1997). The fleshy tuberous root has a white succulent interior, which is flavourful and can be eaten raw. All yam bean species are diploid with a basic chromosome number of n = 11, and interspecific hybridisation between all cultivated yam bean species results in fertile and vigorous hybrids (Sørensen, 1996; Heredia, 1996; Grüneberg et al., 2003). *P. erosus*, also called by the common name "jicama", is a favourite food of Central America and South-east Asia, and is becoming popular as a salad vegetable in the US (NRC, 1979; Hoof and Sørensen, 1989; Sørensen, 1996; Sørensen et al., 1997).

Yam bean *P. erosus*, the first *Pachyrhizus* species described scientifically by Linnaeus in 1753, is believed to be native in South-western Mexico (NRC, 1979). Archaeological evidence reveals that this species was grown by the early civilisations of Mexico and Central America, such as the Aztecs and the Mayas (NRC, 1979; Sørensen, 1996). The Spaniards probably introduced the yam bean *P. erosus* to Southeast Asia via the Philippines in the 16th century (NRC, 1979;

Sørensen, 1996). Since then, the cultivation spread to Indonesia and the rest of the Far East as well as into parts of the Pacific (Sørensen, 1996; Hoof and Sørensen, 1989; Sørensen, et al., 1997). The French botanist and explorer, Perrotet, collected samples of the species from Mollucas islands (East Indonesia) in 1821 (Sørensen, 1996).

Yam bean *P. erosus* shows high yields, even without mineral fertiliser application (Heredia-Garcia 1994; Nielsen and Sørensen., 2000). Yields vary according to cultivation practices, plant density, the cultivar used and field irrigation applied. Cultivation practices however depend on socio-economic factors like labour, resource availability, markets and the farming system in which the crop has to fit (Grum, 1990). Tuber yield was about 27 t ha^{-1} in Mexico (Rivas, 1998), 30-40 t ha^{-1} in Ecuador (Arevalo, 1998b), 20-60 t ha^{-1} in Venezuela (Espinoza, 1998), and 50 t ha^{-1} in Brazil (de Melo et al., 1994). In Malaysia, yields of 7-10 t ha^{-1} have been reported (Sørensen, 1996), and in Thailand of 18-24 t ha^{-1} (Ratanadilok et al., 1998). In experimental trials, yields were 80 or 90 t ha^{-1} in the Philippines, Indonesia, and in Mexico (NRC, 1979). Removal of flowers results in higher tuber yield (Adjahossou and Ade, 1998) and biomass production (Arevalo, 1998a).

Yam bean *P. erosus* is an attractive alternative crop for cultivation on poorer soils. The crop has an efficient symbiosis with bacteria (*Bradyrhizobium* strains) and even enrich the soils with the residual nitrogen due to rhizobia bacteria in the root nodules of the plant (NRC, 1979; Grum and Sørensen, 1996; Castellanos et al., 1997). Moreover, root colonisation with arbuscular mycorrhizal fungi (AMF) supports yam beans growth under strong phosphate shortage (Grum, 1998).

Nitrogen fixation of around 200 kg ha^{-1} was recorded in Mexico over two seasons for *P. erosus* (Badillo and Castellanos, 1996; Castellanos et al., 1997). These levels were not affected by flower removal, and were more than twice that fixed by *P. ahipa* (Castellanos et al., 1997). Yam bean can be grown under small amount of additional supply of nitrogen fertiliser (Belford, et al., 2001). Traditionally, in Central and South America yam beans are inter-cropped with maize and common beans (Sørensen, 1996; Sørensen et al., 1997; Castellanos et al., 1997). Flower removal improves adaptation of the species to drought conditions (Diouf et al., 1998). Such characteristics suggest *P. erosus* as an important component of low-input farming system on marginal lands.

Yam bean *P. erosus* is grown in nearly all tropical countries of America, Africa, Asia and the Pacific (Sørensen, 1996; Rivas, 1998; Paull et al., 1988; Sen et al., 1996; Grum, 1990). The habitat of *P. erosus* is along forest edges and in scrub vegetation in areas with an annual dry season, on soil types ranging from deep clay to sandy loam. The species is found from 0 to 1750-m a.s.l., with the majority of records from 500 to 900 m a.s.l., with a rainfall from 250-500 mm to over 1500 mm (Sørensen, 1996). The optimal day/night temperature is about 30/20°C (Grum, 1990; Sørensen, 1996). Well-drained, sandy, alluvial soils are preferred in cultivation (Sørensen, 1996) while excess rainfall causes lower yields and tuber rot (Vaz et al., 1998). The photothermal sensitivity in *P. erosus* was analysed in many studies. In Hawaii, Paull et al. (1988) observed a significant overlap between flowering and tuberization during short days under field conditions. During long days, tuber growth is initiated after 4-6 weeks. Flowering was initiated when the daylight approaches 12.5 hours. During short days, there is an increase of tuber growth (Cotter and Gomez, 1979; Sørensen,

1996). All cultivated yam beans are propagated by seeds and grown as an annual crop, eventhough the plants have a perennial habit (Sørensen, 1996). Today, yam bean *P. erosus* is cultivated almost on the entire Indonesian archipelago (*pers. observation*).

Nutritional properties of *P. erosus* tubers are interesting. Based on 100 g fresh tuber weight, the tuber consists of 78 - 94 % water, 4.6 - 14.9 g carbohydrates, 1.0 - 2.2 g protein and 0.1 - 2.2 g lipids (from several authors compiled by Sørensen, 1996). Based on tuber dry matter weight, starch content is up to 68 % (de Melo et al., 1994). The amylose content of yam bean tuber is about 23 %, which is higher than the mean values found in cassava starch of 17%, but viscosity and absolute density of the starch were similar to cassava (de Melo et al., 1994). Based on dry matter weight, protein content in *P. erosus* is about 10 – 12 % (Evans et al., 1977; NRC, 1979; Zanklan, 2003) which is higher than 2.6 % in cassava, 7.2 % in yams, 5.4 % in sweet potato and 9 % in potato (Rehm and Espig, 1991). Two distinct pools of amino acids were found in *P. erosus* tubers, one utilised mostly for protein synthesis and the other probably stored in the vacuole (Vailant and Desfontaines, 1995). The soluble carbohydrates and the pattern of proteins remained unchanged during root development under long-day environment (Vaillant and Pilet, 1998). Removal of flowers results in higher sugar content (Arevalo, 1998a), and protein content (de Oliveira et al., 1999).

A common characteristic of the genus *Pachyrizus* is the presence of an insecticidal compound called rotenone. Mature seeds of *P. erosus* contain approx. 0.5% pure rotenone and 0.5% rotenoids and saponins (Duke, 1981; Sørensen, 1996). Powdered yam bean seed contains insecticides used against

rice weevil *Sitophilus oryzae* (Bhusan and Ghatak, 1991) and rice moth *Corcyra cephalonica* Staint (Ghatak and Bhusan, 1995). Seed extracts are effective against *Callosobruchus analis* (Kardiman and Wikardi, 1997) and tobacco caterpillar *Spodoptera litura* but could not be used as a fumigant (Sahu and Hameed, 1989). Seed extracts are also effective against pepper bug as a major pest for pepper (*Capsicum spp.*) in Indonesia (Alwi and Soetopo, 2000). Because of rotenone the seeds are not suitable for human consumption, though they could be an interesting source of vegetable oil and protein. Mature seed of *P. erosus* contains 30% of vegetable oil (Duke, 1981; Sørensen, 1996). Santos et al., (1996) and Arellano et al., (2001) mentioned seed proteins have an excellent balance of all essential amino acids, except methionine. The major seed protein fractions of *P. erosus* are albumin (31.0 - 52.1 %) followed by globulin (27.5 %- 30.7 %), while the minor fraction is prolamins (0.8 %) (Arellano et al., 2001).

Yam bean *P. erosus* is recently becoming a more interesting crop in Indonesia. Traditionally yam bean is known as a vegetable tuber crop (Sørensen, 1996) for local markets, but recently it is also considered as a promising cash crop for more commercial purposes. Fresh tubers have been trading between islands mostly for vegetable purpose. "Banjar Titih", a traditional vegetable market in Denpasar (Bali), has been supplied with about 30-40 ton of fresh tubers per day in peak season from East and Central Java (A rural-wholesaler, *pers. comm.*). Several commercial ready-food products, i.e. "Pikel" (fermented tubers, vegetables and fruits, in 15-20 % salt solution), yam bean syrup and juice, "manisan bengkuang" (fresh tuber in sugar solution) or "asinan bengkuang" (fresh tuber in salt solution) have been developed. Furthermore, cosmetics using

yam bean tubers on their products, i.e., "yam bean face tonic" (e.g. from Viva Cosmetics) and "yam bean masker" (e.g. from Mustika Ratu), "yam bean powder" (Indonesia: *Bedak dingin*), a traditional-natural cosmetics powder consisting of rice meal and yam bean starch, with spices and flowers, are commercialised in Java and Sumatra. Fermented yam bean leaves contain high crude protein and low crude fibre percentage and can be used with up to 12 percent in the broiler diet (Nuraini et al., 2000; Nuraini, 2000).

1.2. Germplasm variability

Yam bean *P. erosus* has a herbaceous vine with great variation in the shape of the leaflets, from dentate to palmate (Sørensen, 1996). Variation on morphological characters of the pods are also found within the species, as well as variation in seed colour, ranging from olive-green to brown or reddish brown (Sørensen, 1988, 1990, 1996). Wild specimen of *P. erosus* were clearly separated from the cultivated landraces (Døygaard and Sørensen, 1998). Hernandez (1992) has differentiated three groups within 40 accessions *P. erosus* of the Centro Agronomico de Investigaticon y Ensenanza (CATIE) collection based on 13 qualitative and 6 quantitative traits. The first group was dominated by Mexican accessions, the second was dominated by South American accessions and the third group was mixed of Mexican accessions with accessions from Costa Rica, Rep. of Dominica, and Mauritania. However, accessions from Thailand and Macao were included into the second group. Furthermore, a phylogeny study based on molecular markers conducted by Estrella et al. (1996) showed that the genus *Pachyrhizus* was separated into two main branches based on ecological adaptation. Areas with clear annual dry

seasons in Central America are probably the origin of *P. erosus*, whereas the tropical and sub-tropical rain forest and Andean valleys are centre of diversity for *P. tuberosus* and *P. ahipa*.

The variation of the tuber dry matter content is generally low in the yam bean genpool. The average tuber dry matter content was 13.5 % in *P. erosus*, 20.5 % in *P. ahipa* and 21.1 % in *P. tuberosus* (from various authors compiled by Sørensen, 1996). An exception was found in *P. tuberosus* (Chuin type) from the tropical lowlands of Peru, which has a tuber dry matter content between 24 – 28% (Sørensen et al., 1997; Grüneberg et al., 1998). Forsyth and Shewry (2002) found that in *P. ahipa* tuber dry matter content was 20-25 % of dry weight. In recent studies Zanklan (2003) observed in sun dried sample tubers from unpruned and pruned plants dry matter content of 30% and 31.9% in *P. tuberosus*, 20.7% and 21.6 % in *P. erosus*, 22.3% and 23.0 % in *P. ahipa*, respectively. Zanklan (2003) concluded that there was no effect of pruning on tuber dry matter content. Genetic variation for tuber dry matter content was found within *P. tuberosus* and *P. ahipa* populations, but *P. erosus* showed no significant genetic variation for this trait (Zanklan, 2003).

1.3. Assessment of genetic diversity by morphology, agronomic traits and Random Amplified Polymorphic DNA (RAPD) markers

Morpho-agronomic traits are widely used for plant classification (Stuessy, 1990; Bretting and Widrlechner, 1995). Morphological traits are generally simple, rapid and inexpensive to score, even from preserved specimens (Bretting and Widrlechner, 1995; Arbizu et al., 1997). Hernandez (1992) reported that the flower and vegetative growth traits are the most important factors in classifying

the different accessions of *P. erosus*. Zanklan (2003) evaluated 71 morpho-agronomic data to describe the relationship between the three cultivated yam bean species. Zanklan (2003) suggested to discard the traits which without correlation with one of the first five principal components. Tapia and Sørensen (2003) using 70 morpho-agronomic trait to characterise genetic variation within *P. tuberosus* mentioned that the most useful traits to describe the differences within the species were the shape and type of terminal leaf lobe, growth habit, days to flowering and to physiological maturity.

Genetic variation analysis based on RAPD marker have been widely used in many crop species (Lashermes et al.,1993; Jarret and Austin, 1994; Phippen et al., 1997; Blattner and Mendez, 2001; Carvalho and Schaal, 2001; Maciel et al., 2001; Kump and Javornik, 2002; Ayana et al., 2000; Fu et al., 2002; Gustine et al., 2002; Kaundun and Park, 2002; Sonnante et al., 2002; Massawe et al., 2003). Comparison between morpho-agronomic traits and RAPD markers with discrepancies results have been reported (Brustin and Charcosset, 1997; Duarte et al., 1999; Steiner and Santos, 2001; Cheng et al., 2002; Dahlberg et al., 2002; Bruschi et al., 2003; Wen and Hsiao, 1999). Nevertheless, only Estrella et al., (1998) reported the usefulness of molecular marker techniques to assess the variation and relationships between and within species of yam beans.

1.4. Objectives of the study

Present knowledge on the status of the yam bean in Asia is restricted to the general information provided by Sørensen (1996), Hoof and Sørensen (1989), and Ratanadilok et al., (1994). There are no published data regarding to

cultivation status and genetic diversity based on morpho-agronomic traits and RAPD marker of yam bean in Indonesia. Therefore integrated efforts of collecting the germplasm, recording the cultivation and processing knowledge are important. Investigating genetic diversity based on morpho-agronomic traits and RAPD markers would give benefit to germplasm utilization. The objectives of the present study were to record the cultivation status and to collect yam bean landraces in Indonesia, to analyse morpho-agronomic trait diversity of the yam bean landraces, and to compare the diversity of the yam bean based on morpho-agronomic traits with RAPD marker analysis.

II. EXPERIMENT RESULTS

2.1. Cultivation status of yam bean (*Pachyrhizus erosus*) in Indonesia

2.1.1. Abstract

The yam bean (*Pachyrhizus erosus*) is a legume root crop. It is usually known as a vegetable crop and is frequently used in many Southeast-Asia countries but so far the information about the crop in Indonesia is limited. During a collection trip in Indonesia, a survey was conducted to record its cultivation status. Eight islands were visited, 80 informants were interviewed and 110 landraces were collected. Yam bean is mostly used as a vegetable crop, and the crop is planted under various cropping systems. In West Indonesia yam bean is mainly cultivated as a sole crop and occasionally on commercial scale. In East Indonesia under stress conditions due to drought and low soil fertility, yam bean is predominantly intercropped with maize and cassava. Early maturing yam bean cultivars are preferred in Western regions while late maturing genotypes are cultivated in Eastern regions. Estimated yam bean tuber yield according to information from growers ranged from 10 to 70 t ha^{-1} in West Indonesia, and from 10 to 50 t ha^{-1} in East Indonesia.

2.1.2. Introduction

The genus *Pachyrhizus* (yam bean) is of neotropical origin and comprises three cultivated species that are closely related: *P. erosus* (L.) Urban, *P. tuberosus* (Lam.) Spreng., and *P. ahipa* (Wedd.) Parodi. All three species are exclusively used for its tuberous roots and always propagated by seeds (Sørensen 1996, Sørensen et al., 1997). The tuber is mainly known as a vegetable due to its succulent flesh tuber (Sørensen, 1996) but "Chuin" variety (within *P. tuberosus*) which has higher tuber dry matter is used as a starch source (Sørensen, 1996; Grueneberg et al., 1998). *P. erosus* is the yam bean species that has been successfully introduced into South-east Asian countries and is widely used as vegetable crops in many countries (Hoof and Sørensen, 1989; Sørensen, 1996; Sørensen *et al.*, 1997)

There is no recent information available regarding cultivation status of yam bean in Indonesia. Yam bean was collected from Mollucas islands (East Indonesia) in 1821 (Hoof and Sørensen, 1989; Sørensen, et al., 1997; Sørensen, 1996), but no further information on the crop in Indonesia has been published. Siemonsma and Piluek (1994), NRC (1979), and Sørensen and Hoof (1996) gave an indication that yam bean is a secondary use vegetable and is traditionally found in vegetable markets in Indonesia. Furthermore, there are no passport data of the available yam bean cultivars which are cultivated in different regions of Indonesia. Therefore field survey on the cultivation status and collection of yam bean germplasm in Indonesia were important.

The objectives of this study were to document current cultivation status and to collect the local landraces of yam bean *P. erosus* in different geographic regions of Indonesia. The updating report on cultivation status would provide information on the role and importance of the crop, while the available landraces collected from different ecogeographic regions provide the materials for improvement yam bean cultivars in Indonesia.

2.1.3. Methods

Several initial contacts with institutions and contact persons were established to optimize the field survey and collection trip. Later it was clear that statistic data concerning to cultivation status, agronomic performances, as well as area of cultivation of yam bean in Indonesia were absence. Hence, initial identification of "promising area of cultivation" has to be done by interview with street vendors or with vegetable sellers in traditional markets in every region surveyed.

A questionnaire (Ørting et al., 1996) was used to record the cultivation practices, breeding and selection as well as consumption and knowledge available about processing of yam beans. Since the economic importance of yam bean was also recorded, therefore, informants in this field survey study were not only farmers, but also street vendors selling the crop, traders in local markets, and so-called "rural wholesalers". The questionnaire could be completed in about two hours since the very different "mother tongues" between Indonesian islands, therefore local translators were important in this survey. The collected accessions were obtained from farmers who cultivated the yam bean.

The soil pH has been measured from every region where the yam beans are collected. The soil samples were taken from five different spots within a field. The soils were then mixed and a 500 gram from them was taken and stored in a plastic bag before sent to the soil biology laboratory in Bogor Agricultural University for soil pH measurements.

Field surveys were carried out in the late dry season from August to October 2001 in Java, Bali, Timor, Sumba, Flores, and Sulawesi and, during the wet season from January to February 2002, in Sumatra and Kalimantan. Collection trip in Sumatra has been done by Indonesian counter part (Dr. Iswandi Anas) from Bogor Agricultural University. Since cultivation practices applied by farmers in Java and Sumatra were more intensive, results are presented separately for the Western regions (Java and Sumatra) and the Eastern regions (Bali, Sumba, Flores, West Timor, Sulawesi, and Kalimantan). The collection sites of yam bean (*P. erosus*) in Indonesia are shown in Figure 2.1.

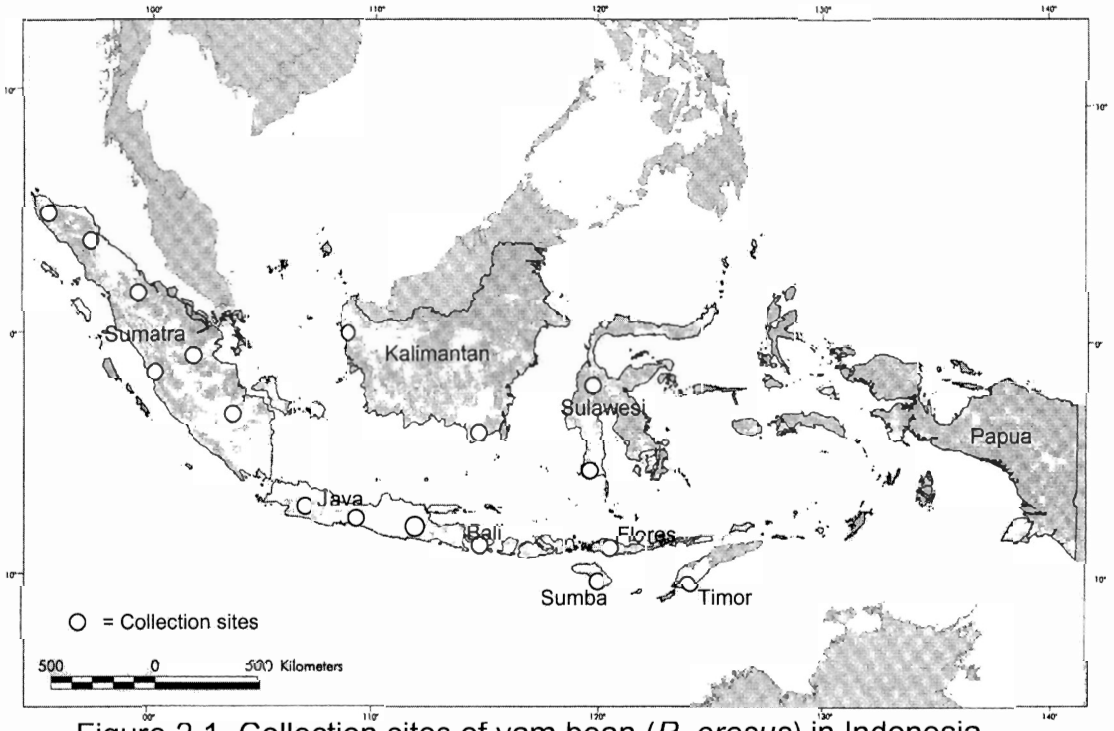

Figure 2.1. Collection sites of yam bean (*P. erosus*) in Indonesia

2.1.4. Results

During the survey, 81 informants were interviewed as well as 110 yam bean landraces were collected. Several local names for yam bean were recognised e.g., "Bengkuang", "Besusu", and "Huwihiris" in East, Central, and West Java, respectively. "Bengkuang" is also well known in Sumatra, Bali, Sulawesi, as well as in West Kalimantan, whereas the names "Uas" or "Bose" are used in West Timor, Sumba and Flores.

Table 2.1. Collection site characteristics where the yam bean is grown in Indonesia

Island	Province	Localities	Altitude (m asl)	Rainfall (mm/year)	Soil pH
Sumatra	Aceh	Kuala Simpang	87	1900	5.2
	North Sumatra	Brohol, Baktikarya, Padang bulan	25-50	1900-2000	4.0-5.4
	Riau	Bangkinang	60	1900	4.3
	West Sumatra	Kuranji, Padang, Pauah, Lubuak Munturun, Limau Manih	150-200	1500-2300	3.9-4.3
	Jambi	Muara Bungo	100-150	1500-2000	3.9-5.0
	South Sumatra	Palembang	25	2250	4.5
Java	West Java	Abean, Arjasari, Bojonghaluang, Cibeduk, Cipete, Katulampu, Papuaran, Palasari, Tanjungmekar,	200-600	1200-3500	5.1-7.3
	Central Java	Kedungbulan, Kali Gede, Linggasari, Mulyasari, Situgede, Siti Bentar, Tanjung Sembir, Tersobo, Winong	10-25	2000-4400	6.2-6.5
	East Java	Bumiayu, Gunungronggo, Jatipehlandak, Jatisari, Kadipaten, Krenceng, Lemahputih,	10-440	700-2600	5.7-7.3
Bali	Bali	Pecatu, Penebel, Senganan, Tajen	50-600	1000-1800	6.6-7.6
Kalimantan	West Kalimantan	Pontianak, Siantan tengah, Pasir Panjang, Sijangkung I & II	3-300	2400-3200	4.5-5.0
	South Kalimantan	Parit Tokaya	20	2500	5.3
Sulawesi	South Sulawesi	Bontobiraeng, Bontolangkang	25-50	700-1800	4.5-4.8
	Central Sulawesi	Bale, Bobo, Pekoare	6-200	1800-3200	6.3-6.7
West Timor	East Nusatenggara	Benhutu, Eonbesi, Oof	300-800	780-1000	7.2-7.7
Sumba	East Nusatenggara	Keretana, Nggalu, Wangga, Weetebula	12-300	600-1050	6.1-7.4
Flores	East Nusatenggara	Enturia, Borokanda, Riaraja, Wolotopo	100-600	850-1100	6.9-7.8

Yam bean in Indonesia is cultivated under various soil pH and rainfall conditions. (Table 2.1). In Western regions, especially in Central Java and West Sumatra, yam bean is mostly cultivated at locations that are traditionally known by the rural-wholesalers as production centres. In these areas, different cultivation practices with respect to rainy and dry seasons were recognised.

Some farmers in Java explained that during the rainy season, yam bean is cultivated with a drainage system so that the plant would not be flooded by excessive water. In the late rainy season, yam bean is planted after rice (*Oryza sativa* L.) harvest to utilise the residual soil moisture. In the Eastern regions, yam bean is mainly cultivated in rain-fed areas during the late rainy season (Table 2.2.).

In both regions, planting periods started from June to December and, hence, the harvest may take place at diverse times (Table 2.2.). Hence, the average cultivation period for tuber production was not the same in all areas. Farmers in the Eastern regions applied mineral as well as organic (dung) fertilizer in relatively small amounts and tilled the fields less as compared to the cultivation practices recorded in the Western regions. Estimated average tuber yields in the Eastern regions were lower than in the Western regions. Additionally, most informants reported that there were no significant yield losses due to pests, diseases or cattle. The only potential pests causing tuber damage were rats, and wild pigs in localities bordering forests.

Table 2.2. Cultivation area and practices, tuber yields and market prices in the Eastern and Western regions of Indonesia.

	East Indonesia				West Indonesia			
	No. of informants	Averages	Minimum	Maximum	No. of informants	Averages	Minimum	Maximum
Yam bean cultivation area (m^2)	14	910	100	5 000	35	880	65	5 000
Land preparation depth (cm)	29	25	0	45	35	25	10	40
Start of planting	25	June	January	December	34	July	March	December
Plant distance between row (cm)	23	30	10	100	35	25	10	100
Plant distance within row (cm)	23	20	10	50	35	20	5	30
Seed planting depth (cm)	20	2,5	2	5	13	3	2	5
N fertilization (kg Urea ha^{-1})	5	81	30	150	29	86	0	400
P fertilization (kg TSP ha^{-1})	16	16	0	100	29	67	0	357
K fertilization (kg KCl ha^{-1})	16	0	0	0	29	7	0	180
Organic fertilizer (kg dung ha^{-1})	15	440	0	2 000	31	600	0	6 670
Reproductive pruning (frequency)	15	1.5	0	3	13	2	0	4
Cultivation period (months)	29	5	4	12	35	4.5	4	6
Estimated tuber yield (t ha^{-1})	6	25	10	50	31	35	10	70
Tuber sold to local market (%)	20	68	10	90	12	80	70	100
Tuber price in local market (ID Rp kg^{-1})	21	1 650	650	3 750	34	650	200	1 500
Tuber price (US$ kg^{-1})*		0.17	0.07	0.39		0.07	0.02	0.16

* Exchange rate assumption US$ 1.00 was equivalent to ID Rp. 9 500,00

Average cultivation areas in both regions did not differ much but cropping systems were different. In Western regions, 26 out of 35 informants planted yam bean as a monocrop following rice. Only on very rare occasions (9 from 35 farmers), yam bean was grown together with papaya (*Carica papaya* L.), chilli (*Capsicum annuum* L. var. *annuum*), or teak (*Tectona grandis* L.). In contrast in Eastern regions, 20 from 21 farmers cultivated yam bean intercropped mostly with maize (*Zea mays*) and cassava (*Manihot esculenta*). Farmers in West Timor intercropped also yam bean with cotton (*Gossypium* spp.). Furthermore the tilling practices was less intensive in Eastern regions since only 5 from 23 farmers tilled the field compared to 25 out of 35 farmers in Western regions. Irrigation was not applied in the Eastern regions.

In all areas visited yam bean was regarded as a vegetable crop. If compared to other tuber crops grown in Indonesia, like sweet potato and cassava, yam bean is a minor crop. Instead of for vegetable, tubers are also used for a skin whitening and smoothing agent for traditional cosmetics as well as for modern cosmetics industries, especially in Java. In Eastern regions (Flores and West Timor), flowers and immature pods are also used as vegetables. Nevertheless in the localities known as production centre areas in Central Java (Pemalang, Prembun, Kendal) and West Sumatra (Padang), yam bean is cultivated intensively. In such regions, most fresh tubers are sold to local markets through rural-wholesaler. In the Eastern regions most of the tubers are sold directly by the farmers at local markets.

Farmers maintain the yam bean in both the Western and in the Eastern regions. In the Western regions, the informants also buy new seed from their neighbors or collectors every planting season (Table 2.3).

Table 2.3. Seed origin, multiplication and breeding efforts.

Practices	Western regions		Eastern regions	
	No. of informants	Yes	No. of informants	Yes
Seed self maintained	11	6	25	23
Seeds multiplication every season	11	10	17	11
Seeds buy every season	11	5	17	3
Seed exchanges between farmers within the village	11	6	17	10
Tuber exchanges between farmers within the village	11	0	17	2
Tuber direct selection	11	8	17	7
Seed selection	11	1	17	1
Vigorous plant/vegetative selection	11	0	17	6

Yam bean is a perennial plant but it is cultivated as an annual tuber crop. Although the crop is usually propagated by seed, selected tubers are often used to maintain seed production. Fresh tubers were selected at the harvesting time from the fields. Tubers of 400-500 g with round shape were mostly selected by the farmers for seed productions. The most popular strategy in all areas studied was to grow selected tubers in specially designated places surrounding the field where individual plants are trellised. Alternatively, tubers were planted under fruit trees within home gardens to produce seed. In some localities in the Soe highland of West Timor, farmers collected yam been seeds from naturalized plants in the forest close to the village. Such naturalized materials from forests

are not easy to classify as wild yam bean, and further investigations are needed to determine the phylogeny of the species. In both regions, half of the informants exchanged seeds, but tuber exchange was recorded to be very rare.

Several yam bean selection strategies based on indigenous knowledge are applied. In the Western regions, tubers are often selected based on tuber size and shape. Stems of these selected tubers are cut to allow the new buds to grow for seed production. Farmers in the Western regions (especially in West Sumatra, West and Central Java) prefer early-maturing plants, intermediately sized tubers (400–500 g), in combination with less fiber and sweet tuber taste. In West Timor, Flores, and Sumba, farmers preferred late-maturing plants and more succulent flesh, but larger tubers (800–1000 g). The second strategy is a selection based on good shape and healthy status of the seeds. The third strategy, which is applied in the Eastern regions only (Sumba, Flores, West Timor, South Sulawesi), is to select vigorous plants within populations in the field, i.e., having strong and erect stems, broad green and healthy leaves, and producing large pods. The selected vigorous plants are then tagged for seed production.

2.1.5. Discussion

Yam bean (*P. erosus*) was commonly cultivated on eight islands surveyed suggesting the suitability of the crop for various cropping systems in Indonesia. The yam bean is grown in all regions despite the differences in soil properties as well as annual precipitation.

Considering to the cultivation area and cultivation practices, yam bean in Indonesia is a typical crop for small-scale farming, whereas in Mexico yam bean is extensively grown for large-scale of commercial production (Sørensen et al., 1997). Nevertheless, also in Mexico yam bean is often intercropped with maize and beans (Sørensen, 1996). In South Americas, yam bean is intercropped with chilli (*Capsicum spp.*), sesame (*Sesamum orientale* L.), groundnut (*Arachis hypogaea* L.), or tomato (*Lycopersicon esculentum* Miller) (Sørensen *et al.*, 1997). Yam bean is a valuable crop both in intercropping and crop in rotation due to the residual nitrogen supplied to other crops (Sørensen et al., 1997; Castellanos et al., 1997; Belford et al., 2001). Intercropping systems may minimize the risk of yield loses of major crops due to drought, as it is explained by most of informants in the Eastern regions where the rainfall is the major constraint.

The estimated of the average population densities were 166 666 to 200 000 plants ha^{-1} in East and West Indonesia, respectively. However such density were higher compared to 95 500 plant ha^{-1} in Malaysia, 133 000 plant ha^{-1} in Maharastra, India, or 110 000 plant ha^{-1} in Nayarit and Guanajato Mexico (Sørensen, 1996). Such high plant densities, especially in Western regions (Sumatra and Java), were due to the strategy of producing marketable intermediate sized tubers of 400–500 g. These different values of population densities of yam bean *P. erosus* from South-east Asian landraces compared to

their ancestral accessions from Central America may be the result of selection under differences agricultural practices and ecological conditions.

The estimated average tuber yield ranges from 25 to 35 ton ha^{-1} from the Eastern and in Western regions of Indonesia, respectively, were considerably higher than those from Malaysia (7–10 ton ha^{-1}; Sørensen, 1996), Thailand (18–24 ton ha^{-1}; Ratanadilok et al., 1994), or Sierra-Leone (22.8 ton ha^{-1}; Belford et al., 2001). But all these figures were much lower than Brazil (50 ton ha^{-1}; de Melo et al., 1994), Tonga (77–125 ton ha^{-1}; Nielsen and Sørensen, 2000), or Mexico (100–145 ton ha^{-1}; Heredia-Garcia, 1994).

The established selection strategies based on indigenous knowledge have proved successful to establish the landraces that fit to local needs. Nevertheless, since the selection based only on several tubers from generation to generation for seed production, it can also be expected to lead to a narrower genetic basis of the landraces within a particular region in Indonesia. However, seed harvested from the whole population without selection, that also applied in some localities within given regions in Indonesia, could preserve the genetic variation within population. A method involving the harvest of only one pod per plant through the entire population for seed production in South America (Grüneberg, 1998) could be more suitable to maintain the full range of genetic variation within any single landrace.

The low dry matter content in yam bean tubers, on average 13%, based on results from various authors compiled by Sørensen (1996), leads to the yam bean being consumed raw or fresh as a vegetable or fruit crop. In the Western regions, beside the vegetable market, there is an established cosmetics industry for tubers. Yam bean face tonic, yam bean masker, and yam bean cosmetic powder are natural cosmetics developed from yam bean starch. In the Eastern regions, there is no established market for yam bean tubers. Nevertheless, the market prices always fluctuate depending on the seasonal demand in both regions.

The result have generally established a collection of 110 yam bean accessions from 8 Indonesian islands visited. Yam bean is mostly used as a vegetable crop, and the crop is planted under various cropping systems. The diversity of these materials from very different ecological regions will be investigated in further studies reported in chapter 2.2 and 2.3.

2.2. Genetic diversity of yam bean (*Pachyrhizus erosus*) revealed by morpho-agronomic traits

2.2.1. Abstract

The yam bean (*Pachyrhizus erosus*) is a tuberous legume which is cultivated in South and Central America and in many countries of Southeast Asia. The objective of this study was to estimate the genetic diversity among yam bean landraces collected from different islands of Indonesia based on morpho-agronomical traits. Thirty-one selected yam beans accessions from diverse ecological regions of Indonesia, i.e. Sumatra, Java, East Nusatenggara (ENT) and 9 accessions from South and Central America were examined. The yam bean was grouped into four groups, i.e., America, Sumatra, Java and ENT, to perform genetic variation by partition anova analysis within and between groups. Field trials were performed at two locations near Bogor, Indonesia, in a randomised block design with two replications. The partition anova revealed no significant different for all traits within American yam bean, while significant differences for some traits within groups of Sumatra, Java and ENT were observed. Significant genetic variances were found between groups for 9 out of 15 traits analysed. Time to flowering, time to first pod development, petiole length, leaf width and leaf length have played an important role in the classification of the yam bean population. Cluster analysis showed that accessions from Sumatra were clearly separated from accessions from Java and ENT. Furthermore, American accessions were separated from Indonesian yam bean materials.

2.2.2. Introduction

Yam bean (*P. erosus*) is originated from Central America. The Spaniards probably introduced the yam bean to Southeast Asia via the Philippines in the 16th century (NRC, 1979; Sørensen, 1996). The plant then spread to Indonesia and the rest of the Far East as well as into parts of the Pacific (Sørensen, 1996; Hoof and Sørensen, 1989; Sørensen, et al., 1997). The Asiatic yam bean cultivars are possibly derived from introduction from the Meso-American region (Estrella et al., 1986). Today yam beans are widely grown in many islands of Indonesia as a vegetable crop (Sørensen, 1996; Hoof and Sørensen, 1989; Sørensen, et al., 1997). Due to evolutionary forces the yam bean in Indonesia may have genetic differentiation to its ancestor landraces of Central and South America. A species may show morphological variations as adaptation to different selection pressure (Nevo et al., 1986; Morrison and Weston, 1985; Hageman and Fahselt, 1990).

Morphological and agronomic traits are traditionally used to identify the differences within the genotypes of a crop. Both traits might be not significantly distinct within a species level and unstable due to environmental influences, but until recently, plant classification was based nearly exclusively on such traits (Stuessy, 1990; Bretting and Widrlechner, 1995). Recording the morphological traits are generally simple, rapid and inexpensive to score, even from preserved specimens (Bretting and Widrlechner, 1995). Morphological characterisation by grouping accessions according to their morphological similarities and by cluster analysis proved to be accurate and reliable tools to identify morphotypes in Andean root and tuber crops (ARTC) collections (Arbizu et al., 1997).

Morpho-agronomic traits can be used to distinguish the variation between yam bean. Hernandez (1992) reported that the flower and vegetative growth traits are the most important factors for classifying the different accessions belonging to the Mexican yam bean *P. erosus*. According to Hernandez (1992), number of nodes of the main stem, length of stem, number of leaves were closely associated with the flower characters, i.e. inflorescence per stem, date of flower initiation and duration of flowering. Zanklan (2003) used 71 morpho-agronomic data to construct the phylogeny relationship between three cultivated of American yam bean species. The *P. erosus* could be clear separated from other groups of *P. ahipa* and *P. tuberosus* (Zanklan, 2003). Based on morphological traits, wild specimens on *P. erosus* were clearly separated from the cultivated ones (Døygaard and Sørensen, 1998). The objective of this study was to analyse morpho-agronomic trait diversity of the yam bean landraces.

2.2.3. Materials and methods

Plant materials

Forty yam bean landraces consisting of 31 accessions from Indonesia and 9 accessions from South and Central America were examined (Table 2.4.). All accessions were cultivated materials, and were considered as representatives of the variability formed within different geographic areas in Indonesia and in Central and South America regions.

Table 2.4. Acessions code, area of origin, altitude, and grouping of the acessions based on area of origin

No.	Acc. Code	Area of origin	Altitude (m asl)	Grouped
1	EC033	Yucatan, Mexico	100	America
2	EC533	Macao	Unknown	America
3	EC041	Jutiapaca, Guatemala	1100	America
4	EC550	Guanajuato, Mexico	1750	America
5	ECKew	Mexico	Unknown	America
6	EC104	Yucatan, Mexico	100	America
7	EC557	Guanajuato, Mexico	1500	America
8	EC040	Jutiapa, Guatemala	1100	America
9	EC114	Para, Brazil	Unknown	America
10	B-26/NS	P. Siantar, North Sumatra	50	Sumatra
11	B-27/NS	Medan, North Sumatra	25	Sumatra
12	B-28/R	Bangkinang, Riau, Sumatra	60	Sumatra
13	B-29/WS	Kuranji, West Sumatra	200	Sumatra
14	B-30/WS	Lubuak Munturun, West Sumatra	600	Sumatra
15	B-31/WS	Limau Manih, West Sumatra	200	Sumatra
16	B-33/J	Muara Bungo, Jambi, Sumatra	150	Sumatra
17	B-137/Ac	Aceh, Sumatra	87	Sumatra
18	B-138/Ac	Aceh, Sumatra	87	Sumatra
19	B-141/NS	North Sumatra	Unknown	Sumatra
20	B-130/WS	Padang, West Sumatra	200	Sumatra
21	B-39/WJ	Kedunghalang, West Java	600	Java
22	B-42/WJ	Tanjung Mekar, West Java	700	Java
23	B-43/WJ	Arjasari, West Java	800	Java
24	B-54/WJ	Batujajar, West Java	500	Java
25	B-55/CJ	Tersobo, Central Java	25	Java
26	B-56/CJ	Prembun, Central Java	25	Java
26	B-57/CJ	Kembaran, Central Java	25	Java
28	B-132/CJ	Prembun, Central Java	25	Java
29	B-58/EJ	Tajinan, East Java	1000	Java
30	B-59/EJ	Panggungrejo, East Java	100	Java
31	B-61/EJ	Kepung, East Java	100	Java
32	B-63/EJ	Wringin Anom, East Java	20	Java
33	B-74/ENT	Kupang, West Timor	300	East-Nusatenggara
34	B-77/ENT	Molu Utara, West Timor	800	East-Nusatenggara
35	B-80/ENT	Ndona, Flores	600	East-Nusatenggara
36	B-86/ENT	Enturia, Flores	600	East-Nusatenggara
37	B-89/ENT	Pandawai, East Sumba	300	East-Nusatenggara
38	B-90/ENT	Pahungalodu, East Sumba	300	East-Nusatenggara
39	B-91/ENT	Laratama, West Sumba	12	East-Nusatenggara
40	B-94/ENT	Kupang, West Timor	20	East-Nusatenggara

Field plot design

Field trials were performed from January to May 2003 at two locations, i.e., Leuwikopo and Yasmin, in Bogor district, Indonesia. The locations were located at 250 m a.s.l., and differed mostly on soil properties. Luewikopo has soil pH of 4.8 with soil texture of 8.45% sand, 51.29 % silt, 40.26 clay. Location Yasmin has soil pH of 5.2 with soil texture of 8.09 % sand, 39.59 % silt, 52.32 % clay. Experimental design was a randomised block with two replications at each location. At the first location, each plot consisted of 4 rows of 10 plants each and a plot size of 2 m by 2.5 m. The distance between plots was 0.75 m. At the second location, each plot consisted of 1 row of 10 plants each and a plot measured 1 m by 2 m. Two rows were spaced 0.5 m apart and the distance between plants within a row was 0.25 m. A single seed was sown per hole at a depth of about 2 cm. There were no application of irrigation, reproductive pruning, fertilizer or pesticide.

Morphological traits measurements

The morphological data (13 traits) measurements have been done four months after seed sowing. While two agronomic traits, i.e., time to flowering and time to first pod development were measured according to the appropriate stages. Codes and procedures of recording for each character are listed in Table 2.5. Data recording is based on the IPGRI descriptor list for *Ipomea batatas* (sweet potato), with small modifications. Data on single plant basis were recorded on 5 randomly individuals within the two center rows at the first location. At the second location, samples were taken from 5 plants in the center of each row.

Table 2.5. Agronomic and morphological characters evaluated, code and procedure of measurement

Observations	Code	Procedure of recording
Time to flowering	TF	in days – from sowing to 50% of plants flowering within center rows
Time to first pod development	FPD	in days – from sowing to first pod development – min. 2 cm pods length within 2 center rows
Inflorescence length	IL	in cm – at time of full flowering – 5 plants within plot center (5 inflorescence per plant).
Number of pods per inflorescence	PNIL	in number - counted at 4 months after sowing – 5 plants within plot center
Stem diameter	SD	in cm - at 4 months after sowing – 5 plants within plot center (5 internode per plant).
Internode length	ITL	in cm - at 4 months after sowing – 5 plants within plot center (5 internode per plant).
Petiole length	PL	in cm - at 4 months after sowing – 5 plants within plot center (5 petiole per plant).
Leaflet length	LL	in cm – at 4 months after sowing – 5 plants, within plot center (6 leafs per plant)
Leaf width	LW	in cm – at 4 months after sowing – 5 plants, within plot center (5 leafs per plant)
Pod length	PL	in cm – at 4 months after sowing – 5 plants within plot center (5 pods per plant).
Pod width	PW	in cm – at 4 months after sowing – 5 plants within plot center (5 pods per plant).
General outline of leaf	LO	in scores - at 4 months after sowing Rounded to almost divided (scores from 1 to 5: 1=cordate, 2=triangular, 3=hastate, 4=lobed, 5=almost divided – 5 plants within plot center (5 leaflets per plant).
Leaf green colour	LC	in scores - very light to very dark (5 scores) – at 4 months after sowing – 5 plants within plot center
Lateral leaflet peak	LP	in scores - entire to very deep (scores from 1 to 5: 1=no lateral peak, 2=very slight peak, 3=slight, 4=moderate, 5=deep) – at 4 months after sowing - 5 plants within plot center (5 leaflets per plant).
Stem Colour	SC	in scores - green to mostly purple. Scores from 1 to 5: 1= green, 2= green with few purple spots, 3= green with many purple spots, 4= green with many dark purple spots, 5= mostly purple - at 4 months after sowing - 5 plants within plot center.

Statistical Analysis

The accessions were grouped according to collection sites to analyse the diversity within and between group of accessions. Group 1 consisted of Central and South American yam beans, group 2 consisted of Sumatra yam beans, group 3 accessions from Java, and group 4 accessions from East Nusatenggara. The PLABSTAT software version 2N package (Utz, 1997) was used to analyze the partitioning ANOVA within and between groups. Genetic diversity between accessions was determined by multivariate analysis. In a first step all 15 traits mean values across replications and location were standardized to arrange the data sets for multivariate statistics by principal component and cluster analysis. The NTSYSpc sofware Version 2.10q Applied Biostatistics Inc. (Rohlf, 2001) was used to determine principal components, corresponding eigenvalues and proportions of eigenvalues, the scores of the principal components, as well as the cluster analysis. The relationships of accessions were presented by plotting the scores of the first, and second principal components. The resulting matrix of Euclidean distances was used to produce a UPGMA cluster dendrogram. Pearson coefficient correlation by SigmaStat sofware was performed to analyze the correlation of principal components with mean values of traits.

2.2.4. Results

Genetic variances within and between groups

The yam bean accessions showed significant genetic variation for different traits within each group (Table 2.6). Nevertheless, group 1, consisting of accessions from Central and South American showed non-significant genetic variance for all traits. Yam bean accessions collected from Sumatra (group 2) showed significant

genetic differences for petiole length (PL), time to first pod development (FPD), and steam colour (SC). Group 3 consisting of accessions from Java showed significant differences on inflorescence length (IL), petiole length (PL), time to flowering (TF), and stem colour (SC). Within group 4 (acessions from East Nusatenggara) only pod number per inflorescence (PNIL) showed a significant genetic variation. Mean values of maturity (TF and FPD) of the East-Nusatenggera (ENT) accessions (group 4) were higher compared to the three other groups. The ENT accessions show a later maturity compared to the other groups

The significant differences of some traits between groups were observed, i.e., pod width (PW), pod length (PL), leaf width (LW), leaflet length (LL), time of flowering (TF), time to first pod development (FPD), leaf green color (LC), stem Color (SC), lateral leaflet peak type (LP) (Table 2.6.). It is however indicated that Indonesian yam beans were different from some traits to their ancestor landraces from the Americas. Table 2.6 also shows accessions from Sumatra and America with larger pod size and smaller leaf sizes. Accessions from ENT poses smaller pod size but larger leaf size.

Table 2.6. Mean values and mean squares from analysis of variance for four regional groups from the combined analysis over two locations

Traits	Mean values				Mean squares				
	America (N=9)	Sumatra (N=11)	Java (N=12)	East Nusa Tenggara (N=8)	Between groups	America (N=9)	Sumatra (N=11)	Java (N=12)	East Nusa Tenggara (N=8)
Inflorescence length (IL)	32.9	29.9	32.0	33.5	83.19	100.20	55.39	179.54**	82.96
Pod number per inflorescence (PNIL)	3.6	3.9	3.7	3.9	0.00	2.78	0.80	2.23	4.88*
Pod width (PW)	1.3	1.3	1.2	1.2	0.104**	0.014	0.010	0.026	0.014
Pod length (PL)	11.6	11.5	10.9	10.6	7.97**	0.37	0.53	0.55	0.35
Stem diameter (SD)	0.4	0.4	0.4	0.4	0.0054	0.0003	0.0036	0.0038	0.0033
Internode length (ITL)	11.6	11.9	12.3	11.9	2.59	10.62	2.92	4.73	1.98
Petiole length (PTL)	8.9	8.7	9.2	8.9	1.99	1.91	2.47*	3.80**	1.23
Leaf width (LW)	8.9	8.5	9.2	8.8	4.33**	1.75	1.51	1.80	0.76
Leaf length (LL)	7.8	7.9	8.8	8.8	13.49**	1.33	1.91	1.63	0.67
Time to flowering (TF)	72.4	73.4	81.6	89.0	2026.15**	382.36	460.18	621.34*	323.07
First pod development (FPD)	84.8	85.6	90.0	98.8	1317.65**	391.58	484.02*	354.52	284.77
Leaf colour (LC)	2.2	2.5	2.3	2.4	0.63**	0.13	0.08	0.04	0.09
Stem colour (SC)	1.9	2.9	2.0	1.8	11.19**	0.36	1.81**	0.96**	0.35
Leaf outline (LO)	3.9	3.9	3.9	3.8	0.019	0.015	0.020	0.020	0.015
Leaf Peak (LP)	1.5	1.6	1.7	1.7	0.238**	0.075	0.032	0.059	0.026

*,** Significant at the 0.05 and 0.01 levels of probability, respectively

Table 2.7. Minimum, maximum, means, standard deviation, heritability and means squares from the combined analysis over two locations for all 40 accessions

Traits	Min	Max	Mean	Std.Dev	Heritability	MS			
						G	L	GxL	Error
Inflorescence length (IL)	18.0	41.6	31.91	5.16	50.6	106.685*	657.587+	52.623+	36.282
Pod number per inflorescence (PNIL)	2.2	6.1	3.78	0.74	18.3	2.257	39.799	1.844	1.219
Pod width (PW)	1.1	1.4	1.24	0.07	8.5	0.023	0.047	0.021**	0.010
Pod length (PL)	10.1	12.2	11.16	0.51	15.6	1.043	21.114+	0.879	0.828
Stem diameter (SD)	0.3	0.4	0.39	0.03	5.3	0.003	0.037	0.002	0.003
Internode length (ITL)	9.8	14.4	11.97	1.09	0.0	4.816	11.698	5.071*	2.812
Petiole length (PTL)	7.8	10.8	8.92	0.79	61.1	2.470**	15.824	0.959	0.747
Leaf width (LW)	7.7	10.2	8.86	0.64	41.9	1.670*	0.452	0.970	0.936
Leaf length (LL)	6.8	9.8	8.33	0.69	57.9	1.947**	2.617	0.818	0.668
Time to flowering (TF)	63.0	102.8	78.83	12.09	56.9	585.521**	5110.393*	251.892**	84.595
First pod development (FPD)	72.3	111.3	89.42	10.69	60.6	456.893**	222.590	179.614**	53.158
Leaf colour (LC)	1.9	2.8	2.36	0.17	31.4	0.122	0.009	0.084	0.068
Stem colour (SC)	1.5	3.9	2.20	0.66	86.7	1.731**	0.233	0.229	0.261
Leaf outline (LO)	3.7	3.9	3.86	0.06	0.0	0.017	0.011	0.022	0.018
Leaf Peak (LP)	1.4	2.0	1.65	0.13	27.6	0.063	0.001	0.045*	0.026

*,** Significant at the 0.05 and 0.01 levels of probability, respectively

The combined ANOVA over two locations showed significant genetic variations for seven morpho-agronomical traits (Table 2.7). Within those six traits, time to flowering (TF) and time to first pod development (FPD), width (PW), and lateral leaflet peak (LP) have been influenced by the G X L interaction. Leaf properties (leaf width, colour, outline and peak) performed a similar variation for all yam bean accessions over two locations.

Correlation between15 morpho-agronomic traits

Time to flowering (TF) and FPD showed a highly positive significant correlation (Table 2.8). Both traits have highly negative significant correlation to pod width (PW), pod length (PL), but they have a positive significant correlation with petiole length (PTL), and leaf length (LL). Leaf width (LW) and leaf length (LL) showed positive correlation with inflorescence length (IL), pod length (PL), stem diameter (SD), internode length (ITL), and petiole length (PTL). It indicated that the longer the time to flowering and time to first pod development, the longer the inflorescence length, petiole length, and leaf length, but the smaller the pod width and pod length. Therefore, earliness (FP and FPD) in this yam bean population is positive correlated with bigger pod size, but negative correlated with vigorous vegetative traits.

Table 2.8. Coefficients of correlation between 15 morpho-agronomic traits on 40 yam bean landraces

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	PNIL	0.28														
3	PW	-0.16	0.03													
4	PL	0.02	0.08	0.64**												
5	SD	0.15	0.03	0.05	0.07											
6	ITL	0.19	0.37*	0.11	0.12	0.12										
7	PTL	0.39*	0.32*	-0.17	-0.13	0.50**	0.12									
8	LW	0.43**	0.18	-0.31*	-0.10	0.56**	0.45**	0.69**								
9	LL	0.36*	0.22	-0.44**	-0.48**	0.43**	0.37*	0.65**	0.83**							
10	TF	0.31*	0.20	-0.62**	-0.61**	0.11	-0.17	0.55**	0.28	0.56**						
11	FPD	0.34*	0.28	-0.57**	-0.57**	0.14	-0.15	0.545**	0.30	0.56**	0.95**					
12	LC	-0.15	0.09	0.21	-0.06	0.01	0.13	-0.27	-0.26	-0.09	-0.14	-0.05				
13	SC	-0.21	0.02	0.29	0.34*	0.01	0.23	-0.26	-0.28	-0.37*	-0.34*	-0.32*	0.44**			
14	LO	0.08	0.01	0.16	0.28	0.15	0.16	-0.17	0.04	-0.17	-0.40**	-0.28	0.08	0.08		
15	LP	0.27	0.06	-0.34*	-0.45**	-0.09	-0.07	0.04	0.09	0.36*	0.37*	0.29	-0.01	-0.27	-0.27	
		IL	PNIL	PW	PL	SD	ITL	PTL	LW	LL	TF	FPD	LC	SC	LO	LP

*,** Significant at the 0.05 and 0.01 levels of probability, respectively

Principal component analysis

To consider 15 traits simultaneously, a principal component analysis was performed (Table 2.9). The first four components accounted for 67.6 % of the total variation. The first component accounted for 32.9 % of the total variation, and is mainly influenced by pod size (PL and PW; negative values), LL and LW (positive value), and maturity properties (TF and FPD; positive values). The second component exploring 16.9 % of the total variation primarily because of LW and ITL. The third component contributed 9.9 % to the total variation mostly by LC (negative value).

Table 2.9. Eigenvector matrix of 15 traits for the four principal components in 40 yam bean accessions

Traits	PC-1	PC-2	PC-3	PC-4
Inflorescence length (IL)	0.48	0.32	0.01	0.50
Pod number per inflorescence (PNIL)	0.26	0.36	-0.47	0.49
Pod width (PW)	-0.64	0.38	-0.01	0.09
Pod length (PL)	-0.60	0.53	0.21	0.25
Stem diameter (SD)	0.31	0.57	0.16	-0.53
Internode length (ITL)	0.07	0.66	-0.41	0.12
Petiole length (PTL)	0.72	0.40	0.14	-0.07
Leaf width (LW)	0.68	0.59	0.17	-0.12
Leaf length (LL)	0.85	0.28	-0.08	-0.16
Time to flowering (TF)	0.85	-0.31	-0.13	-0.02
First pod development (FPD)	0.83	-0.23	-0.17	-0.03
Leaf colour (LC)	-0.25	0.01	-0.77	-0.32
Stem colour (SC)	-0.50	0.21	-0.51	-0.25
Leaf outline (LO)	-0.29	0.41	0.13	0.01
Lateral leaflet peak (LP)	0.45	-0.35	-0.21	0.25
Proportion (%)	*32.94*	*16.99*	*9.96*	*7.72*
Cumulative (%)	*32.94*	*49.94*	*59.90*	*67.63*

The pattern of divergence between 40 accessions for the first two components is shown in Figure 2.2. The space is divided into four quadrants. In quadrant I were mostly occupied by accessions from Sumatra, and quadrant-IV were occupied by

almost all of American accessions. In other hand, all the accessions from ENT were plotted in quadrant-II and quadrant-III. The accessions from Java were mostly occupied the quadrants similar to accessions from ENT.

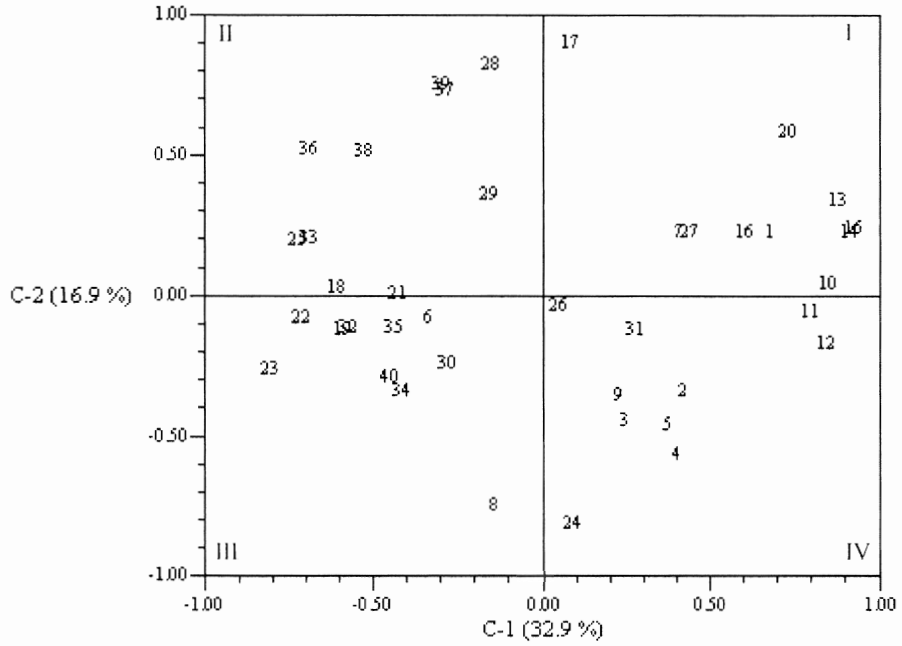

Figure 2.2. Pattern of divergence between 40 accessions for the first two components

Pearson correlation coefficient of the traits

Pearson correlation coefficient for principal components was performed to have a clear relation of each trait of the accession for the total pattern of variations (Table 2.10). The first component contributed of 32.9 % of total variations was correlated with 12 traits, which primary to pod size (pod width and pod length; the highest positive values), and the maturity (time to flowering and time to first pod developed; the highest negative values). The second components gave 16.9 % of total variation was negatively correlated with seven traits, mainly to stem

diameter and leaf width (two highest negative values). While the third (9.6 % of total variation) and the fourth (7.7 % of total variation) components were significantly correlated to five and three traits, respectively. The Pearson correlation coefficient however implied that the variation within the yam bean accessions due all traits except PNIL, SD, and ITL (the first principal component).

Table 2.10. Pearson correlation coefficient for principal components analysis for 15 traits of 40 yam bean accessions

Traits	Pearson correlation coefficient for principal components			
	PC-1	PC-2	PC-3	PC-4
Inflorescence length (IL)	-0.42*	-0.03	0.30*	-0.47*
Pod number per inflorescence (PNIL)	-0.08	-0.06	0.12	-0.57*
Pod width (PW)	0.71*	-0.15	0.04	-0.08
Pod length (PL)	0.71*	-0.28	0.28*	-0.22
Stem diameter (SD)	-0.19	-0.63*	0.09	0.26
Internode length (ITL)	0.14	-0.26	-0.54*	-0.58*
Petiole length (PTL)	-0.46*	-0.57*	0.22	-0.19
Leaf width (LW)	-0.39*	-0.74*	-0.18	-0.28
Leaf length (LL)	-0.66*	-0.44*	-0.37*	-0.13
Days to flowering (TF)	-0.88*	0.21	0.23	-0.01
First pod development (FPD)	-0.83*	0.21	0.24	0.01
Leaf colour (LC)	0.33*	0.38*	-0.26	-0.06
Stem colour (SC)	0.53*	0.21	0.07	-0.07
Leaf outline (LO)	0.45*	-0.32*	0.02	0.25
Lateral leaflet peak (LP)	-0.49*	0.30*	-0.48*	0.12

Plot of the first and second component scores for four groups of yam bean accessions is shown Figure 2.3. The first and the second principal components showed that accessions from Java and ENT were relatively closer compared to accessions from Sumatra and America.

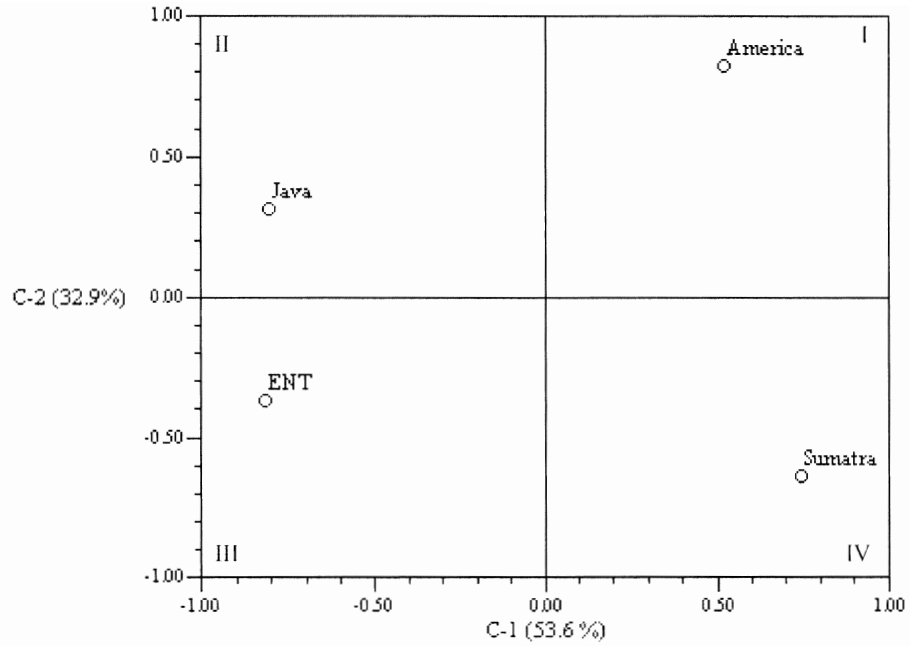

Figure 2.3. Plot of the first and second component scores for four groups of yam bean accessions

Cluster analysis

A dendrogram of the 40 yam bean accessions is shown in Figure 2.4. There were mainly two separate clusters, cluster 1 is consisting of the yam bean accessions collected from Sumatra and cluster 2 of the landraces from Java and from ENT. Two American accessions (EC033 and EC557) were clustered with Sumatra accessions (cluster 1), while the other American accessions were clustered with accessions ENT and Java (cluster 2). Two West Java accessions (B39/WJ and B42/WJ) and B74/ENT were separated from those two clusters.

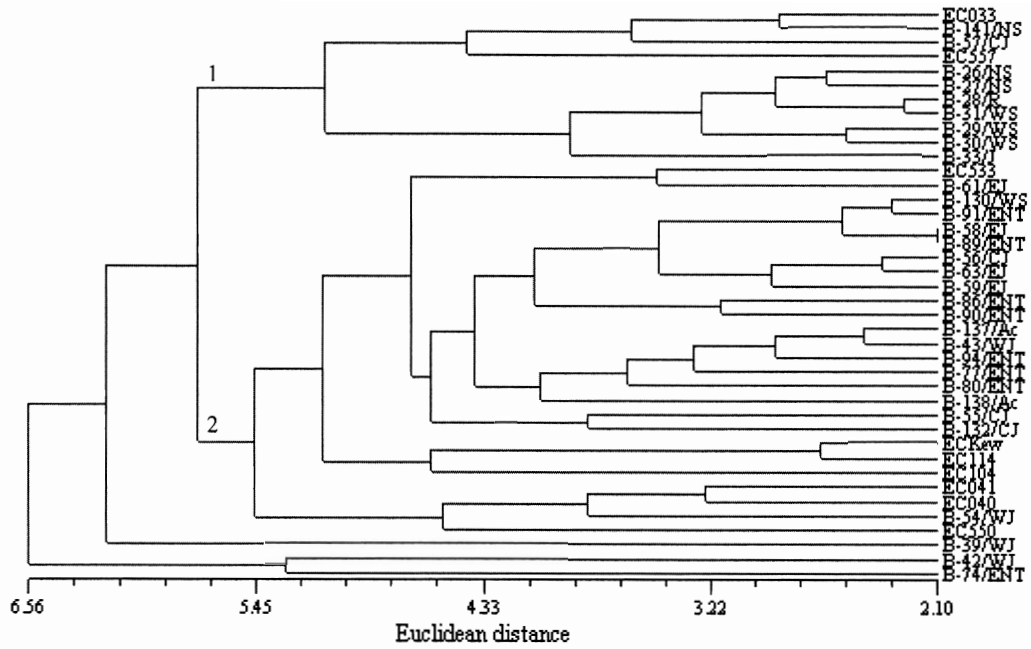

Figure 2.4. Grouping of 40 yam bean accessions based on 15 morphological traits using Euclidean distances and UPGMA clustering

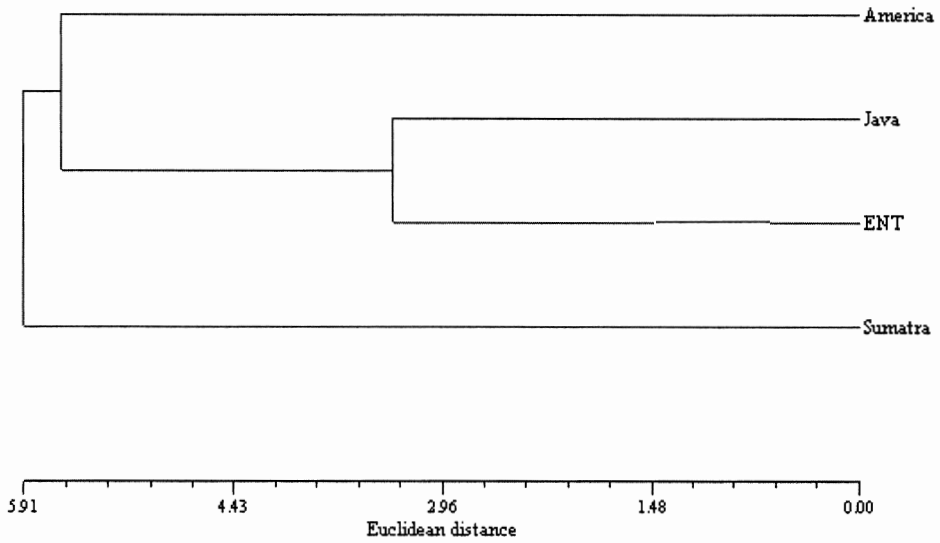

Figure 2.5. Grouping of four groups of yam bean accessions based on 15 morphological traits using Euclidean distances and UPGMA clustering

Figure 2.5 showed that group accession from America was located in the middle of two sub-groups accessions from Sumatra, and from Java and ENT. It is implied that American accession was not completely separated from Indonesian materials.

2.2.5. Discussion

The result generally implied that significant differences of morphological traits between the four groups of yam bean were observed. It is suggested a considerable large genetic diversity between groups. Nevertheless, the differences of such traits within groups were not obvious. The yam bean landraces within East Nusa Tenggara (ENT group) performed more similarity characteristics compared to landraces from other Indonesian groups. All the ancestor landraces (American group) performed similar properties of all traits observed. Furthermore, American yam beans performed similar leaf properties (colour, outline, and peak) except for the leave lobes, with all the Indonesian materials. This observation of leave properties did not agree with Sørensen (1998) who mentioned *P. erosus* has great variation in the outline of leaflets. Nevertheless, accessions from Guatemala (EC040 and EC041) could be distinguished from other accessions based on their deeply lobed leaves, as also mentioned by Sørensen (1996).

The existence of two sub-groups within Indonesian yam bean, i.e., from Sumatra and from Java (together with ENT) could be a sign that introduction yam bean from South and Central America to Indonesian regions (Sørensen 1988; Estrella

et al., 1998) have been done at different times. It is also possibly that yam bean has been isolated during a long period of cultivation within Sumatra island, separately from other Indonesian islands. Nevertheless, the similarity of accessions from Java and ENT is possibly caused by introductions via neighboring villages or communities between those regions. Obviously the lack of reports on cultivation knowledge and ethnobotanical uses of yam bean in Indonesia makes confirmation of such hypotheses difficult. The only report of cultivation yam bean *P. erosus* from Southeast Asia was from Malaysia (Sørensen, 1998) and from Thailand (Ratanadilok et al., 1994).

Pearson correlation coefficient described that nearly all the traits played important roles in grouping the accessions. Yam beans from Sumatra were considered as potential source of earliness, while ENT accessions considered as source of lateness. Most of Java materials posses similar traits to ENT accessions. Lateness and earliness were the two different arrays of selection strategies that applied by the farmers in eastern and western region of Indonesia, respectively (see also chapter before – Cultivation status of yam bean *P. erosus* in Indonesia). In the eastern regions of Indonesia (e.g. ENT) which are commonly dry areas, farmers preferred a longer maturity of yam bean with bigger and watery tubers. While in Western Indonesia (e.g. Sumatra), earliness was a preference by most farmers. The marketable tuber size in western region, especially in Sumatra, characterised by small and sweet tubers. Farmer knows that to produce smaller marketable tubers, the earlier maturity was better than the late ones. In Mexico, under rich soil and longer day, commercial tubers are commonly harvested after three months (NRC, 1979).

Accessions from ENT were considered as source of vigorous vegetative growth, while the Sumatra and American plant materials provided bigger pod size (PW and PL). All these vigorous elements (IL, PTL, LW, LL) which is found in ENT materials showed positive correlation. It means all these four traits can be combined. Nevertheless, it will be more difficult to combine more vigorous traits with earlier FP and FPD since they showed negative correlations.

It is generally apparent that the genetic variation of morphological traits between yam bean landraces from different islands of Indonesia is considerably large. The population is consisting of two sub-groups, one is Sumatra landraces and the other is landraces from Java and ENT regions. Genetic variation of some traits between yam bean from Indonesia to its ancestral landraces from South and Central America has been observed.

2.3. Comparative assessment of genetic diversities based on RAPD markers and morpho-agronomic traits in yam bean (*Pachyrhizus erosus*)

2.3.1. Abstract

Estimating genetic diversity based on morpho-agronomic traits and molecular markers of yam bean may provide better utilisation of germplasm. The usefulness of Random Amplified Polymorphic DNA (RAPD) markers as predictors for genetic distance in yam bean was evaluated by comparing the RAPD and multivariate analysis approaches. Fifteen morphological traits, and 100 RAPD bands were used to classify the accessions. The relatedness of genetic distances resulted from morpho-agronomic and RAPD markers among the accessions were measured using the correlation method. The general pattern of diversity agreed between the analysis of morpho-agronomic traits and RAPD markers. Indonesian *P. erosus* material is differentiated into two sub-groups, i.e., West Indonesia (accessions from Sumatra) and East Indonesia (accessions from the eastern islands, including from Java). Both methods also showed that Indonesian yam bean *P. erosus* accessions were different from the American *P. erosus* ancestor materials. Nevertheless, the correlation between distances based on morpho-agronomic traits and RAPD markers was negatively significant but rather low (r = - 0.22).

2.3.2. Introduction

Yam bean (*Pachyrhizus erosus* (L.) Urban) is an important vegetable tuberous root crop in the tropics. The species has a long history of cultivation in Indonesia since it was introduced into the Philippines by the Spaniards in 16th century from Central America (Hoof and Sørensen, 1989; Sørensen, 1996; Sørensen *et al.*, 1997). Yam bean displays a range of interesting traits which is suiting to low-input farming system, to drought regions, and to various cropping pattern. The extent distribution and nature of genetic variation, cultivation practices and selection might provide a new diversity within yam bean gene pool collected from Indonesia compare to its ancestor from Central America. Information on genetic variability and distance is valuable to select parents with large genetic distance, to enhance the efficiency of germplasm collection and conservation, as predictors for heterosis, and to predict the historical process of the diversity (Dumolin-Lepegue et al., 1997; Burstin and Charcosset, 1997; Bruschi et al., 2003).

Morpho-agronomic as well as molecular markers are widely used to estimate the genetic variation within and among population of many crops (e.g. Zang et al.,1998; Maciel et al., 2001; Hernandez, 1992; Jarret and Austin, 1994; Lashermes et al., 1993; Ayana et al., 2000; Massawe et al., 2003). The relationship between diversity at marker loci and morphological differentiation has been intensively investigated (Brustin and Charcosset, 1997; Duarte et al., 1999; Steiner and Santos, 2001; Cheng et al., 2002; Dahlberg et al., 2002; Bruschi et al., 2003; Wen and Hsiao, 1999). However, in most cases no significant correlation between the two distances were reported (e.g. Dahlberg et

al., 2002; Bruschi et al., 2003; Duarte et al., 1999; Steiner and Santos, 2001). Information concerning to association between diversity on DNA level and loci controlling morphological traits is necessary to evaluate the importance of genetic drift and natural selection of a population (Merila and Crnokrak, 2001). Morpho-agronomic traits has been used to clasify the genus *Phachyrizus* (Døygaard and Sørensen, 1998; Hernandez, 1992; Tapia and Sørensen, 2003) as well as by RAPD markers (Estrella et al., 1996). Nevertheless, no report has been attributed and published in relation of molecular and morphological diversity analysis within yam bean *P. erosus* population from Indonesia.

This study was subjected to estimate the genetic relationship and to compare phylogenetic grouping of the yam bean population collected from Indonesia based on morpho-agronomic and RAPD markers. The aim of the comparison was to evaluate the usefulness of RAPD markers as predictors of morpho-agronomical variability in the yam bean populations.

2.3.3. Materials and methods

Plant materials

Fourty-two selected yam bean *P. erosus* accessions consisting of 33 yam bean accessions from Indonesia, six accessions from South and Central America, and one accession from Thailand were examined (Table 2.11). All accessions were cultivated materials, and were considered as representatives of the variability formed within different geographic areas in Indonesia and in America.

Table 2.11. Selected yam bean landraces used, area of origin and altitude

No.	Acc. Code	Area of origin	Altitude (m asl)
1	EC033	Yucatan, Mexico	100
2	EC041	Jutiapaca, Guatemala	1100
3	EC550	Guanajuato, Mexico	1750
4	EC557	Guanajuato, Mexico	1500
5	EC040	Jutiapa, Guatemala	1100
6	EC114	Para, Brazil	Unknown
7	B-26/NS	P. Siantar, North Sumatra	50
8	B-27/NS	Medan, North Sumatra	25
9	B-28/R	Bangkinang, Riau, Sumatra	60
10	B-29/WS	Kuranji, West Sumatra	200
11	B-30/WS	Lubuak Munturun, West Sumatra	600
12	B-31/WS	Limau Manih, West Sumatra	200
13	B-33/J	Muara Bungo, Jambi, Sumatra	150
14	B-35/WK	Pontianak, West Kalimantan	10
15	B-39/WJ	Kedunghalang, West Java	600
16	B-42/WJ	Tanjung Mekar, West Java	700
17	B-43/WJ	Arjasari, West Java	800
18	B-54/WJ	Batujajar, West Java	500
19	B-55/CJ	Tersobo, Central Java	25
20	B-57/CJ	Kembaran, Central Java	25
21	B-58/EJ	Tajinan, East Java	1000
22	B-59/EJ	Panggungrejo, east Java	100
23	B-61/EJ	Kepung, East Java	100
24	B-63/EJ	Wringin Anom, East Java	20
25	B-64/SSw	Bontolangkamng, South Sulawesi	50
26	B-65/SSw	Bontobiraeng, South Sulawesi	50
27	B-67/CSw	Tawaeli, Central Sulawesi	200
28	B-71/B	Penebel, Bali	50
29	B-74/ENT	Kupang, West Timor	300
30	B-77/ENT	Molu Utara, West Timor	800
31	B-80/ENT	Ndona, Flores	600
32	B-84/ENT	Borokanda, Flores	600
33	B-86/ENT	Enturia, Flores	600
34	B-89/ENT	Pandawai, East Sumba	300
35	B-90/ENT	Pahungalodu, East Sumba	300
36	B-91/ENT	Laratama, West Sumba	12
37	B-93/CM	Ciang Mai, Thailand	Unknown
38	B-94/ENT	Kupang, ENT	20
39	B-130/WS	Padang, West Sumatra	200
40	B-132/CJ	Prembun, Central Java	25
41	B-137/Ac	Aceh, Sumatra	87
42	B-138/Ac	Aceh, Sumatra	87

Multivariate analysis based on Morpho-agronomic traits

Field trials were performed from January to August 2003 at two locations, i.e., Leuwikopo (first location) and Yasmin (second location), in district of Bogor

Indonesia. The detail of the field experimental design, morpho-agronomic traits observed as well as statistical analysis can be seen in the chapter 2.2 before (Genetic diversity of yam bean (*Pachyrhizus erosus*) revealed by morpho-agronomic traits).

RAPD analysis

Seeds of accession were sown in a 1:1 compost/sand mixture contained in 8-cm diameter pots. Pots were placed in a glasshouse with temperature maintained at 18°C and day/night periods of 16/8 h under illumination of lamps (*Phillips*; 400 W). Leaves of each accession were harvested from a single healthy 8-week-old plantlet. Each sample consists of 0.1 g leaf material. The sample was immediately put in a 1.5 ml sterile tube (Micro Test Tube Safe-Lock 1.5 ml), and was frozen in liquid nitrogen (-196 °C) before kept at -30 °C.

DNA isolation was done using the Nucleon PhytoPure Kit for small samples (Amersham, 1997) according to Nucleon Extraction and Purification Protocols. After adding TE-buffer, the DNA was kept at 4 °C for one week to achieve full resuspension. DNA concentration was measured by a Fluorometer with the fluorochrome dye Hoechst 33258. DNA samples were then diluted to a standard concentration of 12.5 ng/µl. Amplification conditions of RAPD fragments are as follows: the 25 µl RAPD reaction mix contained 2 µl (12.5 ng) template DNA; 15.8 µl sterile distilled water; 1 µl (10 mM) of a single decanucleotide; 0.5 µl (10 µM) dNTPs; 3 µl (25mM) $MgCl_2$; 0.2 µl (= 5 U/µl) Tag-DNA-polymerase and 2.5 µl reaction 10X buffer provided by the manufacturer. A set of 49 selected primers (Operon Technologies, Inc.) was employed (Table 2.12). The restricted ligated DNA samples were placed in 0.2 ml tubes Thermo-Strip (9 tubes/strp).

RAPD reactions were accommodated in disposable 96-well plates placed on a MJ Research thermocycler (PTC-100 MJ research inc.) programmed as follows: one initial cycle of 30 s denaturation at 94°C; 45 cycles of 30 s at 94°C, 60 s of annealing at 35°C (RAMP 35°C with 0.4°C/sec) and 120 s extension at 72°C. A final cycle of 5 min at 72°C was used to complete extension of any remaining products, and PCR product were stored at 4°C before electrophoresing the final products. Amplification products were separated in 1.5 % agars gels in 1x TAE buffer (80 V for 4.5-5 h) and detected by staining with ethidium bromide for 15 min. Gels were de-stained in a water bath for 30 minutes, and then photographed under UV light and photodocumented with a gel image analysis system (EASY STORE software).

Any position on the gel contains at least one DNA amplification product, called "fragment". A given fragment contains scoreable amplification products will be termed as "band". The strongly present of polymorphic band was scored as (1), and the absent of such a band was scored as (0). To facilitate scoring across gels, a 1 kb DNA ladder (250-10000 bp; MBI Fermentas, Inc.) was used. Genetic similarity values (GS values) according to Jaccard similarity index was used applying the software NTSYSpc ver. 2.10q. (Rohlf, 2001).

Preliminary studies involved screening of 250 primers and 49 primers showing reproducible polymorphism, were selected. A total of 100 polymorphic bands produced from these 49 primers was included in the analysis (Table 2.12).

Table 2.12. List of primers used in the RAPD analysis

No.	Primer name	Sequence (5' - 3')	No. of polymorphic Bands	No.	Primer name	Sequence (5' - 3')	No. of polymorphic Bands
1	A-08	GTGACGTAGG	2	26	U-12	TCACCAGCCA	2
2	A-11	CAATCGCCGT	3	27	U-14	TGGGTCCCTC	1
3	A-19	CAAACGTCGG	5	28	U-17	ACCTGGGGAG	2
4	B-14	TCCGCTCTGG	2	29	AG-08	AAGAGCCCTC	1
5	C-08	TGGACCGGTG	2	30	AG-13	GGCTTGGCGA	4
6	C-20	ACTTCGCCAC	2	31	AH-03	GGTTACTGCC	2
7	D-05	TGAGCGGACA	1	32	AI-19	GGCAAAGCTG	2
8	H-04	GGAAGTCGCC	1	33	AI-20	CCTGTTCCCT	1
9	H-08	GAAACACCCC	3	34	AJ-01	ACGGGTCAGA	2
10	H-14	ACCAGGTTGG	1	35	AJ-02	TCGCACAGTC	2
11	H-19	CTGACCAGCC	2	36	AJ-03	AGCACCTCGT	2
12	H-20	GGGAGACAT	2	37	AJ-05	CAGCGTTGCC	2
13	Q-05	CCGCGTCTTG	3	38	AK-06	TCACGTCCCT	1
14	Q-13	GGAGTGGACA	2	39	AK-20	TGATGGCGTC	1
15	Q-14	GGACGCTTCA	1	40	AL-04	ACAACGGTCC	3
16	R-13	GGACGACAAG	1	41	AL-06	AAGCGTCCTC	2
17	R-15	GGACAACGAG	1	42	AL-11	GTCACGTCCT	2
18	R-20	ACGGCAAGGA	2	43	AL-20	AGGAGTCGGA	1
19	S-12	CTGGGTGAGT	2	44	AM-01	TCACGTACGG	1
20	S-13	GTCGTTCCTG	1	45	AM-04	GAGGGACCTC	1
21	T-06	CAAGGGCAGA	1	46	AN-09	GGGGGAGATG	3
22	T-12	GGGTGTGTAG	1	47	AN-10	CTGTGTGCTC	3
23	U-01	ACGGACGTCA	1	48	AN-13	CTTCCAGGAC	3
24	U-03	CTATGCCGAC	5	49	AN-17	TCAGCACAGG	6
25	U-08	GGCGAAGGTT	3				

Comparison between morpho-agronomic and RAPD markers

The relationship of the Euclidean distance constructed from morpho-agronomic data and the Jaccard similarity developed from RAPD polymorphic bands were determined by the product moment correlation derived from Mantel Z using MXCOMP command of NTSys program, version 2.10q (Rohlf, 2001).

2.3.4. Results

Morpho-agronomic analysis

All the 15 morpho-agronomic traits were simultaneously considered to assess the pattern of variations in a principal component analysis. The pattern of variation of each trait for principal component analysis is shown in Table 2.13.

Table 2.13. The eigenvalues of the correlation matrix for 15 morpho-agronomic traits

Traits	PC-1	PC-2	PC-3	PC-4
Inflorescence length (IL)	0.48	0.27	0.21	0.63
Pod number per inflorescence (PNIL)	0.21	0.42	0.56	0.36
Pod width (PW)	-0.68	0.40	-0.14	0.06
Pod length (PL)	-0.62	0.53	-0.12	0.34
Stem diameter (SD)	0.36	0.43	-0.14	-0.29
Internode length (ITL)	-0.11	0.65	0.21	-0.25
Petiole length (PTL)	0.58	0.59	-0.13	0.03
Leaf width (LW)	0.57	0.69	-0.25	-0.18
Leaf length (LL)	0.76	0.41	-0.12	-0.32
Time to flowering (TF)	0.87	-0.22	0.25	0.07
First pod development (FPD)	0.83	-0.21	0.36	0.04
Leaf colour (LC)	-0.28	0.06	0.67	-0.45
Stem colour (SC)	-0.54	0.14	0.48	-0.25
Leaf outline (LO)	-0.34	0.39	0.15	0.19
Lateral leaflet peak (LP)	0.51	-0.29	-0.04	-0.03
Proportion (%)	*29.77*	*16.42*	*11.34*	*10.09*
Cumulative (%)	*29.77*	*46.20*	*57.54*	*67.64*

The principal component analysis indicated that the first four components have eigenvalues greater than 1 and accounted for 67 % of the total variation (Table 2.13). The first component is characterized by time to flowering (TF), time to first pod development (FPD), leaf length (LL), pod width (PW), and pod length (PL), and accounts 29.7 % of the total variation. The second component is characterized by leaf width (LW) and internode length (ITL) and accounts 16.4 %

of the total variation. It is apparent that TF, FPD, PW, PL, and LL played essential role in the classification of the yam bean.

The pattern of divergence between the 42 accessions for the first two components is shown in Figure 2.6. The accessions were occupied all the four quadrants. In quadrant I (both first component and second component performed positive values) were mostly occupied by accessions from Sumatra, and quadrant IV (first component positive; and second component negative) were occupied by almost all of American accessions. In other hand, all the accessions from eastern region of Indonesia were plotted in quadrant-II and quadrant-III. The Accession from Java was spread mostly in same quadrant with accessions from ENT.

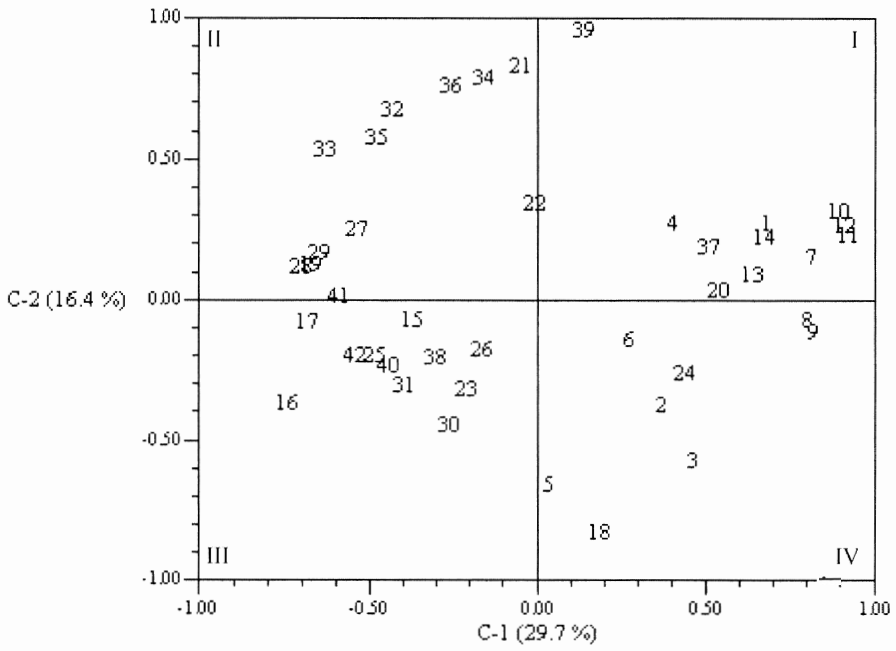

Figure 2.6. Matrix plot of the first and second components scores for 42 yam bean accessions

Grouping the 42 yam bean accessions could be grouped into four main clusters (Figure 2.7) Two Mexican accessions (1, 6) and a Brazilian accession (4) were in first cluster, while two Guatemalan accessions (2, 5) are grouped together with a Mexican accession (3) and two accessions from Java (18, 40) in cluster 3. Cluster 2 is dominated by the accessions from Sumatra, whereas cluster 4 mainly includes the eastern Indonesian yam beans. The accessions from Java are spread over cluster 2, 3 and 4. A clear separation among the yam bean accessions collected from Indonesia, i.e., the Sumatra landraces (cluster 2) and the landraces collected from eastern Indonesia (cluster 4) was detected.

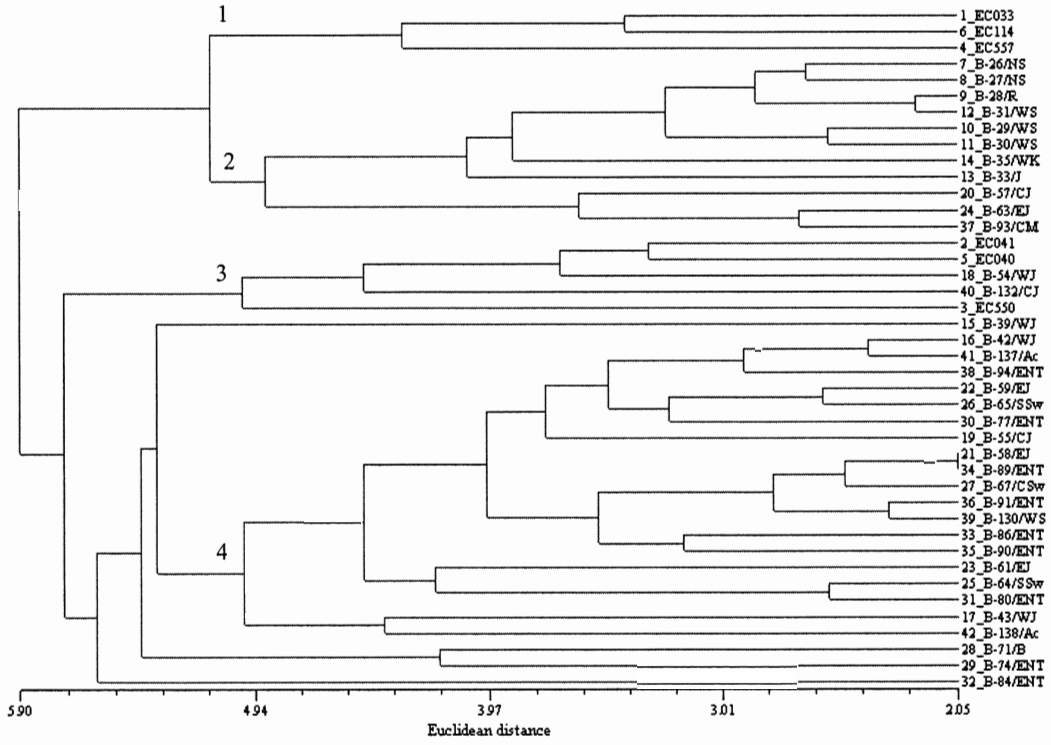

Figure 2.7. Grouping of 42 yam bean accessions based on 15 morphological traits using Euclidean distance and UPGMA clustering

RAPD analysis

The first two principal co-ordinates encompassed 26.7 % of the total variation (Table 2.14). Since the first two principal components exceed more than 25 %, cluster analysis would be less sensitive and less reliable than PCA and PcoA for detecting pedigree relationship among genotypes (Melchinger, 1993).

Table 2.14. Result of principal co-ordinate analysis, eigenvalue from RAPD markers on 42 yam bean genotypes

PCo-i	Eigenvalue	Percent	Cumulative
1	2.42	14.23	14.23
2	2.12	12.49	26.72
3	1.39	8.18	34.91
4	1.25	7.36	42.27
5	0.93	5.47	47.75
6	0.78	4.58	52.33
7	0.69	4.09	56.42
8	0.53	3.12	59.55
9	0.51	3.04	62.59
10	0.45	2.69	65.29

Principal Co-ordinate Analysis (PCoA) was performed to assess the pattern of variations (Figure 2.8.). The American landraces were clearly separated from most of the Indonesian landraces (upper right in quadrant I). Yam bean landraces collected from Sumatra occupied quadrant IV. Whereas most of landraces from Java and other islands can be found in quadrant II.

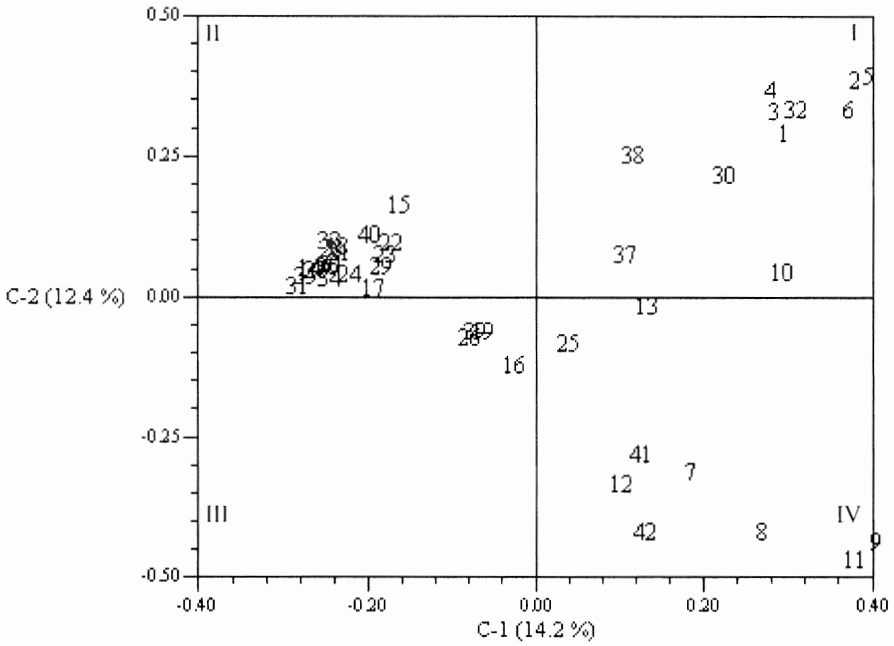

Figure 2.8. Association among 42 yam bean landraces revealed by Principal Co-ordinate analysis of 100 RAPD markers.

The cluster analysis summarised as a dendrogram, showing the relationships among the landraces (Figure 2.9). The accessions are grouped into 7 clusters. In cluster 1, one Mexican landrace originated from Lowland Yucatan (1) showed a closed similarity to some accessions from West Timor (30, 38), two accessions from Sumatra (10, 13) and from Chiangmai Thailand (37). In cluster 2, two Sumatra landraces (12, 39) were grouped with two landraces from South Sulawesi (25, 26) and one from Central Java (19). In cluster 3, landraces from Bali, Kalimantan, Flores, West-Timor and Sumba, are gathered together with most landraces from Java. In cluster 4, consisting of 4 Sumatra landraces. In cluster 5, one accessions from Flores (32) seems to be closely related to two accessions originated from Guatemala highlands (2, 5) and to one accession from Para-Brazil (6).

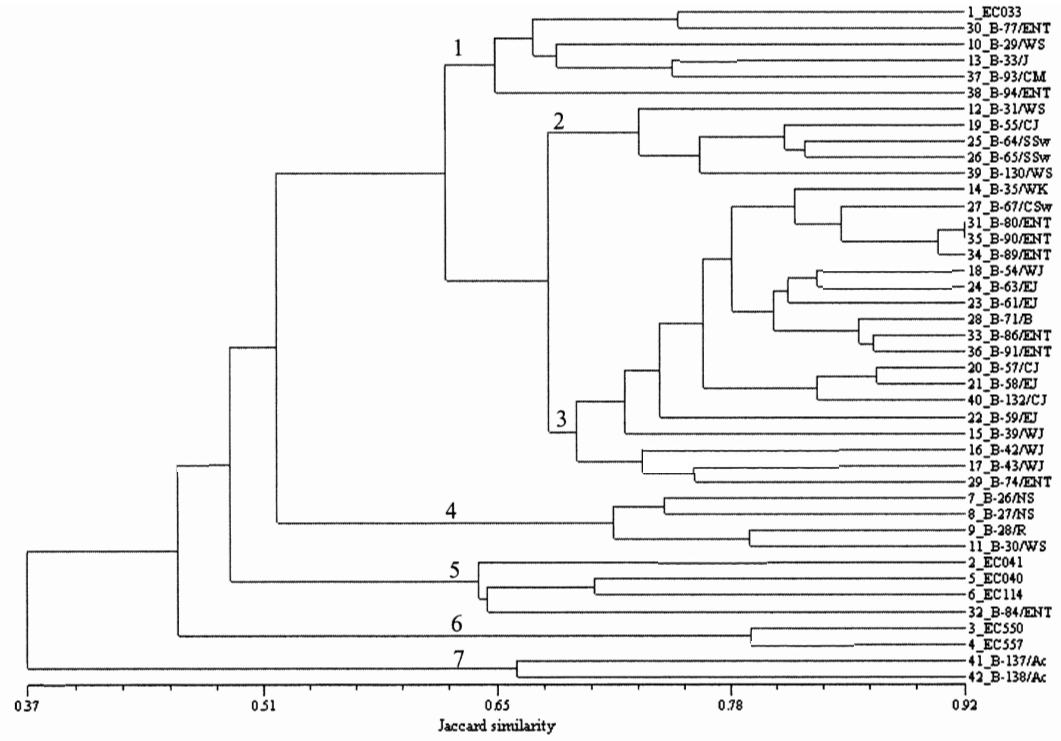

Figure 2.9. Grouping of 42 yam bean landraces based on 100 RAPD markers using Jaccard coefficient of similarity and UPGMA clustering

The South American materials (2, 5, 6) in cluster 5 were separated from Mexican Guanajuato-higland accessions (3, 4) in cluster 6. Two accessions from Aceh Sumatra (41, 42) in cluster 7 seem to be remote accessions from other yam bean groups.

Most landraces from Sumatra (cluster 4) are not related to those from the eastern islands (cluster 3). Therefore, two sub-groups within Indonesian yam bean landraces have been detected, i.e., sub-group of Sumatra landraces and sub-group of landraces from eastern regions (including Java). These two groups were detected both in RAPDs analysis and in morpho-agronomic analysis.

Correlation between morphological traits and RAPD markers

The 2-way Mantel test, showed that there was a significant but very small correlation between the genetic similarity estimated from molecular marker and the differences estimated from morphological variation (Matrix correlation: r = -0.22; Approximate Mantel t-test: t = -2.3969; Prob. random Z < observed Z: p = 0.0083), as shown in Figure 2.10.

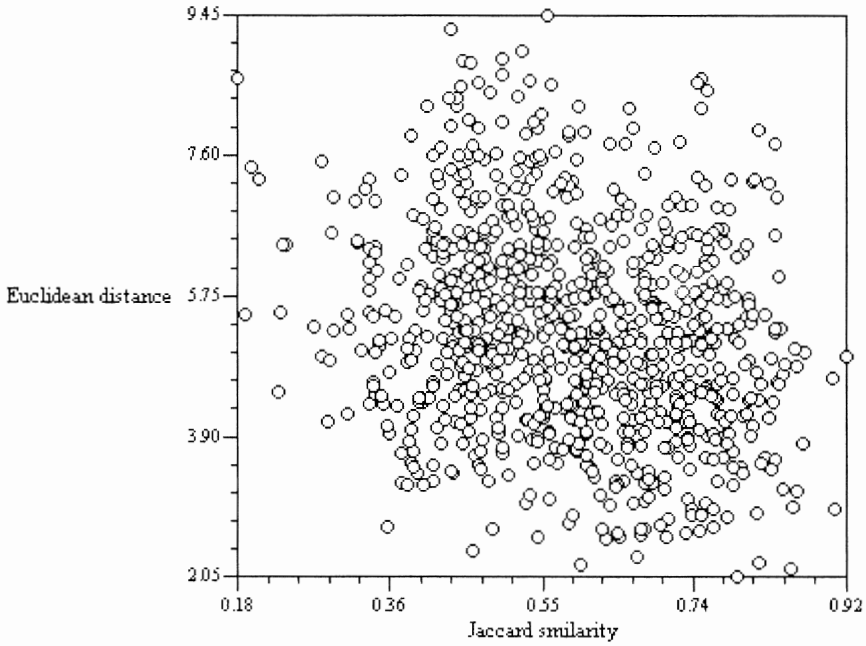

Figure 2.10. Matrix comparison between morpho-agronomic traits (Euclidean distance) and RAPD markers (Jaccard similarity) of 42 yam bean accessions.

2.3.5. Discussion

The number of clear RAPD polymorphic bands per each primer on yam bean *P. erosus* in this study (average 2.01) is relatively smaller compare to assessment result on the same species, i.e., 4.2 fragment polymorphism per primer conducted by Estrella et al., (1996). This average number of fragment polymorphism per primer value is also very small compare to sweet potato (*Ipomea batatas)* (5.9 by Zhang et al., 1998; 7.6 by Dhillon and Ishiki, 1999). The number of polymorphic bands in this study was also smaller compared to 20.3 polymorphic bands in common beans (*Phaseolus vulgaris* (L.); Maciel et al., 2001), or 9.2 polymorphic bands in wild sorghum (*Sorghum bicolor* spp. *Verticilliflorum*; Ayana, et al., 2000). Nevertheless, RAPD markers in this study have been demonstrated as useful for the assessment of the variation at the DNA level in yam bean landraces. The limited number of strong RAPD polymorphic bands per primer in yam bean observed in this study suggested that RAPD profiles were not found sufficient reproducible. Insufficient reproducibility of RAPD profiles have been observed by Ewards (1998) and Jones et al. (1998).

Both multivariate analysis based on morpho-agronomic traits and RAPD markers analysis showed that yam bean cultivars from Indonesia are substantially different from their ancestors in Central and South America after centuries of isolated evolution in distinct agro-ecological environments. The differences in climatic conditions between American continent to Indonesian archipelago, and different selection based on local preferences and the differences in cultivation practices resulted new adaptation of the yam bean during a long period of cultivation in Indonesia.

An absence of a strong correlation between the RAPD and morpho-agronomic variations in this study showed no clear relationship between distances computed from quantitative traits (morpho-agronomic traits) and distances estimated from individual loci (RAPD). This result is similar to those reported in other species (Person and Gustavson, 2001; Dahlberg et al., 2002; Bruschi et al., 2003; Duarte et al., 1999; Steiner and Santos, 2001; Blattner and Mendez, 2001). But congruent relationships between two methods of analysis were also reported sometimes (Lerceteau et al., 1997; Wen and Hsiao, 1999).

Several studies have been reported concerning the discrepancies between morphological data and RAPD markers relationship (e.g. Duarte et al., 1999; Steiner and Santos, 2001; Dahlberg et al., 2002; Bruschi et al., 2003). RAPD, as other molecular markers, is considered to be neutral, and hence to provide no direct assessments of the fitness of a given trait. The magnitude of correlation coefficient between phenotypic and marker distances depends on the association between the number of loci involved in the variation of quantitative traits. Theoretical and experimental study conducted by Burstin and Charcosset (1997) proved that phenotypic and molecular distance relationship display a triangular shape. Low marker distances are systematically related to low phenotypic distances, while high marker distances correspond to either low or high phenotypic distances. Due to such property, non-linear relationship between both distances will increase as the number of QTLs contributed in the variation of the traits for phenotypic distance increase. Furthermore, linkage disequilibrium between marker loci and the QTLs involved in the traits considered for quantitative distance estimation would affected the relationship between quantitative and markers distances.

Bruschi et al., (2003) also reported a non-significant correlation between molecular variation and leaf morphological variation among Italian *Quercus petraea* population. RAPD variation is based directly on DNA variation, and as a result, a change in repetition sequences can result in a change in RAPD pattern. They also added that such relationship because of low gene flow would provide different adaptive differences, and hence, concordance between molecular and morphological variation would occur.

Most morphological traits are controlled by several genes, which may highly be influenced by environments. Dahlberg et al. (2002) found a non significant correlation between the variation among seed morphology and RAPD marker in sorghum germplasm. They argued that seed morphology is possibly controlled by many genes and also highly influenced by the environment. Genes from such seed traits are also possibly only a sample of a small region of the genome. Since DNA profiling is a refined random evaluation of the genome, and RAPD samples the DNA markers randomly distributed throughout the genome, it may required the full genome to be identified and genes sequenced before a true measure of genetic variation can be measured.

The degrees of differentiation in quantitative traits typically exceed that observed in neutral marker genes. Merila and Crnokrak (2001) conclude such a phenomenon from several empirical data comparative studies. Phenotypic data may consist primarily of limited trait measurements, and did not represent a random sampling of gene effects. Or phenotypic expression may be influenced by many non-genetic factors that are not expected to effect DNA marker pattern. Some of the phenotypic traits have been selected under domestication. Hence,

phenotypic similarity may not reflect evolutionary relationships across the entire genome that are more likely to be detected by molecular markers.

RAPD could detect a wider genome, coding as well as noncoding regions, rather than morphological analysis (Person and Gustavsson, 2001). Since most of the genome is composed of noncoding DNA, hence the majority of the amplified fragments are from these regions. It is suggesting a non-significant correlation between quantitative traits and molecular markers.

The absence of a relationship between the genetic and the morphologic classification. Steiner and Santos (2001) mentioned that such condition in adaptive ecology of *Lotus corniculatus* suggested the specific traits with adaptive value might have accumulated in habitats subjected to similar ecological conditions, with less regard for genetic origins of the progenitors. Johns et al., (1997) also mentioned that deviation between genetic and morphologic classifications in common bean landraces may be due to the conservation of morphologic traits under natural selection in similar environments.

RAPD method is not able to identify recessive mutation (absence band scores) in heterozygote, since the method is designated to analysis dominant (present band) or recessive (absence band) genes. Person and Gustavsson (2001) found that high level of morphological variation observed in lingonberry (*Vaccinium vitis-idaea* L.) seedling due to somatic mutation could not be detected by RAPD. Nevertheless, Wu et al., (2002) have developed co-dominant RAPD markers which closely linked to two morphological traits in rice (*Oryza sativa* L.). This co-dominant RAPD marker approach makes the RAPD markers to be more efficient.

The result of the present study have generally revealed that morphological variation is not closely related to RAPD based differences in yam bean. Many factors discussed above may contributed to the lack of strong correlation between phenotypic and molecular distances. Nevertheless, both methods showed that Indonesian accessions consisted of two main groups, one from Sumatra and the other from Java and eastern islands. American yam bean accessions were different from Indonesian yam beans.

III. CONCLUSIONS

- A collection of 110 yam bean accessions from eight islands in Indonesia has been established.
- Two main cultivation practices for yam bean (*P. erosus*) was recorded, i.e., monocroping and intercroping systems.
- The yam bean was mostly used as a vegetable crop, and the tuber was mainly consumed raw due to its succulent flesh.
- The genetic diversity between yam bean landraces from different islands of Indonesia was considerably large.
- The yam bean landraces from Sumatra island were genetically distinct from Java and from other Eastern regions of Indonesia.
- The yam bean from Indonesia and their ancestral landraces from South and Central America were genetically different.
- RAPD markers and morpho-agronomic traits analysis were only slightly negative correlated.

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V. SUMMARY

The yam bean (*Pachyrhizus erosus* (L.) Urban) is grown in almost the entire Indonesian archipelago. The species is traditionally known as a vegetable tuber crop for local markets in Indonesia, but recently the plant became an interesting crop for commercial and industrial purposes – a close relative to *P. erosus* from Amazonian Peru so called Chuin cultivars of *P. tuberosus* are used and processed like cassava. So far the knowledge about the genetic diversity of this crop in Indonesia is limited. The yam bean can become more attractive to agriculture of Indonesia through collecting germplasm, recording the cultivation and processing knowledge, and investigating the genetic diversity of the species. Estimating genetic differentiation of yam bean based on morpho-agronomic and molecular markers may provide useful information for better utilizing the germplasm. Therefore, field survey studies and genetic diversity analysis of yam bean landraces from Indonesia have been conducted with the objectives :

(i) to record the cultivation status and to collect yam bean landraces in Indonesia,

(ii) to analyse morpho-agronomic trait diversity of yam bean landraces, and

(iii) to compare the diversity of yam bean based on morpho-agronomic traits and on RAPD marker analysis.

Three different experiments have been conducted. The first was a field survey and collection trip on 8 major islands of Indonesia to document current cultivation status and to collect local landraces from different geographic regions of Indonesia. Eighty informants were interviewed and 110 yam bean accessions

have been collected. Yam bean is locally grown on all eight visited islands of Indonesia, and considered as a vegetable crop and the tuber is mainly consumed raw due to its succulent flesh. In West Indonesia (Sumatra and Java) yam bean is mainly cultivated as a sole crop and occasionally on commercial scale, whereas in East Indonesia (especially in Sumba, Timor, and Flores) yam bean is predominantly intercropped with maize and cassava. In West Indonesia consumers preferred earlier maturity genotypes with relatively small and sweet tubers. In Eastern regions of Indonesia with mainly dry conditions and local-traditional markets, genotypes with late maturity, more succulent flesh and bigger tubers are preferred. In West Indonesia yield ranged from 10 to 70 t ha^{-1}, and in East Indonesia the yield was from 10 to 50 t ha^{-1}. However, yam bean seems to be well adapted under various cropping systems and may be well suited to increase soil fertility.

In the second experiment the genetic diversity among the yam bean accessions was estimated under field conditions. In field trials at two locations near Bogor, Indonesia, the genetic diversity was analysed based on morpho-agronomic traits. Forty selected yam bean accessions consisting of 31 yam bean landraces from diverse ecological regions of Indonesia, and 9 accessions from South and Central America were examined. The experimental design was a randomized block with two replications at each location. Analysis of variances and multivariate analysis of 15 morphological traits were performed. Euclidean distances were calculated and the resulting distance matrix was used for a UPGMA cluster analysis. Correlations between mean values of the traits and principal components were estimated. Analysis of variances (ANOVA) and multivariate analysis (principal component and cluster analysis) of 15 morpho-

agronomic traits showed genetic diversity among the yam bean landraces. Principal component analysis showed that the first four components accounted for 67.6 % of the total variation. The first component accounted for 32.9 % of the total variation, and was correlated mainly with time to flowering, time to first pod development, pod size, and leaf length. The second component contributing of 16.9 % of total variation was mainly associated with leaf width and internode length. The third component contributed 9.9 % to the total variation and was associated with leaf colour. Cluster analysis showed that accessions collected from Sumatra tend to be separated from accessions from Java and East Indonesia. American yam beans were different from Indonesian yam beans.

The objective of the third experiment was to compare the diversity measured by morpho-agronomic traits with diversity measured based on RAPD markers. This analysis has involved 38 selected yam bean accessions from Indonesia, 6 accessions from South and Central America, and one accession from Thailand. The relationship between the Euclidean distance estimated from morpho-agronomic data and the Jaccard similarity index calculated from RAPD polymorphic bands were determined by correlation analysis. A preliminary study on RAPD markers involved screening of 250 primers. From these screening, 49 primers, which showed reproducible polymorphic products, have been selected. In total 100 polymorphic bands from these 49 primers were included in the analysis. The average number of clear polymorphic bands per primer was 2.01, which is relatively small. Principal component analysis (PCA), principal co-ordinate analysis (PCoA) and cluster analysis were performed to assess the pattern of variation. RAPD markers and morpho-agronomic traits variation showed a negative correlation. Nevertheless, the general pattern of genetic

diversity of yam bean analysis based on both methods was similar. Most of landraces from Java and eastern islands of Indonesia were clearly separated from Sumatra. Furthermore, American landraces were differed from most of the Indonesian yam beans.

VI. ZUSAMMENFASSUNG

Die Yam Bohne (*Pachyrhizus erosus* (L.) wächst nahezu im gesamten Indonesischen Archipel. Die Art ist traditionell bekannt als Gemüsewurzelfrucht auf den lokalen Märkten Indonesiens, aber seit kurzen wird die Yam Bohne auch als eine interessante Kulturart zur kommerziellen und industriellen Nutzung betrachtet, so wird eine enge Verwandte von *P. erosus* - der sogenannte "Chuin" Typ von *P. tuberosus* aus dem Amazonas Gebiet von Peru - genutzt und verarbeitet wie Cassava. Bislang ist gibt es wenig Information zur genetischen Diversität der Yam Bohne in Indonesien. Diese vernachlässigte Kulturart könnte für die Landwirtschaft Indonesiens attraktiver gemacht werden durch die Sammlung von Herkünften, Erfassung der Anbau- und Verarbeitungstechniken und Untersuchung der genetischen Diversität. Die Schätzung der genetischen Differenzierung der Yam Bohne auf Basis von morpho-agronomischen und molekularen Marker dürfte nützliche Informationen liefern für eine bessere Nutzung dieser genetischen Ressource. Hierzu wurden Feldstudien und genetische Diversitätsanalysen von Yam Bohnen Landrassen aus Indonesien durchgeführt mit den Zielen:

1. Erfassung des Kultivierungsstatus und Sammlung von Yam Bohnen Landrassen in Indonesien,
2. Analyse der morpho-argonomischen Diversität von Yam Bohnen Landrassen und
3. Vergleich der Diversität der Yam Bohne auf Basis von morpho-agronomischen und RAPD Marker Analysen.

Drei verschiedene Experimente wurden durch geführt. Die erste Untersuchung war eine Feldstudie und Sammlungsreise auf acht Hauptinseln Indonesiens um den gegenwärtigen Kultivierungsstatus der Yam Bohne zu dokumentieren und um lokale Landrassen aus verschiedenen geographischen Gebieten Indonesiens zu sammeln. Achtzig Informaten (Farmer und Landhändler) wurden interviewt und 110 Yam Bohnen Herkünfte wurden gesammelt. Die Yam Bohne wird lokal auf allen acht bereisten Inseln Indonesiens angebaut und wird als Gemüsekultur betrachtet, die hauptsächlich roh konsumiert wird aufgrund des hohen Wassergehaltes der Wurzelknolle. In West Indonesien (Sumatra and Java) wird die Yam Bohne hauptsächlich als Einzelfrucht angebaut – gelegentlich in größerem kommerziellen Maßstab –, während in Ost Indonesien (insbesondere in Sumba, Timor und Flores) die Yam Bohne vorherrschend im Mischanbau mit Mais und Cassava kultiviert wird. In West Indonesien bevorzugen die Konsumenten frühreife Genotypen mit relativ kleine und süße Knollen. In den östlichen Regionen Indonesiens – aufgrund von Trockenheit und lokal-traditionellen Marktpräferenzen – werden hingegen spätreife Genotypen mit mehr sukkulenten Fleisch und größeren Knollen bevorzugt. In West Indonesien betrug die Spannweite der Felderträge 10 bis 70 t ha^{-1} und in Ost Indonesien 10 to 50 t ha^{-1}. Es wird die Schlußfolgerung gezogen, dass die Yam Bohne gut an die verschiedensten Anbausysteme angepaßt ist und gut geeignet sein dürfte um die Bodenfruchtbarkeit nährstoffarmer Böden zu steigern.

Im zweiten Experiment wurde die genetische Diversität der Yam Bohnen Herkünfte unter Feldbedingungen geschätzt. In Feldversuchen an zwei Standorten in der Nähe von Bogor Indonesien wurde die genetische Diversität auf Basis morpho-agronomischer Merkmale untersucht. Vierzig ausgewählte

Yam Bohnen Herkünfte – bestehend aus 31 Yam Bohnen Landrassen von unterschiedlichen öko-geographischen Regionen Indonesiens und 9 Herkünfte von Süd- und Zentralamerika – wurden untersucht. Die Versuchsanlage war ein randomisierter Blockversuch mit zwei Wiederholungen an jedem Ort. Fünfzehn morphologische Merkmale wurden erfaßt und mit einer Varianzanalyse sowie multivariater Statistik ausgewertet. Euklidische Distanzen wurden berechnet und die resultierende Distanz Matrix wurde verwendet für eine UPGMA Clusteranalyse. Die Korrelation zwischen den Mittelwerten der Merkmale und den Hauptkomponenten wurden mit dem Pearson Korrelationskoeffizienten geschätzt. Die Varianzanalyse (ANOVA) und die multivariate Analyse (Hauptkomponentenanalyse und Clusteranalyse) für die 15 morpho-agronomischen Merkmale zeigte genetische Diversität für einige morpho-agronomische Merkmale der Yam Bohnen Landrassen. Die Hauptkomponenten-analyse zeigte, dass die ersten vier Hauptkomponenten 67.6 % der Gesamtvariation erklärten. Die erste Hauptkomponente erklärte 32.9 % der Gesamtvariation und war hauptsächlich korreliert mit Reifezeit, Hülsenlänge und Blattlänge. Die zweite Hauptkomponente erklärte 16.9 % der Gesamtvariation und war hauptsächlich assoziiert mit Blattbreite und Internodiumlänge. Die dritte Hauptkomponente erklärte 9.9 % der Gesamtvariation und war korreliert mit der Blattfarbe. Die Clusteranalyse zeigte, dass die in Sumatra gesammelten Herkünfte dazu tendierten von den Herkünften Javas und Ost-Indonesien getrennt zu sein. Amerikanische Yam Bohnen Herkünfte und Indonesische Yam Bohnen Herkünfte war verschieden.

Das Ziel der dritten Untersuchung war ein Vergleich der geschätzten Diversität mit morpho-agronomischen Merkmalen und der geschätzten Diversität auf Basis

von RAPD Markern. Diese Untersuchung umfasste 38 ausgewählte Yam Bohnen Herkünfte von Indonesien, sechs Herkünfte von Süd- und Zentralamerika und eine Herkunft aus Thailand. Die Beziehung zwischen der Euklidischen Distanz – geschätzt aus morpho-agronomischen Daten – und dem Jaccard Ähnlichkeit Index – berechnet aus polymorphen RAPD Banden – wurde mit einer Korrelationsanalyse ermittelt. Voruntersuchungen für RAPD Marker umfassten ein Screening von 250 Primern. Aus diesem Screening wurden 49 Primer ausgewählt die reproduzierbare Bandenprodukte zeigten. Insgesamt wurden 100 polymorphe Banden von diesen 49 Primern erzeugt und für die statistisches Analyse verwendet. Die mittlere Anzahl klarer polymorpher Banden pro Primer war 2.1 (eine relativ gering Anzahl im Vergleich zu anderen Arten). Hauptkomponentenanalyse (PCA), Hauptkoordinaten-analyse (PCoA) und Clusteranalyse wurden verwendet um das Muster der Variation zu erfassen. RAPD Marker und morpho-agronomische Merkmalsvariation zeigten keine starke Korrelation. Dennoch das generelle Muster der genetischen Diversität der Yam Bohnen Analyse auf Basis beider Methoden war ähnlich. Die meisten Landrassen von Java und Ost-Indonesiens waren getrennt von den Landrassen aus Sumatra. Amerikanische Landrassen war klar getrennt von den meisten Indonesischen Landrassen.

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Curriculum Vitae

Name	Agung Karuniawan
Nationality	Indonesia
Date of birth	1.11.1966
Place of birth	Sidoarjo, East Java, Indonesia
Sex	Male
Marital status	married, two sons
Official address	Department of Agronomy, Faculty of Agriculture, Padjadjaran University, Campus Jatinangor, Bandung 40600, Indonesia.
Email address	akaruni1@yahoo.de

School education

1972-1979	Primary school (SDN Kemerakan, Sidoarjo, Indonesia)
1979-1982	Secondary school (SMP N I Krian, Sidoarjo, Indonesia)
1982-1985	High secondary school (SMAN Krian, Sidoarjo, Indonesia)

University education

1985-1990	Under graduate program, Padjadjaran University, Bandung, Indonesia, Faculty of Agriculture. (Degree: Sarjana Pertanian equal to BSc).
1995-1997	Graduate program at the Integrated Tropical and Sub-Tropical Agricultural and Forestry Sciences, Georg-August University, Göttingen, Germany. (Degree: MSc.agr.).
2000-2004	Studying for obtaining Ph.D. degree at Institute of Agronomy and Plant Breeding, Georg-August University of Göttingen, Germany.

Professional employment

Since 1991	Teaching Assistant, Department of Agronomy, Faculty of Agriculture, Padjadjaran University, Bandung, Indonesia.