Agronomic performance and genetic diversity of the root crop yam bean (*Pachyrhizus spp.*) under West African conditions

Doctoral Dissertation

Submitted for the degree of Doctor of Agricultural Sciences
of the Faculty of Agricultural Sciences
Georg-August University Göttingen
Germany

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Göttingen, July 2003

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Date of oral examination: 17 July 2003

To my parents, brothers and sisters

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List of Abbreviations

AC	Andean yam bean (*P. ahipa*) accession
CC	Chuin Cultivar group of the Amazonian yam bean (*P. tuberosus*)
EC	Mexican yam bean (*P. erosus*) accession
G	Genotype
ha	Hectar
INRAB	Institut National des Recherches Agricoles au Bénin
L	Location
NIA	Experimental station at Niaouli (Benin)
NIRS	Near Infrared Reflectance Spectroscopy
PC	Principal component
PCA	Principal Component Analysis
R	Replication
SON	Experimental station of “Centre Songhai“ in Porto-Novo (Benin)
t	Ton
TC	Amazonian yam bean (*P. tuberosus*) accession

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1. Introduction and literature review

1.1 Background and objectives

Many thousand plant species have been used for several purposes by human. About 100 have been developed into important crops (Hill et al., 1998) and only few of these crops have been intensively and widely used in the world´s agriculture. This has lead to the shrinking or erosion of agricultural biodiversity and at the same time to an increasing level of vulnerability of food suply. These concerns have generated growing interest in the research on “underutilized“ crops.

Root and tubers are second in importance for human nutrition after cereals. Conventional root crops such as cassava, sweet potatoes, yam and taro are seriously deficient in protein and when used as the main source of nourishment, the local population, especially weaning children, often suffers from protein deficiences. Already in 1979 the FAO (1979) pointed out that due to the unawareness of legume root crops extension agents recommend farmers to cultivate conventional root crops which often imperil good protein food sources.

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

The yam bean is attractive for agronomy and plant breeding. As a root crop it might provide high yields as well as high yield stability (Grüneberg, pers. comm.) and as a legume it will produce protein rich food and improve sustainability in cropping systems (NRC, 1979). However, the plant has been known until recently as a

vegetable crop only because the tuberous roots are usually consumed raw due to their high moisture content. Sørensen et al. (1997) observed a new yam bean type within *P. tuberosus* which is consumed like manioc and this so-called Chuin type from Peru has a high dry matter content (Grüneberg et al., 1998). This has lead to the conclusion that the yam bean might be developed into a widely adapted protein rich staple legume root crop. Grüneberg et al. (2003) reported that *P. ahipa* can be hybridized with the *P. tuberosus* Chuin type resulting in fertile and vigorous hybrids. The starch may be of good quality in regard to the digestibility and consists essentially of amylopectine (Bergthaller et al., 2001). The seeds of the yam bean are not used due to the high rotenone content (about 1% seed weight), even so the seeds are an interesting source of high palmitic acid oil (Santos et al., 1996, Grüneberg et al., 1999). According to Santos et al. (1996) a rotenone reduction from 1% to 0.06% is achieved by heating and solvent extraction.

In West African countries as in many other world regions there is the need to increase the production of high quality food and the sustainability of cropping systems. A broadly adapted high dry matter yam bean which can be used like cassava could be a crop which might help to fulfill these needs, especially in Sub-Saharan Africa where in some areas root crops are a major source of nourishment. So far investigations on the yam bean germplasm as well as on the possibility to incorporate high dry matter from the Chuin type into the remaining yam bean genepool is limited. There are numberous reports and poster presentations on the yam bean (Sørensen,1996; Sørensen et al., 1996, Sørensen et al., 1997; Nielsen et al, 1999; Nielsen et al., 2000), but many of these investigations have focused only on few accessions or were not carried out at several locations. It is still necessary to evaluate a broad range of yam bean accessions under field conditions in order to obtain more detailed information about the climatic zones where yam beans can be grown, the agronomic potential of accessions as well as the genetic diversity within the yam bean genepool. Informations on genetic distances within and between the species may assist to get a better understanding of the phylogenetic relationships between yam bean species and the amount of

diversity within each yam bean species. This information is helpful to make decision concerning parental material for further breeding programs.

Breeding research over the past decade has developed techniques which assist the breeder to take the most promising material into his multi-locaton field trials. Traditionally genetic diversity has been estimated mainly for morphological traits. This is still the most widely used method to group genebank material. However, the measurement of many morphological traits is laborious and time consuming.

Near Infrared Reflectance Spectroscopy (NIRS) can offer an interesting alternative to estimate genetic diversity. It is cheap, rapid and does not require to germinate seeds. So far, NIRS is mainly used as alternative to wet chemistry procedures for determining concentrations of major classes of chemical compounds in organic materials. The methods utilizes reflectance signals resulting from bending and stretching vibrations in molecular bonds between carbon, nitrogen, hydrogen, and oxygen.

This study consists of three parts:

1. Estimation of the population mean and variation of agronomical traits in the yam bean (*Pachyrhizus spp.*) germplasm in field experiments at two locations in Benin (West Africa). The three cultivated species *P. erosus*, *P. ahipa* and *P. tuberosus* (including Chuin types with high dry matter content) are compared.

2. Estimation of the genetic diversity in the yam bean germplasm between and within the three species. Data from the same field experiments at two locations in Benin are analysed by multivariate methods.

3. Investigations to use NIRS for estimation of genetic diversity by comparing clusters obtained from morpho-agronomic traits and NIRS measurements of whole seeds.

1.2 The genus *Pachyrhizus*

1.2.1 Botanical description, taxonomy and ecogeographical requirements

The name *Pachyrhizus* comes from the Greek *Pachys* = thick(ened) and *rhiza* = root. The genus is taxonomically classified in the family *Fabaceae*, subfamily *Faboidae*, tribus *Phaseoleae* and subtribe *Diocleinae* in close relationship to the subtribe *Glycininae* and *Phaseolinae* (Lackey, 1977; Ingham 1990, Sørensen, 1988, 1996). One of the first botanical references to the yam bean was made by Plukenet in 1696, who described a plant from Mexico as *Phaseolus nevisensis* (Belford, 2000). The present generic name *Pachyrhizus* was originally used by L.C.M. Richard. *Pachyrhizus* is delimited by the short hairs on the adaxial side of the ovary extending almost to the stigma, forming a „beard“ along the incurved style and by the median to subterminal globular process on the adaxial side of the stigma (Sørensen, 1996).

The genus contains five 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. Most likely *P. panamensis* is the common ancestor of *P. ahipa* and *P. tuberosus* and *P. ferrugineus* the ancestor of *P. erosus*. A key to the species is given in Table 1.1.

Table 1.1. Key to *Pachyrhizus* species (Sørensen, 1988)

1. Climbing or trailing vines with entire, dentate or palmate leaflets, occasionally leaflets with differing outline on the same plant; racemes always dibotryoid troughout.....2
1. Erect to semi erect herbs with all leaflets entire; racemes simple or only basally dibotryoid.....5. *P. ahipa*
2. Stems entirely herbaceous; leaflets membranaceous; number of pedicels on lateral axes 4-14; L/W ratio of mature legumes above 8:1.....3
2. Stems woody at base; leaflets subcoriaceous, nearly glabrous; number of pedicels on lateral axes 8-20; L/W ratio of mature legumes below 6:1.....2. *P. ferrugineus*
3. Calyx strigose with brown hairs; floral prophylls lanceolate, strigose with brown hairs, 0,8-3,0 mm long throughout; wing and keel petals glabrate to ciliolate; mature legumes glabrous or strigose with brown hairs.....4
3. Calyx strigose to hirsute with white hairs; floral prophylls linear, strigose with white hairs, 2,8-3,3 mm long throughout; wing and keel petals ciliolate; mature legumes hirsute with white hairs.....3. *P. panamensis*
4. Wing and keel petals glabrous; legume glabrous to strigose at maturity, 6,0-13,0 cm long; seeds flat, square to rounded.....1. *P. erosus*
4. Wing and keel petals ciliolate, rarely glabrous; legume at maturity 13,0-14,0 cm long; seeds plump, reniform.....4. *P.tuberosus*

1. The Mexican Yam Bean (*Pachyrhizus erosus* (L.) Urban)

This cultivated yam bean species is found in Central America as well as South East Asia. The species is named Jicama in Mexico and bang kuang in Indonesia. It has a herbaceous vine with great variation in the outline of the leaflets, from dentate to palmate (Sørensen, 1996). Moreover the species is defined by the lack of hairs on the petals, the number of flowers (4-11) per lateral inflorescence axis by complex

racemes. Morphological characters of the pods are also used to distinguish the species. A number of seed characters are also specific. These include the color, ranged from olive-green to brown or reddish brown (Sørensen, 1988, 1990, 1996).

The photothermal sensitivity in *P. erosus* was analysed in many studies. In Hawai, Paull et al. (1988) observed a significant overlap between flowering and tuberization during short days under field conditions. Field experiments conducted in Guadeloupe revealed the response to different planting dates and tuber growth (Zinsou et al., 1987a, 1987b, 1987c, 1988; Robin et al., 1990: long days; Zinsou and Venthou-Dumaine, 1990; Sørensen et al., 1993; Vaillant and Desfontaines, 1995: short days). The results of these experiments suggested the strong competition between shoot growth, flowering, pod formation and tuber growth. During long days, tuber growth is initiated after 4-6 weeks. Flowering was initiated when the daylength approaches 12.5 hours. During short days, there is an increase of tuber growth (Cotter and Gomez, 1979; Sørensen, 1996). The habitat of wild *P. erosus* is along deciduous forest edges and in shrub vegetation in areas with an annual dry season. The soil types range 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. The optimal day/night temperature is 30/20°C (Grum, 1990; Sørensen, 1996). Well-drained, sandy, alluvial soils are preferred in cultivation (Sørensen, 1996).

2. The Andean Yam Bean (*Pachyrhizus ahipa*)

The local name of this species is Ahipa or Ashipa. It is distinguished morphologically from the other species by being a herbaceous plant with generally entire leaflets, short racemes, which are simple (Sørensen et al., 1997). The wing and keel petals are usually glabrous, but slightly ciliolated specimens have been seen (Sørensen, 1996). The morphological characters of the pods and seeds are also specific. The pods are only slightly dorsiventrally compressed. Both determinate and indeterminate growth habits exist in the species.

The Andean yam bean is daylength neutral and this yam bean species has the most rapid flower initiation. Sørensen (1996) reported start of flowering of 87 days after sowing . So far *P. ahipa* has only been found cultivated. The habitat are cool tropical/subtropical valleys within the altitudinal range of 1800-2900 m a.s.l. (Sørensen, 1996). The crop is adapted to the average temperature within the region of about 16-18°C, where temperature oscillates between a minimum of 0-5°C and a maximum of 30-35°C. In precolombian time it has been cultivated in most Andean highlands but today its distribution is restricted to Bolivia and Northern Argentina. The average annual precipitation in the present day distribution area is between 400 and 700 mm (Sørensen, 1996), however the crop is always irrigated by hill irrigation (Ørting et al., 1996). It is found on farm systems along loamy river banks, though in some cases, sloping hillsides with loamy soil are used. The soil may have a pH of 6-8 (Ørting et al., 1996).

3. The Amazonian Yam Bean (*P. tuberosus*)

P. tuberosus is found cultivated in the tropical lowland to both slopes of the Andean mountain range as well as in the Carribbean. It has many cultivar groups with different local names, e.g. Ashipa, Jíquima, Chuin. *P. tuberosus* has a stem up to 7 m long and is the largest yam bean species. The pods are also larger than those of the other species and are conspicuously compressed between seeds. The seeds are black, black and white mottled or orange-red in color (Sørensen, 1996). The species has retained recent interests because Chuin cultivars from the Ucalali have a high tuber dry matter content (mainly between 26 – 30 %) and are used and processed like cassava (Sørensen et al., 1997, Grüneberg et al., 2003).

Alvarenga and Válio (1989) reporting the effect of different temperature and photoperiodic regimes on the initiation of flowering and tuberous root formation in genotypes belonging to the Ashipa cultivar group, observed that flowering was initiated at daylength of 9-16 hours. Considering the tuberization, the crop may be a short day plant, as the tuberization process occurs at day length below 12 hours (Sørensen, 1996). Day/night temperatures of 30/25°C delay and reduce flowering

and completely inhibit the tuberization. Inversely, day/night temperatures of 25/20°C and 20/15°C were suitable (Alvarenga and Válio, 1989).

P.tuberosus is adapted to sandy or light, well-drained and fertile soils. The species is recorded from 550 to 2000 m a.s.l., with an annual precipitation from 640-5000 mm and temperatures varying between 21.3 and 27.4°C and a soil pH from 4.3 to 6.8 (Munos Otero, 1945; Duke, 1981; Sørensen, 1990; Sørensen et al., 1996). Specificaly, jíquima is recorded at 30-350 m a.s.l. with a precipitation between 450 and 500 mm and a maximum temperature of 31.1-31.6°C, and relative humidity of 90% during the wet season. Ashipa is recorded at 300-2000 m a.s.l., with a precipitation range from 1500-5700 mm and an average temperature of 20.7-25.5°C (minimum temperature: 11.0-13.2°C; maximum temperature: 29.7-35.4°C) and a relative humidity of 84-92% (Sørensen, 1996). Chuins are found at 100-300 m a.s.l., and the annual precipitation is about 3000 mm (Sørensen, 1996)

4. *P. ferrugineus*

P. ferrugineus is a wild yam bean species. It is known from the states of Vera Cruz, Chiapas and Quintana Roo of Mexico as well as Belize and south through the eastern and central parts of Guatemala. It is an herbaceous, basally semi-woody, climbing vine of 1 to 5 m. The tuberous root is woody, up to 60 cm long, with dark brown surface, whitish brown inside. The stems are spirally striated, strigose to glabrate with brown hairs. The woody parts are with prominent lenticels. Leaves subcoriaceous are densely to sparingly strigose, dark green adaxially, light green abaxially. Lateral leaflets are obliquely ovate, entire acuminate, dentate or with 2-4 palmate, shallow to deep and narrow lobes. The terminal leaflet is ovate to rhomboid, entire, dentate, palmately lobed with 3-5 shallow to deep and narrow lobes or rarely linear-oblong with two rudimentary basal lobes. Racemes are strigose with brown hairs. Flowers are blue to dark violet blue. Pods are oblong, strigose to hirsute with brown hairs. Seeds are flat and square to rounded, never reniform, brownish red (Sørensen, 1988).

5. *P. panamensis*

P. panamensis is the second wild species within the genus *Pachyrhizus*. It is known from the Panama Canal Zone in the north, to the forest at Onaca, Santa Marta, Colombia, in the south. It is an herbaceous climbing vine. The root is somewhat elongated, with brown epidermis, greenish white cortex. The stem is striated, strigose with white hairs. Leaves are pilose with white hairs on both surfaces. The lateral leaflets are obliquely ovate, entire or with two shallow lobes. The terminal leaflet is broadly ovate with shallow palmate lobes. Racemes are pilose with white hairs. Flowers are light blue to blue. Legumes are hirsute to sericeous with white hairs, retaining pubescence at maturity. Seeds are rounded, olive green (Sørensen, 1988).

All yam bean species are dipoid with a basic chromosome number of n = 11. Futhermore interspecific hybridizations between all cultivated yam bean species result in fertil and vigorous hybrids (Sørensen, 1996; Grüneberg et al., 2003) so that the cultived yam beans can be considered as one primary genpool. The yam bean is mainly self-fertilizing with an out breeding rate up to 8 % (Grüneberg, pers. Comm.).

1.2.2 Agronomy and breeding

Distribution, Production and Uses

The cultivated species of the yam bean and particularly *P. erosus* are locally grown in nearly all countries of Central and South America as well as South East Asia. With exception of the Chuin type the tuberous roots of the crop are always considered as a vegetable and are most often consumed raw. Tuber production is usually conducted on a small scale. Only from Mexico, the Philipines and Indonesia, commercial production on several hectars are known for *P. erosus*. There is no report that the seeds of the yam bean are used by farmers due to the high rotenone content (about 1% seed weight) and usually pruning of reproductive

parts is conducted in order to increase tuber production. However, if the rotenone could be removed, the seeds provide a good protein food source (Santos et al., 1996) and the seed oil is interesting for the food industry and can be an alternative to groundnut or cotton seed oil (Broadbent and Shone, 1963; Jimenez B., 1994; Grüneberg et al., 1999). Moreover, the rotenone itself can be used because the extracted rotenone and rotenoids have insecticidal effects (Alavez-Solano et al., 1998).

Cultivation

The cultivation practices of the Amazonian, Mexican and Andean yam bean vary greatly among growers. It depends largely on socio-economic setting, labour, resource availability, markets and the farming system into which the crop has to fit (Grum, 1990). However, all cultivated yam beans are propagated by seeds and grown as an annual crop, even though the plants have a perennial habit (Sørensen, 1996). Pruning of reproductive parts is usually conducted for the Mexican and Andean yam bean, but according to Sørensen (1996) for the Amazonian yam bean this cultivation practice is only conducted for the Ashipa cultivar group in the province of Manabi / Ecuador by cutting flowers and up to one third of the vegetative part.

The Mexican yam bean *(P. erosus*) was traditionally intercropped with maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris*) in Central America. According to Heredia (pers. communication) the land area for maize, common bean and yam bean is 35%, 35% and 30 %, respectively. All three species are sown at the same time at end of April and harvest starts with common bean after 90 days, followed by maize after 120 days and yam bean after 140 days. Today the crop is usually monocropped in Mexico often for a larger commercial scale for exportation into the United States of America (Heredia, 1985). The crop is generally not fertilized (Grum, 1990; Sørensen, 1996). Recommended plant density as a monocrop varies from 2.5 to 18 plants/m^2 and depends on the length of the growth period, the

desired size of the tubers and day length at the time of planting (Heredia ,1985; Sahadevan, 1987, Grum, 1990).

The Andean yam bean *P. ahipa* is most often grown as a monocrop and only few cases have been observed where the crop is intercropped with maize (Ørting et al., 1996, Ørting et al., 1998).

The Amazonian yam bean (*P. tuberosus*) with the three main cultivar groups Ashipa, Chuin and Jíquima is generally grown in shifting cultivation by the Indians of the Amazon region (Brücher, 1977; Duke, 1981; Sørensen et al., 1997). The Ashipa cultivar group is grown in areas with a permanently humid climate and mostly on land that is never inundated, i.e. largely infertile, acid and aluminium-loaded uplands (Salick, 1989; Veléz & Veléz, 1993a, 1993b; Sørensen et *al*, 1996). The development of tubers takes place 8-12 months on such soil. But Ashipa grow faster and produce larger tuber on more fertile soil. In the Ashipa cultivar group, reproductive pruning is usually not practised. Ashipas are generally intercropped with plantain (*Musa paradisiaca* L.), cassava and pineapple (*Ananas comosus* (L.) Merill.) (Sørensen, 1996). The Chuin cultivar group is also cultivated in a permanent humid climate, but exclusively on floodplains. It is grown at the higher irregularly or only briefly inundated levees as well as on lower levees, which are flooded for up to 6 months each year, and on river banks, that have not yet been stabilized by vegetation which are flooded for up to 8 months each year. Chuins are intercropped with plantain and cassava at the higher levees and at the lower levees with maize, beans and vegetables. The Jíquima cultivar group is grown in the seasonally dry costal province of Manabí, Ecuador. The crop is monocropped, but also intercropped with chilli (*Capsicum spp.*), sesame (*Sesamum indicum* L.), groundnut (*Arachis hypogaea* L.) and tomato (*Lycopersicon esculentum* L.) (Sørensen, 1996). Today the crop has nearly disappeared in this region and was only found at three locations during a field survey study in 1994 (Estrella, Orting and Grüneberg, pers. communication).

There are many reports of yam bean tuber yield, which are summarized in Table 1.2. Nevertheless, a comparison of all three cultivated species of yam bean has not been published so far. For West Africa the only data available are yield estimates from 10 *P.erosus* accessions in Senegal (Annerose and Diouf, 1998) and 15 *P. erosus* accessions in Sierra Leone (Belford, 2000, Belford et al., 2001).

Breeding

The yam bean has bisexual flowers and is mainly self-pollinating. Some cross fertilizations occur depending on the availability of pollinators, mainly bumblebee species (Sørensen, 1996). All species with the exception of *P. ferrugineus* have been demonstrated to be compatible, resulting in fertile interspecific hybrids (Grum, 1990).

Recurrent selection involves cycles of crossing and selection. This method has been most widely used in cross-fertilizing species, but was also applied in self-fertilizing species (Wricke and Weber, 1986). The main advantages of recurrent selection is that more recombination is permitted by repeated crosses, so undesirable genes are not fixed and can eventually be discarded (Grum, 1990). The major disadvantage is that the method is labour intensive, requiring large numbers of crosses. The starting population may be the F2 generation of a single cross or the result of multiple crosses. The large number of mating designs in a recurrent selection programme stretch from random mating to the diallel selective mating system proposed by Jensen (1970). A recurrent selection programme can be an ongoing programme from which lines can be continuously extracted for evaluation as new varieties. New lines can also be added to the programme as required (Jensen, 1988).

Table 1.2. Literature overview of yield performance in *Pachyrhizus spp.*

Species	Experiment	Number of accessions	Country	Yield (t ha^{-1})	Authors
P. ahipa	Field survey Study	8	Bolivia	Mean: 28.5 Range: 10 – 74	Ørting et al. 1996
	2 row plot at two locations	15	Spain	Mean: 22.60 Range: 13.98 – 43.88	Velasco et al. 2001
P. tuberosus	Pruning practices	6	Kingdom of Tonga (South Pacific)	Mean: 23.3 Range: 7.4 – 27.7	Nielsen et al. 1999
P. erosus	Three years field trials	60	Kingdom of Tonga (South Pacific)	Mean: 93.8 Range: 77.0 – 125.9	Nielsen et al, 2000
P. erosus	6 row plot	15	Sierra Leone	Mean: 14.37 Range: 10.19 - 22.87	Belford et al. 2001
P. erosus	Multi-locations trial 4 row plot	10-14	Senegal	-	Annerose and Diouf 1998
P. erosus	5 row plot	8	Thailand	Mean: 27.84 Range: 20.24 – 35.56	Ratanadilok et al. 1998

According to Grum (1990), the self-fertilizing nature of the genus gives inbred homozygous lines as the input and output of breeding. This plays an important role in the statistics of inheritance and influences the methods and stages of selection. The diploid (2n=22) nature of the species (Sørensen, 1988) makes it easier to evaluate recombinant lines after a cross, as recessive alleles will show up in smaller groups of progeny than they would with higher levels of ploidy. The breeding methods that are suitable for *Pachyrhizus* are those methods generally applicable to self-fertilizing crops with the exception of the bulk method (Grum, 1990). The bulk method utilizes natural selection for reproductive capacity, which probably would be at the expense of tuber growth. The economically best results of breeding would be obtained by the simultaneous selection for number of viable seeds and tuber yield, weighing the two characters according to their economic values, thus obtaining a single value for selection (Grum, 1990).

Cross populations between *P. ahipa* and the Chuin types of *P. tuberosus* have been developed by Grüneberg et al. (2003). 24 populations from crosses between *P. ahipa* and *P. tuberosus* have been tested at two locations and unpruned field trials in Indonesia at Bogor and Bandung with 20.1 t ha^{-1} tuber yield and 22.6% tuber dry matter content across all populations and means of 30.1 t ha^{-1} tuber yield and 27.7% tuber dry matter content for the best population (Grüneberg pers. comm.). Breeding of yam bean is concerned above all with the development of types with high dry matter content and also with the development of types with high quality traits, e.g. starch and protein content of the tubers.

1.2.3 Chemical Composition and Nutritional Value

Menezes and Oliveira Nunes (1955) report the composition of the tuber of *P. tuberosus* of a local genotype belonging to the Ashipa cultivar group. In this study 100 g fresh weight of tuberous root contained 90.4 g water and 100 g dry matter contained 10.4 g nitrogenous compounds / proteins, 0.9 g lipids, 79.4 g non-nitrogenous compounds/starches/sugars, 6.4 g fibres and 2.9 g minerals. Duke (1981) has reported the presence of adenine and choline in the tuber.

Several studies reported the nutritive value of the tuber of *P. erosus* (Nag et al., 1936; Rattan and Sen, 1941; Porterfield, 1951; Martínez, 1956; Aguilar, 1958; Wu and Flores, 1961; Purseglove, 1968; National Research Council, 1979; Duke, 1981; USDA, 1984; Tadera et al., 1984; Sahadevan, 1987; Hoof and Sørensen, 1989; Ratanadilok and Thanisawanyangkura, 1994). These reports indicated, that *P. erosus* tubers are a source of proteins, lipids, starch, sugars, fibres, ash, minerals and vitamins. Schmar et al. (1987) studied the structural changes in yam bean tuber as a result of microwaving. The tubers used in the study were found to have increased digestibility, compared with fresh tuber.

The nutritive value of *P.ahipa* was less studied. Bergthaller et al. (2001) suggested *P. ahipa* as a new source of starches.

The yam bean pod size and thousand seed weight is comparable to those of *Phaseolus* beans, but the presence of high levels of isoflavonoids (rotenoids and pachyrhizide) makes pods and seeds unsuitable for human consumption (Alvarenga and Válio, 1989; Scramin, 1994). The characteristic of yam bean *Pachyrhizus* is the presence of the isoflavonoid called rotenone, an insecticidal compound, in the mature seeds. Moreover, Duke (1981) reported adenine, choline, rotenone and saponine in the mature seeds. Lepage et al. (1946) studied the toxic effect of the constituents (rotenone and pachyrhizine) of *P. tuberosus* seeds on aphids (*Aphis brevicoryne var. brassicae* (L.). Hansberry et *al* (1947) reported the toxic effect of the seed extract of *P. tuberosus* on larvae of the mexican bean weevil.

The insecticidal and fungicidal properties of yam bean seeds have been widely studied (Sillevoldt, 1899a, 1899b; Boorsma, 1910; Peckolt, 1920; Nag et al., 1936; Hwang, 1941; Liu and Hsu, 1941; Hansberry and Lee, 1943; Norton, 1943; Plank, 1944; Norton and Hansberry, 1945; Lepage et al., 1946; Meijer, 1946; Hansberry et al., 1947; Jakobs, 1949; Simonitsch et al., 1957; Holz and Hong, 1964; Ollis, 1964; Krishnamurti and Seshadri, 1966; Krishnamurti et al., 1970; Sahu and Hameed, 1983; Bortolato et al., 1985; Magalhaes et al., 1987; Magalhaes et al., 1988;

Jimenez B., 1994). These studies found out that the rotenone has high insecticidal properties.

Scramin (1994) and Villar and Válio (1994) pointed out the ecological advantages for the species to contain rotenoids in the seeds and leaves, as the compounds are likely to have a protective effect against insect predators. Moreover the positive insecticidal effect of seed extract of *P. erosus* has been studied (Walker and Anderson, 1943; Adjahossou and Sogbenon, 1994; Halafihi, 1994).

The characterization and quantification of the amino acids composition of *P. tuberosus* seeds compared with soya bean seeds was reported by Sales et al. (1990). The seeds are rich in both proteins and lipids/oil (Grüneberg et al., 1999). Yam bean seeds are characterized by high oil (about 20 to 28 %) and protein (about 23 to 34 %) contents. Seed oil contains high concentrations of palmitic (about 25-30 % of the total fatty acids), oleic (21-29 %) and linoleic (35-40 %) acids. The levels of linolenic acid are very low, from 1.0 to 2.5 %. Total tocopherol content of the seeds was relatively low in *P. erosus* (from 249 to 585 mg kg^{-1} oil) and *P. tuberosus* (from 260 to 312 mg kg^{-1} oil) compared with the levels found in *P. ahipa* under identical conditions (508 to 858 mg kg^{-1} oil) (Grüneberg et al., 1999). Other studies also showed the chemical composition and quality of the *P. erosus* oil (Cruz, 1950; Broadbent and Shone, 1963; Jimenez B., 1994; Santos et al., 1996). These studies agree that if the insecticidal compounds are removed, the oil has a composition comparable with that of groundnut and cottonseeds oil.

1.2.4 Biological Nitrogen Fixation

The genus has an efficient symbiosis with nitrogen-fixing *Rhizobium* and *Bradyrhizobium* bacteria. No additional supply of nitrogen fertilizer is therefore required. The crop allows a sustainable land-use system from both an ecological and a socioeconomic standpoint. Studies pointed out the efficiency of the biological nitrogen fixation under greenhouse and field conditions. Castellanos et al. (1997) reported the first field test quantifying the actual amount of nitrogen fixed by two

accessions of *P. ahipa* (58-80 kg N / ha) and three cultivars of *P. erosus* (162-215 kg N / ha). 50 % of the N harvested (800 Kg protein / ha) were accumulated in the tuber in *P. erosus*. The amount of N recorded in the residue (hay) of *P. erosus* was 120-150 kg, which is twice the amount recorded in the *P. ahipa* residue and is higher than the quantity recorded in practically all grain legumes (Sørensen, 1996). One must mention that the plant population of both species was 110.000 plants / ha and the plants were reproductively pruned.

2. Evaluation of the root legume yam bean (*Pachyrhizus spp.)* under West African Conditions

2.1 Introduction

The name yam bean is used to designate the genus *Pachyrhizus* in particular the three cultivated species: Amazonian yam bean (*P. tuberosus*), Mexican yam bean (*P. erosus*), the Andean yam bean (*P. ahipa*). However, crosses between all cultivated yam beans result in fertil and vigorous hybrids (Grum, 1990; Grüneberg et al., 2003) so that the species can be considered as one primary genepool. Unlike its relatives the soybean and the *Phaseolus* beans the yam bean is exclusively used for its tuberous roots. The tuber contains a relatively high protein content (8 to 18% of dry matter), which is three to five times higher compared to traditional root crops such as potato, cassava and yam (Velasco and Grüneberg, 1999). The seeds of the yam bean are not used due to the high rotenone content (about 1% of seed weight), though the seeds have a high protein (26 to 32% of seed weight) and oil content (22 to 26 % of seed weight) (Santos et al., 1996; Grüneberg et al., 1999). In small scale and commercial production reproductive parts are usually pruned to increase tuber production. The tuber of yam bean is used as a vegetable and is characterized by a high moisture content, usually more than 80 % of fresh tuber weight. The exception is the Chuin cultivar group of *P. tuberosus* from Amazonian Peru with a moisture content between 68 to 72 % of fresh tuber weight (Sørensen et al., 1997).

The yam bean has low demands for nitrogen fertilisers, as it has an efficient symbiosis with rhizobia for the fixation of nitrogen (Castellanos et al., 1997). The crop is also associated with mycchoriza, which facilitates the supply of phosphor for the plants. The low inputs required make *Pachyrhizus* a highly suitable crop for the small farmer. According to Castellanos et al. (1997), a substantial amount of the fixed nitrogen is returned to the soil. The crop therefore forms an integral part of a

sustainable land use system, both in an ecological sense and from a socio-economic standpoint (Grum and Sørensen,1998).

Agronomical data on the yam bean germplasm is limited and restricted to field experiments with few accessions. This study was conducted to evaluate the yam bean germplasm comprising all three cultivated yam bean species as well as the Chuin cultivar group which may be of interest to diversify West African agro-ecosystems. The study was conducted at two diverse sites in Benin and the effect of reproductive pruning was examined.

2.2 Materials and Methods

Plant material

A total of 34 accessions representing better agronomic types from diverse ecogeographical backgrounds were used for the present study. The accessions consist of 14 *Pachyrhizus ahipa* lines, 14 *P. erosus* accessions and 6 *P. tuberosus* accessions. The *P. ahipa* material was selected from single plant progenies out of 13 accessions. At least one genotype was selected out of each accession. In *P. ahipa*, genotypes were designated by accession and progeny line number respectively. From AC214, two lines (AC214-109 and AC214-110) were selected. No selection was carried out for the *P. erosus* and the *P. tuberosus* material. An overview of the accessions is given in Table 2.1.

Seed multiplication was done from June 2000 to January 2001 at the “Centre Songhai“ in Porto-Novo (Benin). 4 to 8 plants were used for the multiplication to have sufficient seeds for the following evaluation.

Field experiments

The germplasm was grown in 2001/2002 at the “Centre Songhai“ station in Porto-Novo and at the experimental station of INRAB (Institut National des Recherches Agricoles du Bénin) in Niaouli. The soil was well drained at both stations and is sandy red loam. The experiments were carried out between June 2001 and January 2002. The characteristics of the two locations are presented in Table 2.2.

Table 2.1. List and passport data of accessions tested, (CC = Amazonian yam bean (*P. tuberosus*) Chuin cultivar group, TC = Amazonian yam bean (*P. tuberosus*) Ashipa cultivar group, EC = Mexican yam bean (*P. erosus*) and AC = Andean yam bean (*P. ahipa*)).

Accession code	Collector(s)	Country	Region	Longitude	Latitude
CC353	Jensen & Thirup	Peru	Requena	73°53´W	5°05´S
CC354	Jensen & Thirup	Peru	Requena	73°59´W	4°59´S
CC355	Jensen & Thirup	Peru	Requena	73°50´W	5°07´S
CC361	Huanta	Peru	Requena	73°53´W	5°05´S
CC362	Huanta	Peru	Rio-Ucayali	73°12´W	3°42´S
TC118	Hyvert	Haiti	Nord Este	72°19´W	19°50´N
EC006	Sørensen	Mexico	Oaxaca	96°42´W	17°03´N
EC032	N.N.	Mexico	Yucatan	89°01´W	20°48´N
EC033	N.N.	Mexico	Yucatan	88°49´W	20°42´N
EC040	Sørensen	Guatemala	Jutiapa	90°01´W	14°12´N
EC041	Sørensen	Guatemala	Jutiapa	90°01´W	14°12´N
EC042	Sørensen	Guatemala	Jutiapa	90°02´W	14°03´N
EC104	CATIE no 17137	Mexico	Yucatan	88°58´W	20°13´N
EC114	N.N.	Brazil	Para	51°51´W	3°23´S
EC204	Sørensen	Mexico	Vera Crux	96°57´W	19°25´N
EC253	Hue Anh.	Vietnam	Tan An	106°39´E	10°59´N
EC533	Cheang Keong	Macau	-	113°54´E	22°2´N
EC550	CAEB	Mexico	Guanajuato	100°53´W	20°31´S
EC557	INIFAP/CIFAP	Mexico	Guanajuato	100°53´W	20°31´N
ECKEW	(1)	Mexico	-	-	-
AC102	Sørensen	Bolivia	(2)	64°43´W	21°31´S
AC201	Ørting & Grüneberg	Bolivia	Luribay	67°38´W	17°00´S
AC202	Ørting & Grüneberg	Bolivia	Luribay	67°38´W	17°00´S
AC203	Ørting & Grüneberg	Bolivia	Luribay	67°36´W	17°00´S
AC205	Ørting & Grüneberg	Bolivia	Machaca	66°53´W	17°8´S
AC208	Ørting & Grüneberg	Bolivia	Machaca	66°53´W	17°6´S
AC209	Grum & Grüneberg	Bolivia	Tirata	67°46´W	16°46´S
AC213	Ørting	Bolivia	Irupana	67°27´W	16°34´S
AC214	Grüneberg	Bolivia	Arce	67°35´W	16°49´S
AC215	Grüneberg	Bolivia	Arce	67°34´W	16°44´S
AC216	Grüneberg	Bolivia	Arce	67°34´W	16°44´S
AC524	(3)	-	-	-	-
AC525	Valio	Bolivia	Ayopaya	66°10´W	17°23´S

(1) ECKEW obtained from botanical garden KEW, England; (2) = AC102 obtained from market in Tarija; (3) = AC524 from Jardin Botanique, Meisen, Belgium, no. 0494 of unknown origin (growth type of AC524 similar to AC102). N.N. = Unknown

Experimental design was a completly randomised block with two replications at both locations. Separate blocks were conducted for each treatment (pruned or unpruned) and each species (*P. tuberosus, P. erosus, P. ahipa*). An illustration of the field plan for this design is given in Tables A.1 and A.2. Each plot consisted of 4 rows of 6 plants each and a plot measured 1.25 m by 2.25 m. The distance between plots was 1 m . Two rows were spaced 0.75 m apart and the distance between plants within a row was 0.25 m. Two seeds were sown per hole at a depth of about 2 cm. Thining of the plants to one per hole was done five weeks after sowing. Irrigation was done at the station „Centre Songhai“ in Porto-Novo, during a period comprising the dry month August. At Niaouli, no irrigation was applied. Weeds were removed every two weeks. Two pickets were erected to maintain the plant upright. No fertiliser or pesticide was applied. For the treatment reproductive pruning, all flowers were removed one time each week.

For blocks without reproductive pruning 31 agronomical characters were measured which are listed in Table 2.3. This table presents the traits, codes and procedures of recording for each character. The IPGRI descriptors lists for *Phaseolus spp.*, *Vigna spp.* and *Ipomoea batatas* (sweet potato) were used with minor modifications. Data on single plant basis were recorded on six randomly selected competitive individuals within the two center rows.

For blocks with reproductive pruning the following 12 agronomical characters were measured (Table 2.3): TUBY, TDMY, VLW, BIOM, DM, HIT, DSLI, DTN, NTP, PRO, CAR, C/N.

Table 2.2. Description of experimental sites

	Songhai	Niaouli
Region	Ouemé – Porto-Novo	Atlantique
Longitude	02°37 E	02°18 E
Latitude	06°29 N	06°66 N
Institution	Experimental station of "Centre Songhai", Porto-Novo	Experimental station of "Institut National des Recherches Agricoles au Bénin (INRAB)" in Niaouli
Average temperature	28.1°C	27.2 °C
Max. temperature month	32.3°C	31.8 °C
Min. temperature month	23.8 °C	22.5 °C
Annual rainfall	976.9 mm	1101.3 mm
Rainfall periode 1	March to July	March to July
Rainfall periode 2	September to November	September to November
Rainfall in period 1	755.5 mm	767.4 mm
Rainfall in period 2	212 mm	333.9 mm
Soil type	Sandy loam red	Sandy loam red
Soil pH	7.1	6.6
Irrigation	Irrigation: yes	No irrigation
Sowing date	22.06.2001	20.06.2001
Inoculum	No	No
Harvest date	20 January 2002	20 January 2002

Table 2.3. Agronomical characters evaluated, code and procedure of measurement

Characters	Code	Procedure and time of recording
Tuber fresh yield	TUBY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows, fresh weight
Tuber dry matter yield	TDMY	TDMY = TUBY x DM
Vines and Leaves Dry Weight	VLW	t ha^{-1} – at physiological maturity- 24 plants from 4 rows, sun dried
Seed yield	SEEY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Pod yield	PODY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Total biomass	BIOM	BIOM = TDMY + VLW + PODY
Harvest-index for tuber (T) and seed (S) yield	HIT HIS	HIT = (TDMY / BIOM) x 100 HIS = (SEEY / BIOM) x 100
Total harvest-index	HITOT	HITOT = ((TDMY + SEEY) / BIOM) x 100
Shell weight	SHEL	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Begin of flowering	BF	Days from sowing to begin of flowering
Time of flowering	TF	days from sowing to 50% of plants flowering within center rows
Time of emergence	TE	days from sowing to 50% of plants emergence within center rows
Thousand seed weight	TSW	in g – at physiological maturity – measured on two samples of 100 seeds
Tuber dry matter content	DM	In % - measured on sun dried samples
Time of maturity	TM	days from sowing to physiological maturity – 80% dry pods within 2 center rows
Damage of stem and Leaves by Insects	DSLI	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of stem and Leaves by Fungi	DSLF	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of tubers by Nematodes	DTN	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of tubers by Insects	DTI	Scores from 0 to 6; 0 = no damage. 6 = high damage
Early vigor (width of first leaf)	EV	in mm – at time of development of third leaf– 6 plants within plot center and two measurements per plant
Period of flowering	PF	Days from begin of flowering to end of flowering - 6 plants within plot center
Start of climbing	SC	days from sowing to begin of climbing – 6 plants within plot center
Plant height	PH	in cm – at time of full flowering – 6 plants within plot center
Plant type	PT	Scores from 3 to 9 (3=erect; 5=semi-erect, 7=spreading, 9=extremely spreading)
Number of pods per plant	PN	counted – at harvest – 6 plants within plot center
Number of tubers per plant	NTP	Counted – at harvest – 6 plants within plot center
Seed number per pod	SNP	counted – at harvest - 6 plants within plot center (6 pods per plant)
Protein content	PRO	In % TDMY - elementary analysis – calculated from N x 6.25
Carbon content	CAR	In % TDMY – elementary analysis -
Carbon / Nitrogen ratio	C / N	Calculated from Nitrogen content (in %) and Carbon content (in %) in tuber dry matter yield

Statistical analysis

Agronomical data were analysed by the software Plabstat (Utz, 1997). Qualitative characters were included in the analysis as if they were expressed on a quantitative scale.

The following model was applied for those traits which were recorded for both treatment levels (with and without pruning of the reproductive parts):

$$Y_{ijkl} = \mu + l_i + t_j + g_k + b_{ijl} + lt_{ij} + lg_{ik} + tg_{jk} + ltg_{ijk} + e_{ijkl}$$

Y_{ijkl} is defined as the observation of genotype k in location i, treatment j and replication l; μ is the overall mean; l_i is the effect of location i; t_j is the effect of treatment j; g_k is the effect of genotype k; b_{ijl} is the effect of replication l in location i and treatment j; lt_{ij} is the interaction effect of location i and treatment j; lg_{ik} is the interaction effect of location i and genotype k; tg_{jk} is the interaction effect of treatment j and genotype k; ltg_{ijk} is the interaction effect of location i, treatment j and genotype k; e_{ijkl} is the interaction effect of location i, treatment j, genotype k and replication l (experimental error).

For those traits which were recorded only without pruning of the reproductive parts the following model was used:

$$Y_{ijk} = \mu + l_i + g_k + b_{ij} + lg_{ik} + e_{ijk}$$

Y_{ijk} is defined as the observation of genotype k in location i and replication j; μ is the overall mean; l_i is the effect of location i; g_k is the effect of genotype k; b_{ij} is the effect of replication j in location i; lg_{ik} is the interaction effect of location i and genotype k; e_{ijk} is the interaction effect of location i, replication j and genotype k (experimental error).

Each species was analysed separately.

2.3 Results

Significant differences between the treatments pruned and unpruned are observed for tuber fresh matter yield, tuber dry matter yield, total biomass and harvest index for tuber dry matter yield in all species (Table 2.4). Tuber dry matter content, vine and leaves dry matter yield, damage of tubers by nematodes, damage of tubers by insects, number of tubers per plant, protein content of tuber dry matter yield, carbon content of tuber dry matter yield and C/N ratio are not significantly affected by pruning of reproductive parts. *P. erosus* clearly has the highest tuber fresh matter yield under pruned and unpruned conditions (44.6 and 23.3 t ha^{-1}, respectively) compared to *P. tuberosus* and *P. ahipa*, for which results are nearly similar (about 20 and 13 t ha^{-1}, respectively) (Fig. 2.1 and 2.2). The figures 2.1 and 2.2 show the means of tuber fresh matter and tuber dry matter yield, respectively, for accessions from the three species with and without pruning. *P. tuberosus* has the highest tuber dry matter content (about 31% tuber fresh weight) compared to *P. erosus* (about 21% tuber fresh weight) and *P. ahipa* (about 22% tuber fresh weight). For tuber dry matter yield *P. tuberosus* and *P. erosus* have nearly equal tuber yields when no pruning of reproductive parts is conducted (about 4.2 t ha^{-1}). Nevertheless, *P. erosus* has the highest tuber dry matter yield with pruning. An increase of total biomass production occurs if no pruning is carried out for all three species. Nevertheless, the harvest index for tuber dry matter yield increases considerably if pruning is applied with values of 46, 50 and 62% for *P. tuberosus*, *P. erosus* and *P. ahipa*, respectively. For both treatments, the damage of tubers by nematodes was lower in *P. erosus* compared with *P. tuberosus* and *P. ahipa*. The damage of tubers by insects was approximatively the same for *P. tuberosus* and *P. erosus* and was in general lower than in *P. ahipa*, in which most of the tubers were destroyed by insects. Pruning resulted in an increase of 48.1, 91.0 and 60.9% of the tuber fresh matter yield in *P. tuberosus*, *P. erosus* and *P. ahipa* respectively. The increase in tuber dry matter yield was 58.3, 100.5 and 65.8% respectively.

Table 2.4. Effect of pruning on 12 agronomical characters for the Amazonian Yam Bean (*P. tuberosus*), the Mexican Yam Bean (*P. erosus*) and the Andean Yam Bean (*P. ahipa*) obtained from an analysis of variance

Characters		Amazonian Yam Bean (*P. tuberosus*) 6 Accessions		Mexican Yam Bean (*P. erosus*) 14 Accessions		Andean Yam Bean (*P. ahipa*) 14 Accessions	
	MSE	Pruned	Unpruned	Pruned	Unpruned	Pruned	Unpruned
Tuber Fresh Matter Yield (t ha^{-1})	41.64	20.61	13.92**	44.61	23.35**	20.02	12.44**
Tuber Dry Matter Yield (t ha^{-1})	3.75	6.79	4.29**	8.52	4.25**	4.51	2.72**
Tuber Dry Matter Content (%)	11.92	31.91	30.00	21.59	20.77	23.01	22.25
Vines and Leaves Dry matter Weight (t ha^{-1})	2.90	9.12	9.48	7.34	7.41	2.11	2.20
Total Biomass (Dry Matter) (t ha^{-1})	8.45	15.89	18.10**	15.86	24.17**	6.60	9.48**
Harvest Index For Tuber Dry matter Yield (%)	81.97	45.63	23.70**	50.28	17.58**	61.78	28.70**
Damage of Tubers by Nematodes[a]	0.02	2.67	2.67	0.77	0.75	3.34	3.38
Damage of Tubers by Insects[a]	0.47	2.00	1.83	1.93	1.95	4.29	4.21
Number of Tubers per plant	0.07	1.22	1.22	1.30	1.21	1.40	1.51
Protein content of tuber dry matter yield (%)	2.42	10.54	10.70	11.86	11.82	9.02	9.02
Carbon content of tuber dry matter yield (%)	0.12	40.54	40.71	40.68	40.62	40.56	40.60
Carbon / Nitrogen ratio of tuber dry matter yield	13.67	24.37	24.32	22.03	22.14	28.98	28.82

[(a)] Damage by Nematodes and Insects measured on a score from 0-6

MSE= Mean Square of Error (pooled estimate for all species)

(**) significant difference between the treatments pruned and unpruned at the level 0.01

With pruning of reproductive parts, tuber fresh matter and tuber dry matter yield differed clearly under stress and non-stress conditions (Table 2.5). With the exception of tuber dry matter content in *P. erosus* and harvest index for tuber dry matter yield in *P. tuberosus*, the mean of quantitative traits is higher at Songhai than at the stress location Niaouli. In *P. tuberosus* as well as in *P. erosus* and *P. ahipa*, the tubers were more negatively affected by nematodes at Niaouli than at

Songhai. The same is observed for the damage of tubers by insects in *P. tuberosus,* whereas for *P. erosus* and *P. ahipa* the damage of tubers by insects is higher at the non stress location Songhai. The number of tubers per plant did not vary significantly between the two locations for all species. The tuber dry matter content was not affected by locations for *P. tuberosus* and *P. ahipa*, but for *P. erosus* tuber dry matter content increased at the stress location Niaouli. For *P. erosus* and *P. ahipa* the harvest index for tuber dry matter yield was higher at the non-stress location than at the stress location Niaouli but for *P. tuberosus* no difference was observed. There is also no significant difference between the locations for protein content, carbon content and C/N ratio in all species. Protein content of tuber dry matter varies from 9.0% in *P. ahipa* to 11.8% in *P. erosus*.

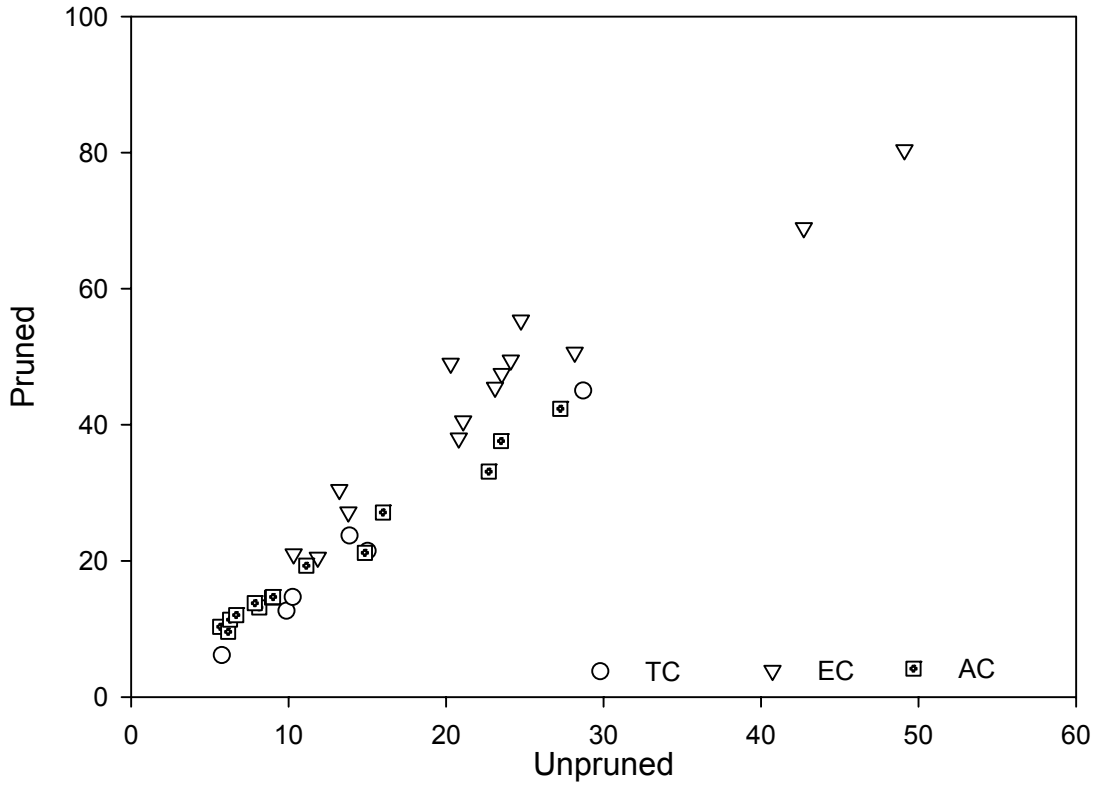

Figure 2.1. Means of tuber fresh matter yield with and without pruning (t ha^{-1})
AC = *Pachyrhizus ahipa*, EC = *Pachyrhizus erosus*, TC = *Pachyrhizus tuberosus*

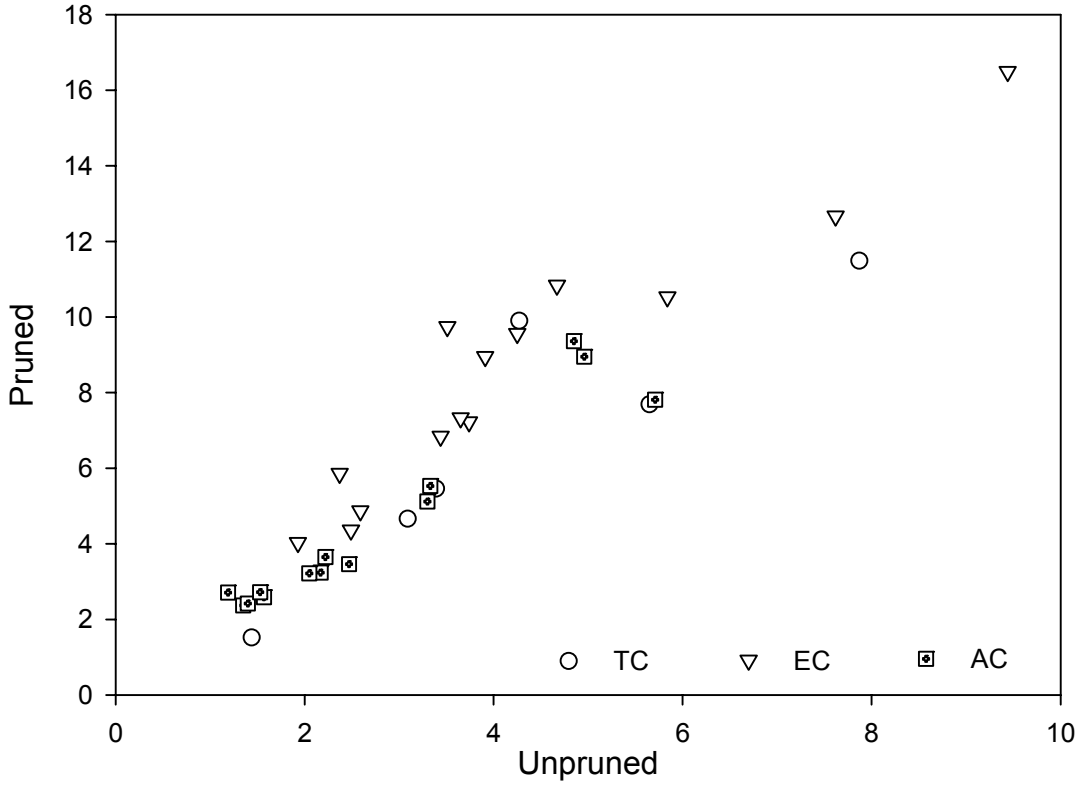

Figure 2.2. Means of tuber dry matter yield with and without pruning (t ha^{-1})
AC = *Pachyrhizus ahipa*, EC = *Pachyrhizus erosus*, TC = *Pachyrhizus tuberosus*

For treatment unpruned, tuber fresh matter yield, tuber dry matter yield, vines and leaves dry matter weight and plant height are clearly higher at the non-stress location Songhai compared to the stress location Niaouli (Table 2.6). There was no significant difference between both locations in *P. tuberosus* and *P. ahipa* for tuber dry matter content. But, in *P. erosus*, the tuber dry matter content was higher at Niaouli than at Songhai. There was no difference in total biomass between Songhai and Niaouli in *P. erosus* compared to *P. ahipa* and *P. tuberosus*, in which the biomass was much higher at Songhai than at Niaouli. Harvest index for tuber dry matter yield did not vary between Songhai and Niaouli in *P. tuberosus*. But there was a variation between both locations for *P. erosus* and *P. ahipa*. The effects of nematodes on the tubers were higher at Niaouli than at Songhai for all species. The tubers were strongly attacked by insects at Songhai with the exception of *P. tuberosus*, which was more affected in Niaouli. No difference was observed for seed yield, pod yield and shell weight in *P. erosus* between the locations compared to *P. tuberosus* and *P. ahipa*. Thousand seeds weight did not vary between the

locations in all species. But a greater variation was observed between the species and *P. tuberosus* had the highest thousand seeds weight. Harvest index for seed yield varied relatively more between the two locations in the Amazonian yam bean (*Pachyrhizus tuberosus*).

Table 2.5. Means of agronomical traits after pruning of reproductive parts measured at two locations in Benin West Africa.

Characters		Amazonian Yam Bean (*P. tuberosus*) 6 Accessions		Mexican Yam Bean (*P. erosus*) 14 Accessions		Andean Yam Bean (*P. ahipa*) 14 Accessions	
	MSE	Non-stress Location Songhai	Drought-stress Location Niaouli	Non-stress Location Songhai	Drought-stress Location Niaouli	Non-stress Location Songhai	Drought-stress Location Niaouli
Tuber Fresh Matter Yield (t ha^{-1})	63.63	32. 52	8. 71**	70. 61	18. 62**	32. 22	7. 81**
Tuber Dry Matter Yield (t ha^{-1})	5.71	10.56	3.06**	12.16	4.88**	7.20	1.81**
Tuber Dry Matter Content (%)	11.67	32.97	31.11	17.38	25.79**	22.70	23.34
Vines and Leaves Dry matter Weight (t ha^{-1})	185.27	15.28	2.96**	8.58	6.11**	2.70	1.51**
Total Biomass (Dry Matter) (t ha^{-1})	11.35	25.84	6.00**	20.74	10.99**	9.86	3.33**
Harvest Index For Tuber Dry matter Yield (%)	70.44	43.64	48.00	60.91	39.66**	70.86	52.81**
Damage of Tubers by Nematodes[a]	0.01	2.33	3.00	0.00	1.54**	0.71	5.96**
Damage of Tubers by Insects[a]	0.10	1.33	2.67*	2.71	1.14**	5.71	2.86**
Number of Tubers per plant	0.03	1.35	1.46	1.27	1.33	1.18	1.26
Protein content of tuber dry matter yield (%)	2.39	10.82	10.26	12.72	11.01	8.98	9.05
Carbon content of tuber dry matter yield (%)	0.06	40.53	40.54	40.59	40.76	40.56	40.56
Carbon / Nitrogen ratio of tuber dry matter yield	14.12	23.77	24.97	20.48	23.59*	29.16	28.80

[(a)] Damage by Nematodes and Insects measured on a score from 0-6

MSE= Mean Square of Error (pooled estimate for all species); (*), (**) significant difference between locations at the levels 0.05 and 0.01 respectively

But in *P. ahipa* and *P. erosus*, there was no significant variation. A moderate variation was noted for total harvest index (tubers and seeds) in all species

between the locations. The plants in all three species had a better early vigor in Songhai than in Niaouli. The genotypes of *P. tuberosus* and *P. erosus* began flowering earlier at Songhai than at Niaouli, while there was no significant difference between locations in *P. ahipa*. No significant difference was also noted concerning time and period of flowering between the locations, but differences were observed between species, with *P. ahipa* starting flowering earlier than the two other species. The genotypes presented the same trend for time of maturity. The stems and leaves of *P. erosus* were more damaged by insects than those of *P. ahipa* and *P. tuberosus*. *P. ahipa* was more damaged by fungi than *P. erosus* and *P. tuberosus*. The pod number per plant did not vary significantly between locations, but a variation between species was noted. Protein content, carbon content as well as the C/N ratio didn´t vary between both locations.

Analysis of variance for tuber fresh matter yield is presented in Table 2.7. With the exception of *P. tuberosus*, the interactions genotype x treatment and genotype x treatment x location were not significant. For *P. tuberosus*, the genotype x treatment and the genotype x treatment x location interactions were significant. The location, treatment, genotypes as well as the interactions treatment x location and genotype x location showed large and significant variation within all the three species. The same observations can be made for tuber dry matter yield. Here the interactions genotype x treatment and genotype x treatment x location were not significant (Table 2.8). But for *P. ahipa*, the genotype x treatment interaction was significant. For the factor location and for tuber dry matter yield, significant variation was observed. For *P. tuberosus* and for the tuber dry matter yield, the genotype x treatment and the genotype x treatment x location interactions were not significant

Table 2.6. Means of agronomical traits without pruning of reproductive parts measured at two locations in Benin West Africa.

Characters		Amazonian Yam Bean (*P. tuberosus*) 6 Accessions		Mexican Yam Bean (*P. erosus*) 14 Accessions		Andean Yam Bean (*P. ahipa*) 14 Accessions	
	MSE	Non-stress Location Songhai	Drought-stress Location Niaouli	Non-stress Location Songhai	Drought-stress Location Niaouli	Non-stress Location Songhai	Drought-stress Location Niaouli
Tuber Fresh Matter Yield (t ha^{-1})	20.55	21.12	6.71**	35.19	11.52*	19.31	5.57**
Tuber Dry Matter Yield (t ha^{-1})	1.47	6.58	1.95*	5.48	3.01**	4.24	1.21**
Tuber Dry Matter Content (%)	12.24	31.66	28.10	16.08	25.45	22.23	22.27
Vines and Leaves Dry matter Weight (t ha^{-1})	2.92	15.98	2.98**	9.20	5.61*	2.94	1.46*
Total Biomass (Dry Matter) (t ha^{-1})	13.74	28.09	8.11*	26.88	21.46	13.81	5.14**
Harvest Index For Tuber Dry matter Yield (%)	36.29	24.96	24.45	21.67	14.29	29.63	23.51**
Damage of Tubers by Nematodes[a]	0.02	2.33	3.00	0.00	1.50	0.79	5.96
Damage of Tubers by Insects[a]	0.07	1.33	2.33	2.71	1.18	5.71	2.71
Number of Tubers per plant	0.02	1.15	1.28	1.18	1.24	1.33	1.70
Seed Yield (t ha^{-1})	0.85	2.86	1.56	5.66	4.69	3.04	1.10**
Pod Yield (t ha^{-1})	4.15	5.53	3.41	12.20	12.84	6.63	2.48**
1000-Seeds Weight (g)	73.29	374.54	376.96	225.85	225.31	318.79	314.92
Shell Weight (t ha^{-1})	1.82	2.67	1.85	6.54	8.14	3.59	1.38**
Harvest Index for seeds (%)	14.93	11.49	17.03**	21.56	21.78	22.91	20.94
Harvest Index Total (%)	33.18	36.45	41.47	43.23	36.07	52.54	44.44
Time of Emergence	2.01	11.17	9.00*	8.39	10.07**	12.04	10.61*
Early Vigour – width of first leave (mm)	17.45	35.38	26.44	38.48	29.49*	44.07	31.03
Start of Climbing	20.69	30.75	45.11	38.83	43.27	34.05	34.84
Begin of Flowering	15.89	73.08	78.08	64.68	71.14	55.29	56.75
Time of Flowering	11.89	83.58	84.00	75.32	78.64*	57.32	59.29
Period of Flowering	19.59	73.33	74.21	56.96	57.17	58.05	57.44
Time of Maturity	10.56	176.42	180.25	170.86	166.21	138.93	134.18**
Damage of Stem and Leaves by Insects[a]	0.20	1.92	1.93	2.68	2.68	2.07	2.25
Damage of Stem and Leaves by Fungi[a]	0.66	0.50	0.50	0.39	0.71	1.21	2.54*
Plant Height (cm)	873.24	431.9	265.8	338.6	305.9**	65.2	51.1
Plant type[b]	0.00	9.00	9.00	8.00	8.00	4.71	4.71
Pod Number per Plant	49.13	24.90	27.26	49.91	55.04^{+}	20.24	34.06**
Seeds Number per Pod	0.26	8.87	9.13*	9.49	9.57	6.97	8.13*
Protein content of tuber dry matter yield (%)	2.42	10.74	10.66	12.68	10.95	8.97	9.07
Carbon content of tuber dry matter yield (%)	0.12	40.56	40.86	40.55	40.68	40.68	40.51
Carbon / Nitrogen ratio of tuber dry matter yield	13.67	24.17	24.47	20.72	23.56	28.89	28.76

(a) Damage by Nematodes, Insects and fungi measured on a score from 0-6;
(b) Plant type measured on a score 3=erect, 5=semi-erect, 7=spreading, 9=extremly spreading.
MSE= Mean Square of Error (pooled estimate for all species); ($^{+}$), (*), (**) significant difference between locations at the levels 0.1, 0.05 and 0.01, respectively

Table 2.7. Variance analysis for tuber fresh matter yield in Andean Yam Bean (*P. ahipa*), Mexican Yam Bean (*P. erosus*) and Amazonian Yam Bean (*P. tuberosus*)

Source	DF	MS	Variance Component	F-test
P. ahipa				
Location (L)	1	10179.82	181.77	17211.41**
Replication in L (R:L)	2	0.59	-0.67	0.03
Treatment (T)	1	1606.92	28.34	82.91**
Genotype (G)	13	650.81	78.92	33.58**
TL	1	797.32	27.78	41.14**
GL	13	318.31	74.73	16.42**
GT	13	26.89	1.87	1.39
GTL	13	22.64	1.63	1.17
RGTL	54	19.38	19.38	
P.erosus				
Location (L)	1	40070.88	713.92	441.25**
Replication in L (R:L)	2	90.81	0.57	1.22
Treatment (T)	1	12657.63	224.69	169.62**
Genotype (G)	13	1547.76	184.14	20.74**
TL	1	5612.83	197.79	75.22**
GL	13	386.19	77.89	5.18**
GT	13	102.07	6.86	1.37
GTL	13	86.06	5.71	1.15
RGTL	54	74.62	74.62	
P. tuberosus				
Location (L)	1	4379.50	181.24	147.56**
Replication in L (R:L)	2	29.67	0.98	1.66
Treatment (T)	1	538.59	21.69	30.16**
Genotype (G)	5	919.95	112.76	51.51**
TL	1	265.01	20.59	14.84**
GL	5	378.35	90.12	21.19**
GT	5	65.26	11.85	3.65*
GTL	5	48.34	15.24	2.71*
RGTL	22	17.85	17.85	

(*) significant at the level 0.05
(**) significant at the level 0.01

Table 2.8. Variance analysis for tuber dry matter yield in Andean Yam Bean (*P. ahipa*), Mexican Yam Bean (*P. erosus*) and Amazonian Yam Bean (*P. tuberosus*)

Source	DF	MS	Variance Components	F-test
P. ahipa				
Location (L)	1	496.15	8.85	4553.31**
Replication in L (R:L)	2	0.10	-0.03	0.09
Treatment (T)	1	89.53	1.57	77.23**
Genotype (G)	13	30.91	3.72	26.67**
TL	1	39.09	1.35	33.72**
GL	13	15.96	3.70	13.77**
GT	13	2.52	0.34	2.18*
GTL	13	1.43	0.13	1.23
RGTL	54	1.15	1.15	
P. erosus				
Location (L)	1	667.04	11.80	109.01**
Replication in L (R:L)	2	6.11	0.03	1.17
Treatment (T)	1	511.51	9.04	97.64**
Genotype (G)	13	60.67	6.92	11.58**
TL	1	161.73	5.58	30.87**
GL	13	30.46	6.30	5.82**
GT	13	5.30	0.01	1.01
GTL	13	4.45	-0.39	0.85
RGTL	54	5.23	5.23	
P. tuberosus				
Location (L)	1	442.22	18.15	68.49*
Replication in L (R:L)	2	6.45	0.06	1.15
Treatment (T)	1	75.11	2.89	13.35**
Genotype (G)	5	66.07	7.55	11.74**
TL	1	26.19	1.71	4.65*
GL	5	28.25	5.65	5.02**
GT	5	7.27	0.41	1.29
GTL	5	3.40	-1.11	0.60
RGTL	22	5.62	5.62	

(*) significant at the level 0.05
(**) significant at the level 0.01

For all three species, the genotypic variance for tuber fresh matter yield was higher than the interaction genotype x location variance, which was more important than the error variance (Table 2.9). For tuber dry matter yield (treatment pruning), the genotypic variance was significant and higher than the interaction genotype x location variance, which was higher than the error in *P. erosus* and *P. ahipa*. The genotypic variance for the trait tuber dry matter content was significant in *P. tuberosus* and *P. ahipa*, and lower than the interaction genotype x location variance in *P. tuberosus*. A wide variation was observed within species for all characters measured, excepted for the damage of tubers by nematodes and insects, the number of tubers per plant, protein content, carbon content of tuber dry matter yield and C/N ratio. However the genotypic and the genotype x location variances were highly significant in *P. erosus* for damage of tubers by nematodes and insects. In these cases, the interaction variance was higher than the genotypic variance. The genotypic variance was significant for protein content as well as C/N ratio in *P. ahipa*.

The variance components of agronomical traits estimated from treatment no pruning of reproductive parts are listed in Table 2.10. The genotypic variance was highly significant for all characters, except the damage of tubers by nematodes, the shell weight, seed yield, pod yield, harvest index for seeds in *P. tuberosus*. The genotype x location interaction variance was lower than the genotypic variance, particularly for tuber fresh matter yield (in all species), tuber dry matter yield (in all species), tuber dry matter content (except in *P. erosus*), vines and leaves dry matter weight (except in *P. tuberosus*), total biomass, harvest index for tuber dry matter yield (in all species), number of tubers per plant (exception: *P. erosus*), thousand seeds weight (in all species), total harvest index (in all species), early vigour (excepted *P. erosus*), start of climbing (excepted *P. tuberosus*), begin of flowering, time of flowering, period of flowering (in all species), time of maturity (exception: *P. erosus* and *P. ahipa*), damage of stems and leaves by insects (in all species), damage of stems and leaves by fungi (except in *P. ahipa*), plant height, pod number per plant (in all species), seeds number per pod (exception: *P. tuberosus*). In *P. ahipa*, the genotypic variance for C/N ratio was highly significant.

Table 2.9. Variance components of agronomical traits estimated from treatment pruning of reproductive parts.

Characters	Amazonian Yam Bean (*P. tuberosus*) 6 Accessions			Mexican Yam Bean (*P. erosus*) 14 Accessions			Andean Yam Bean (*P. ahipa*) 14 Accessions		
	G	GL	Error	G	GL	Error	G	GL	Error
Tuber Fresh Matter Yield ($t\ ha^{-1}$)	178.45**	163.88**	18.25	259.63**	136.85**	124.58	110.32**	112.31**	25.07
Tuber Dry Matter Yield ($t\ ha^{-1}$)	11.34**	8.41^{+}	8.58	9.97**	9.74**	8.34	5.68**	5.48**	1.74
Tuber Dry Matter Content (%)	32.82*	-14.16	36.10	0.47	2.17^{+}	4.31	3.54*	-2.62	10.61
Vines and Leaves Dry matter Weight ($t\ ha^{-1}$)	34.02**	47.45**	2.01	15.48**	3.66*	5.57	0.49**	0.17**	0.15
Total Biomass (Dry Matter) ($t\ ha^{-1}$)	17.45*	33.70*	18.44	19.75**	21.97**	16.44	7.99**	7.07**	2.29
Harvest Index For Tuber Dry matter Yield (%)	375.68**	-18.61	68.32	232.77**	42.78	110.52	73.77**	12.25	35.56
Damage of Tubers by Nematodes[a]	0.36	0.73	0.00	0.25**	0.50**	0.02	0.16**	0.35**	0.01
Damage of Tubers by Insects[a]	0.00	0.73*	0.60	0.43**	1.09**	0.03	0.37	1.45	0.00
Number of Tubers per plant	0.01*	0.00	0.016	0.005	0.01*	0.02	0.01^{+}	0.02^{+}	0.04
Protein content of tuber dry matter yield (%)	1.68	-2.30	11.02	0.49	-0.23	2.50	1.26**	-0.79	2.31
Carbon content of tuber dry matter yield (%)	0.02**	0.03*	0.01	0.02^{+}	0.01	0.07	0.01	-0.02	0.06
Carbon / Nitrogen ratio of tuber dry matter yield	0.21	-0.19	1.58	2.10	0.33	11.48	9.34**	-4.36	16.93

(**) significant at the level 0.01, (*) significant at the level 0.05, ($^{+}$) significant at the level 0.1
(a) Damage by Nematodes and Insects measured on a score from 0-6

Table 2.10. Variance components of agronomical traits estimated from treatment no pruning of reproductive parts.

Characters	Amazonian Yam Bean (*P. tuberosus*) 6 Accessions			Mexican Yam Bean (*P. erosus*) 14 Accessions			Andean Yam Bean (*P. ahipa*) 14 Accessions		
	G	GL	Error	G	GL	Error	G	GL	Error
Tuber Fresh Matter Yield (t ha^{-1})	60.64**	35.07**	10.53	114.38**	22.39*	29.16	49.06**	38.08**	15.08
Tuber Dry Matter Yield (t ha^{-1})	4.74**	2.42*	1.23	3.83**	2.33**	2.40	2.05**	1.98**	0.67
Tuber Dry Matter Content (%)	15.43*	-8.79	20.83	0.46	0.73	7.57	1.49	-1.99	13.54
Vines and Leaves Dry matter Weight (t ha^{-1})	35.63**	47.57**	3.29	17.18**	3.50*	5.17	0.56**	0.21*	0.25
Total Biomass (Dry Matter) (t ha^{-1})	29.19**	17.75*	13.16	29.73**	5.87	23.91	6.18**	3.83**	2.50
Harvest Index For Tuber Dry matter Yield (%)	100.84**	-11.72	48.03	109.84**	21.18^{+}	38.91	66.25**	33.90**	31.85
Damage of Tubers by Nematodes[a]	0.36	0.73	0.00	0.26**	0.53**	0.02	0.15**	0.32**	0.03
Damage of Tubers by Insects[a]	0.16**	0.21*	0.40	0.45**	1.07**	0.02	0.27**	1.30**	0.01
Number of Tubers per plant	0.008*	-0.004	0.03	0.014**	0.02*	0.06	0.03**	0.01	0.10
Seed Yield (t ha^{-1})	0.07	0.47	0.77	1.25**	0.23	1.50	0.32**	0.35**	0.24
Pod Yield (t ha^{-1})	0.26	1.27	3.45	6.91**	2.90	7.60	1.18**	1.44**	0.99
1000-Seeds Weight (g)	1566.71**	-12.94	30.75	256.90**	-8.81	24.69	1105.65**	-64.59	146.84
Shell Weight (t ha^{-1})	0.08	0.15	0.98	2.06**	1.12	3.63	0.34**	0.52**	0.40
Harvest Index for seeds (%)	1.98	27.63	10.97	6.00**	10.85**	8.90	16.64**	18.65*	22.67
Harvest Index Total (%)	131.89**	-2.62	36.79	86.95**	6.49	38.42	15.79**	3.12	23.46

(**) significant at the level 0.01, (*) significant at the level 0.05, ($^{+}$) significant at the level 0.1
(a) Damage by Nematodes, Insects and fungi measured on a score from 0-6;

Table 2.10. Continued

Characters	Amazonian Yam Bean (*P. tuberosus*) 6 Accessions			Mexican Yam Bean (*P. erosus*) 14 Accessions			Andean Yam Bean (*P. ahipa*) 14 Accessions		
	G	GL	Error	G	GL	Error	G	GL	Error
Time of Emergence	0.05*	0.10*	0.06	1.54**	2.37**	1.34	0.23	2.77*	3.16
Early Vigour – width of first leave (mm)	135.31**	-0.60	12.15	6.01**	6.68*	10.53	5.90*	-0.67	17.95
Start of Climbing	0.14	2.36	6.89	-0.14	-3.36	19.78	243.99**	-14.59	29.61
Begin of Flowering	14.14^{+}	-11.29	35.88	30.93**	-0.002	12.52	3.04^{+}	-1.84	12.27
Time of Flowering	28.02**	1.35	9.44	52.48**	7.60*	9.54	8.39**	-2.44	13.13
Period of Flowering	889.81**	-2.95	6.21	88.93**	-2.81	6.49	65.02**	-19.57	40.65
Time of Maturity	258.85**	5.75	8.65	7.30**	10.12**	2.87	23.39**	33.18**	19.78
Damage of Stem and Leaves by Insects[a]	0.22**	-0.04	0.09	0.20**	-0.017	0.03	0.04	-0.04	0.36
Damage of Stem and Leaves by Fungi[a]	0.30	-0.60	1.20	0.27*	-0.23	0.64	0.17*	0.32*	0.41
Plant Height (cm)	4128.70**	-161.77	1412.49	4216.04**	1679.01**	944.68	350.20**	49.31	387.79
Plant type[b]	1.00	0.00	0.00	1.07	0.00	0.00	0.52	0.00	0.00
Pod Number per Plant	91.36**	-6.59	14.48	95.02**	37.91^{+}	77.58	50.07**	33.46**	26.68
Seeds Number per Pod	0.02	0.22*	0.15	0.03*	-0.006	0.11	0.60**	0.33*	0.40
Protein content of tuber dry matter yield (%)	0.13	-1.09	3.10	0.48^{+}	0.01	2.31	0.88**	-0.64	1.76
Carbon content of tuber dry matter yield (%)	0.09^{+}	-0.01	0.18	0.007	0.04	0.10	0.01	-0.01	0.23
Carbon / Nitrogen ratio of tuber dry matter yield	0.35	-6.50	18.14	2.20^{+}	0.11	10.16	9.58**	-4.59	13.27

(**) significant at the level 0.01, (*) significant at the level 0.05, ($^{+}$) significant at the level 0.1
(a) Damage by Nematodes, Insects and fungi measured on a score from 0-6;
(b) Plant type measured on a score 3=erect, 5=semi-erect, 7=spreading, 9=extremly spreading.

The means of the traits within treatment “pruned“ for each accession is given in Table 2.11. The results show the significant variation of the tuber fresh matter and dry matter yield within and between species. The significant variation for these traits between locations was also showed. The variation of tuber dry matter content is not allways significant between the two locations. But the variation between accessions is often clear. Most of all other traits varied significantly between locations, within and between species with the exception of protein content, carbon content and the C/N ratio.

The correlation coefficients of tuber fresh and dry matter yield with important yield components with and without pruning are listed in Tables 2.12 and 2.13 respectively. With pruning, the tuber fresh matter as well as the tuber dry matter yield showed a negative correlation with vines and leaves dry matter weight in *P. tuberosus* and *P. erosus*. Only in the Andean yam bean, one observed a significant positive correlation (r=0.59 for tuber fresh matter yield and r=0.57 for tuber dry matter yield). The tuber fresh matter yield had a negative correlation with tuber dry matter content in *P. tuberosus* (r=-0.26) and *P. erosus* (r=-0.21). Otherwise there was a positive correlation between both characters as well as between tuber dry matter yield and tuber dry matter content. There was a highly positive correlation between the damage of tubers by insects and the tuber fresh matter as well as the tuber dry matter yield in *P. ahipa*. The number of tubers per plant showed a moderate (in *P. tuberosus* and *P. erosus*) and a highly significant positive correlation (in *P. ahipa*) with the tuber fresh matter as well as the tuber dry matter yield. Protein content of tuber dry matter yield showed a positive correlation with tuber fresh and dry matter yields in *P. tuberosus*. There is no correlation between carbon content and tuber fresh matter yield. The opposite observation was made for C/N ratio of the tubers. For the same characters, the same observations can be made in the case of the treatment “without pruning“ (Table 2.13). Seed yield and pod yield have surprisingly a strong and positive correlation with tuber fresh and dry matter yields in *P. tuberosus*. In contrast there was a negative correlation between these traits in *P. ahipa* and *P. erosus*. Time of emergence, harvest index for seeds and pod number per plant showed the same trend like the previous traits.

Only in *P. ahipa* early vigor showed a moderate and positive correlation with the tuber fresh (r=0.26) and dry matter (r=0.24) yields. Begin and time of flowering had a negative correlation with the tuber fresh and dry matter yield in all three species. Period of flowering and time of maturity showed a negative correlation with tuber fresh and dry matter yields in *P. tuberosus* and *P. erosus*. Both characters had adversely a positive correlation with tuber fresh and dry matter yields in *P. ahipa*. Like seed and pod yield, harvest index for seeds was positively correlated with tuber fresh matter and dry matter yield in *P. tuberosus*, and negatively correlated in *P. ahipa* and *P. erosus*.

The correlations of the different traits between one another are presented in Appendix (Tables A3, A4, A5, A6, A7 and A8).

Table 2.11. Means of traits of individual accessions within treatment pruned. (CC = Amazonian yam *bean (P. tuberosus*) Chuin cultivar group, TC = Amazonian yam bean (*P. tuberosus*) Ashipa cultivar group, EC = Mexican yam bean (*P. erosus*), AC = Andean yam bean (*P. ahipa*), locations SON = Songhai, NIA = Niaouli, DTN = damage of tubers by nematods, DTI= damage of tubers by insects, HIT= harvest index for tuber dry matter yield, NTP= number of tubers per plant, PRO= protein content of tuber dry matter yield, CAR= carbon content of tuber dry matter yield, C / N= carbon and nitrogen ratio).

Location	Geno-type	Tuber Fresh Matter Yield (t ha^{-1})	Tuber Dry Matter Yield (t ha^{-1})	Tuber Dry Matter Content (%)	Vine + Leaves Dry Weight (t ha^{-1})	Total Bio-mass Dry Matter (t ha^{-1})	HIT (%)	DTN	DTI	NTP	PRO	CAR	C/N
SON non-stress	CC353	19.07	6.98	36.55	10.76	17.74	39.28	3.00	2.00	1.08	11.25	40.46	22.60
NIA stress		6.24	2.34	36.55	2.50	4.84	46.83	3.00	2.00	1.00	9.53	40.74	26.68
SON non-stress	CC354	74.00	19.37	26.18	13.54	32.91	59.01	3.00	1.00	1.33	10.75	40.36	23.44
NIA stress		16.08	3.61	22.68	2.71	6.31	56.99	3.00	3.00	1.33	10.53	40.79	24.29
SON non-stress	CC355	36.23	13.68	36.92	9.47	23.15	58.25	3.00	2.00	1.08	11.43	40.72	22.23
NIA stress		11.20	6.18	37.56	2.58	8.90	69.48	3.00	1.50	1.25	11.79	40.40	21.38
SON non-stress	CC361	21.17	7.89	37.25	6.70	14.59	54.02	3.00	1.00	1.25	11.81	40.83	21.93
NIA stress		8.20	3.03	37.39	2.38	5.40	55.81	3.00	4.50	1.50	10.03	40.76	25.33
SON non-stress	CC362	35.40	13.06	35.03	14.67	27.73	45.10	2.00	1.00	1.08	10.61	40.38	23.77
NIA stress		7.52	2.33	30.04	2.67	5.00	45.23	3.00	2.50	1.25	10.84	40.19	23.13
SON non-stress	TC118	9.23	2.39	25.89	36.53	38.93	6.14	2.00	1.00	1.25	9.05	40.43	28.67
NIA stress		3.04	0.65	21.43	4.91	5.56	11.69	3.00	2.50	1.25	9.36	40.35	27.64
SON non-stress	EC006	76.80	13.32	17.54	7.94	21.26	62.91	0.00	2.00	1.25	13.65	40.32	18.74
NIA stress		4.30	1.12	25.70	3.41	4.53	24.84	0.00	0.00	1.17	12.06	41.03	21.44
SON non-stress	EC032	86.23	16.35	18.97	14.80	31.15	52.96	0.00	3.00	1.33	12.46	40.22	20.14
NIA stress		11.76	3.13	26.52	9.40	12.53	24.18	1.00	1.00	1.50	11.06	40.81	23.08
SON non-stress	EC033	83.20	10.59	12.68	11.53	22.12	47.68	0.00	2.00	1.25	13.10	40.35	19.40
NIA stress		11.84	3.09	26.09	7.27	10.35	30.38	3.00	2.00	1.08	13.46	40.76	18.90
SON non-stress	EC040	79.30	12.60	15.90	6.54	19.14	65.85	0.00	3.00	1.25	12.03	40.91	21.25
NIA stress		19.68	5.30	26.77	4.87	10.17	51.89	1.00	0.00	1.25	10.36	40.86	24.74
SON non-stress	EC041	79.40	15.00	18.85	7.40	22.40	67.21	0.00	2.00	1.17	12.07	40.60	21.09
NIA stress		21.92	6.05	27.62	7.07	13.12	46.96	3.00	2.00	1.50	9.66	40.79	26.81
SON non-stress	EC042	66.43	12.23	18.53	10.12	22.36	54.81	0.00	2.00	1.42	14.59	40.69	17.42
NIA stress		9.60	2.43	25.43	6.63	9.07	26.55	1.50	1.00	1.25	12.11	41.15	21.21
SON non-stress	EC104	55.73	10.43	18.39	24.33	34.75	28.24	0.00	1.00	1.50	13.48	41.02	19.17
NIA stress		5.20	1.30	24.85	13.01	14.31	9.33	1.00	0.50	1.33	12.00	40.87	21.26
SON non-stress	EC114	88.93	15.65	17.58	11.03	26.68	58.73	0.00	1.00	1.17	13.60	40.68	20.10
NIA stress		21.76	6.02	27.67	5.48	11.50	55.84	2.00	2.00	1.33	11.50	40.87	22.19
SON non-stress	EC204	31.97	7.47	21.97	3.83	11.30	62.91	0.00	4.00	1.33	13.32	40.51	19.14
NIA stress		9.04	2.27	24.78	5.07	7.34	30.09	2.00	2.00	1.17	10.56	40.90	24.56
SON non-stress	EC253	82.50	17.13	20.43	8.36	25.49	66.22	0.00	4.00	1.25	11.67	40.69	21.78
NIA stress		8.48	2.00	23.59	6.50	8.50	23.59	1.00	0.00	1.50	12.03	40.48	21.22
SON non-stress	EC533	89.87	13.92	15.52	3.33	17.25	80.75	0.00	4.00	1.33	9.73	40.77	28.30
NIA stress		70.96	19.06	26.47	3.12	22.18	83.33	2.00	2.00	1.08	10.64	40.62	23.98
SON non-stress	EC550	33.23	5.83	17.55	4.33	10.17	57.35	0.00	3.00	1.25	12.34	40.47	20.76
NIA stress		8.80	2.22	25.79	3.72	5.94	44.11	3.00	2.50	1.50	10.31	40.86	24.86
SON non-stress	EC557	46.53	6.79	14.86	3.50	10.29	66.50	0.00	4.00	1.08	14.13	40.80	18.24
NIA stress		7.80	1.92	24.68	4.81	6.73	28.77	0.00	0.00	1.08	10.30	40.54	24.58
SON non-stress	ECKEW	88.37	12.96	14.63	3.09	16.04	80.65	0.00	3.00	1.17	11.84	40.22	21.21
NIA stress		49.52	12.38	25.10	5.15	17.53	75.33	1.00	1.00	1.83	8.16	40.14	31.38
	LSD.05	11.26	3.38	4.83	2.37	4.76	11.86	0.17	0.46	0.25	2.19	0.36	5.31

Table 2.11. continued

Location	Geno-type	Tuber Fresh Matter Yield (t ha^{-1})	Tuber Dry Matter Yield (t ha^{-1})	Tuber Dry Matter Content (%)	Vine + Leaves Dry Weight (t ha^{-1})	Bio-mass Dry Matter Yield (t ha^{-1})	HIT (%)	DTN	DTI	NTP	PRO	CAR	C/N
SON non-stress	AC102	20.47	4.87	23.66	1.65	6.52	74.26	3.00	6.00	1.58	11.90	40.54	23.06
NIA stress		5.88	1.62	27.42	1.10	2.72	59.30	6.00	3.00	1.25	12.25	40.58	21.11
SON non-stress	AC201	16.13	4.27	26.48	1.99	6.26	68.24	0.00	5.00	1.42	8.31	40.50	30.47
NIA stress		4.50	1.14	25.45	1.40	2.54	45.05	6.00	4.00	1.25	7.34	40.48	34.67
SON non-stress	AC202	54.53	13.14	27.51	3.75	16.72	79.64	0.00	6.00	1.25	8.95	40.17	28.01
NIA stress		11.72	2.86	24.40	1.53	4.39	63.62	6.00	4.00	1.33	7.78	40.15	32.21
SON non-stress	AC203	13.47	3.40	25.30	1.64	5.05	67.53	1.00	6.00	1.33	7.36	40.81	34.59
NIA stress		5.72	1.34	22.56	1.26	2.60	49.68	6.00	2.00	1.50	8.97	40.57	28.66
SON non-stress	AC205	20.13	3.65	18.01	1.99	5.64	64.15	1.00	6.00	1.25	7.77	40.67	32.70
NIA stress		7.44	1.53	20.58	1.36	2.89	53.39	6.00	2.00	1.42	7.94	40.67	32.21
SON non-stress	AC208	23.85	5.22	21.72	3.45	8.67	59.97	1.00	6.00	1.42	8.50	40.76	30.17
NIA stress		5.40	1.22	22.19	2.09	3.31	36.91	6.00	2.00	1.33	8.47	40.69	30.48
SON non-stress	AC209	67.63	14.03	20.74	3.90	17.93	78.25	1.00	6.00	1.25	7.43	40.51	34.26
NIA stress		17.04	3.88	22.94	1.81	5.69	68.18	5.50	5.00	1.67	7.47	40.52	34.02
SON non-stress	AC213	18.33	3.85	20.68	1.23	5.08	75.09	0.00	6.00	1.25	10.83	40.42	23.37
NIA stress		4.36	0.99	20.87	0.82	1.81	49.45	6.00	1.00	1.33	10.50	40.65	24.19
SON non-stress	AC214[a]	45.53	9.23	19.94	2.61	11.84	76.92	0.00	6.00	1.33	9.06	40.45	27.85
NIA stress		8.68	1.82	20.83	1.66	3.48	51.97	6.00	3.00	1.58	8.41	40.31	29.93
SON non-stress	AC214[b]	33.90	8.27	24.24	3.47	11.74	70.19	1.00	6.00	1.33	10.00	40.59	25.82
NIA stress		8.44	1.97	23.28	1.36	3.33	59.22	6.00	4.00	1.67	11.00	40.59	23.09
SON non-stress	AC215	66.00	16.09	24.38	3.98	20.07	80.05	0.00	6.00	1.50	7.58	40.54	32.19
NIA stress		9.20	2.63	28.59	1.94	4.57	56.41	6.00	3.00	2.00	8.89	40.49	28.42
SON non-stress	AC216	19.87	4.47	22.34	3.74	8.21	54.16	1.00	6.00	1.25	10.84	40.73	24.46
NIA stress		4.24	0.97	22.84	2.56	3.53	27.40	6.00	1.00	1.08	10.06	40.77	25.47
SON non-stress	AC524	24.77	6.17	24.99	1.30	7.47	82.50	1.00	6.00	1.42	9.27	40.51	28.65
NIA stress		4.64	1.13	23.83	0.73	1.86	59.22	6.00	2.00	1.17	8.78	40.66	29.80
SON non-stress	AC525	26.43	4.61	17.80	3.10	7.71	59.89	0.00	3.00	1.25	7.88	40.74	32.27
NIA stress		12.14	2.31	21.01	1.57	3.88	59.52	6.00	4.00	1.83	8.67	40.68	29.51
	LSD.05	11.26	3.38	4.83	2.37	4.76	11.86	0.17	0.46	0.25	2.19	0.36	5.31

[(a)] AC214 Line 109 and [(b)] AC214 Line 110.

Table 2.12. Correlation coefficients of tuber fresh matter yield and of tuber dry matter yield with important yield components (Treatment with pruning)

Characters	Amazonian Yam Bean (*P. tuberosus*) 6 Accessions		Mexican Yam Bean (*P. erosus*) 14 Accessions		Andean Yam Bean (*P. ahipa*) 14 Accessions	
	Tuber fresh matter yield	Tuber dry matter yield	Tuber fresh matter yield	Tuber dry matter yield	Tuber fresh matter yield	Tuber dry matter yield
Vines and Leaves dry matter weight	-0.391	-0.601	-0.170	-0.178	0.594*	0.577*
Tuber dry matter content	-0.260	0.090	-0.219	-0.011	0.135	0.312
Damage of tubers by nematodes	0.146	0.118	0.051	0.070	-0.377	-0.339
Damage of tubers by insects	-0.069	-0.101	0.076	0.159	0.705**	0.733**
Number of tubers per plant	0.317	0.145	0.115	0.192	0.542*	0.559*
Protein content of tuber dry matter yield (%)	0.319	0.492	-0.529	-0.610*	-0.395	-0.320
Carbon content of tuber dry matter yield (%)	0.047	0.104	-0.260	-0.196	-0.146	-0.256
Carbon / Nitrogen ratio of tuber dry matter yield	-0.407	-0.592	0.630	0.703**	0.389	0.306

(*), (**): significant at the level 0.05 and 0.01

Table 2.13. Correlation coefficients of tuber fresh matter yield and of tuber dry matter yield with important yield components (Treatment without pruning)

Characters	Amazonian Yam Bean (*P. tuberosus*) 6 Accessions		Mexican Yam Bean (*P. erosus*) 14 Accessions		Andean Yam Bean (*P. ahipa*) 14 Accessions	
	Tuber fresh matter yield	Tuber dry matter yield	Tuber fresh matter yield	Tuber dry matter yield	Tuber fresh matter yield	Tuber dry matter yield
Vines and Leaves dry matter weight	-0.364	-0.453	-0.330	-0.359	0.583*	0.558*
Tuber dry matter content	0.062	0.306	-0.240	-0.081	-0.359	-0.166
Seed Yield	0.908*	0.899*	-0.245	-0.286	-0.044	-0.116
Pod Yield	0.958**	0.904*	-0.302	-0.348	0.176	0.095
Time of Emergence	0.711	0.607	-0.150	-0.191	-0.143	-0.110
Early Vigour	-0.547	-0.658	-0.159	-0.200	0.267	0.242
Begin of flowering	-0.934**	-0.985**	-0.386	-0.417	-0.264	-0.204
Time of flowering	-0.802	-0.805	-0.269	-0.289	-0.209	-0.205
Period of flowering	-0.371	-0.486	-0.042	-0.019	0.585*	0.617*
Time of maturity	-0.509	-0.617	-0.394	-0.385	0.207	0.116
Plant height	0.093	-0.043	-0.404	-0.391	-0.124	-0.156
Plant type	0.000	0.000	-0.115	-0.165	0.225	0.149
Harvest index for seeds	0.303	0.352	-0.542*	-0.539*	-0.735**	-0.767**
Pod number per plant	0.586	0.629	-0.148	-0.218	-0.192	-0.219
Seed number per pod	-0.007	0.088	0.700**	0.668**	-0.121	-0.133
1000-Seeds Weight	-0.534	-0.605	0.260	0.212	0.766**	0.749**
Damage of tubers by nematodes	0.100	-0.098	0.030	0.101	-0.417	-0.379
Damage of tubers by insects	-0.277	-0.350	0.344	0.420	0.492	0.567*
Damage of stem and leaves by insects	0.350	0.435	0.179	0.204	-0.269	-0.301
Damage of stem and leaves by fungi	-0.180	-0.172	-0.159	-0.160	-0.342	-0.345
Number of tubers per plant	0.233	0.277	0.077	0.090	0.649*	0.674**
Protein content of tuber dry matter yield (%)	0.119	0.159	-0.529	-0.607*	-0.371	-0.250
Carbon content of tuber dry matter yield (%)	-0.007	-0.035	-0.068	0.000	0.141	-0.051
Carbon / Nitrogen ratio of tuber dry matter yield	-0.206	-0.282	0.652*	0.728**	0.391	0.254

(*), (**): significant at the level 0.05 and 0.01.

2.4. Discussion

Fresh tuber yield in *Pachyrhizus erosus* was in the average of 14 accessions and two locations about 23 t ha^{-1} without pruning and 45 t ha^{-1} with pruning of the reproductive parts. Taking into consideration that these figures were obtained without application of fertilizer, and when compared with figures from Thailand with yields of 18 - 24 t ha^{-1} (Ratanadilok and Thanisawanyangkura, 1998) and from Sierra Leone with yields of 10 – 23 t ha^{-1} (Belford, 2000; Belford et al., 2001), the production potential of the yam bean in Benin is promising. But in the present study the tuber yield showed a large variation between accessions owing probably to their diverse geographical origins.

Reproductive pruning is enhancing tuber production by avoiding the competition between tuber and pod formation and is often practised in *P. ahipa* and *P. erosus* (Sørensen, 1996). Flower and tuber formation during plant development are almost simultaneous events. Thus, high flower production implies a loss of valuable energy which could be allocated in tubers, resulting in a limited tuber yield (Heredia-Zepeda, 1971; Nielsen et al., 1998). This effect has been demonstrated in the present study as well as in many other field trials. In the study presented here, tuber fresh matter yield increases by 48% in *P. tuberosus*, 91% in *P. erosus* and 61% in *P. ahipa*. These findings are in agreement with those of Heredia-Zepeda (1971), Noda and Kerr (1983), Grum et al. (1994), Caro and Casillas (1998), Vaz et al. (1998), Nielsen et al. (1999) and Belford et al. (2001). An increase of 100%, 900% and 51.5% of the tuber yield was observed respectively by Heredia-Zepeda (1971) in *P. erosus*, Noda and Kerr (1983) in *P. erosus* and Nielsen et al. (1999) in *P. tuberosus*. Pruning has obviously no effect on protein content of tuber dry matter in all three cultivated species. Significant genotype x treatment interaction was observed only for tuber fresh matter in *P. tuberosus* and for tuber dry matter yields in *P. ahipa*. That means, it will be difficult to develop cultivars which do not require pruning.

P. tuberosus has the highest tuber dry matter content (about 30%). This is in accordance with Sørensen et al. (1997). Chuin types of *P. tuberosus* have particularly high dry matter content. *P. erosus* has the lowest tuber dry matter content, but the highest tuber fresh matter yield.

A number of insect pests are reported to cause leaf, tuber and seed damage in *P. erosus*. The nematode *Meloidogyne marioni* (Cornu) Chitwood et Oteifa is mentioned by Duke (1981) as the cause of tuber damage in *P. erosus*. In *P. ahipa*, nematodes may be a problem locally, and the tubers can be completely destroyed by them (Sørensen, 1996). Several nematodes have been reported as being the cause of significant yield reductions in *P. tuberosus* (Sørensen, 1996). Noda et al. (1991) observed serious damage caused by attacks by *Meloidogyne* Goeldi and *Pratylenchus* Filipjev. These effects have been noticed in the present study and the tubers of P. ahipa were more attacked by insects (termites) and nematodes than those of *P. erosus* and *P. tuberosus*. Very serious problems have been observed to be caused by leaf-eating insects in *P. erosus* as well as in *P. tuberosus* and *P. ahipa* (Sørensen, 1996). These problems were also encountered in the present study, in which stems and leaves of *P. erosus* were more attacked by insects than those of *P. ahipa* and *P. tuberosus*. Several fungi have been reported to cause severe damage in *P. erosus*. Sørensen (1996) reported a high mortality rate in young plants as a result of “root attacks“ by *Pythium spp.*, *Corticium spp.* and *Macrophomina spp.* in multilocational field trials in Senegal. Mohanty and Behera (1961) reported a severe leaf spot disease observed in Bhubaneswar, India and succeeded in identifying the fungus as *Cercospora canescens* Ellis et Martin. In the study presented here only the stems and leaves were attacked by fungi and *P. ahipa* was higher affected. This observation may be due to the fact, that the genotypes of *P. ahipa* have determinate growth habit and are most of the time against the humid soil, which makes the attacks by fungi easier. When all five species are cultivated in one location, bean common mosaic virus (BCMV) will infect all three cultivated species and will also infect the wild species *P. panamensis* (Sørensen, 1996). Interestingly in this study, no attack by BCMV in the field was observed.

A total of 31 agronomical characters were used to examine the genetic variation in 34 accessions of yam bean (*Pachyrhizus* DC.). Comparable studies have also been conducted by Hernandez (1992), Belford et al. (2001) and Tapia and Sørensen (2003). But in these cases, the number of accessions used was limited, and only one species (*P. tuberosus*) was studied.

In the present study, there is a great variation within as well as between species. The amount of variation between locations was also high in accordance with previous studies in West Africa (Annerose and Diouf, 1998). The reaction of genotypes differs also with the locations. At the first location (Songhai), where irrigation was applied, most of the traits measured showed higher levels than at location Niaouli. This is also in accordance with Annerose and Diouf (1998). Two genotypes of *P. erosus* (EC533 and ECKEW) showed at the optimal location as well as at the stress location a high level in the expression of the characters tuber fresh and dry matter yields. They showed therefore a remarkable stability over locations.

A significant positive correlation was observed between tuber yield and seed yield (r=0.90 for tuber fresh matter yield and 0.89 for tuber dry matter yield) in *P. tuberosus*. Thus, selection for increased tuber yield would not adversely affect seed yield, and selection for a high seed yield could result in cultivars suitable for tuber production. This finding is interesting, so far the yam bean is multiplied by seeds. Genotypes with high tuber yield as well as seed yield are suitable.

Significant differences between accessions for all variables were detected in both treatments (pruning and no pruning). This is in agreement with Nielsen et al. (2000). In general, the Mexican yam bean (*P. erosus*) showed the highest yield. This in accordance with Ramaswany et al. (1980), Singh et al. (1981), Bhag and Kawalkar (1982), Grum et al. (1994) and Nielsen et al. (2000). Legumes have been grown traditionally on marginal lands of poor fertility. Thus even though the different species of *Pachyrhizus* were domesticated several thousand years ago (Sørensen et al., 1997), they are still grown in edaphic conditions, which are not very different

from those in their native habitats. Thus, natural selection has continued to have a major effect on the evolution of these crops even after domestication in developing countries (Jain and Mehra, 1978). The selection pressure on legumes, such as yam bean, continue to be for adaptations to stress conditions such as drought, poor fertility and competition with other biological systems such as insects, pests and pathogens.

In conclusion, the results showed highly significant differences in all the characters among the accessions, the species, the locations and the interactions genotype x location. Genotypes within *P. erosus* contain genes for high yields and yield components. Two genotypes (EC533 and ECKEW) have been demonstrated as relatively stable over locations for yield and its components. Pruning practices resulted in an increase of tuber yield in all genotypes. These results show the natural competition between tuber production and pod filling process. Genotypes of *P. tuberosus*, particularly within the Chuin cultivar group, contain genes for high tuber dry matter content, while within *P. ahipa* genes for earliness are present.

2.5. Summary

The yam bean (*Pachyrhizus* Rich. Ex DC.) is a tuber bearing legume mainly cultivated in Central and South America as well as in many countries in South East Asia. Seeds are needed to establish the crop, but only the tubers are consumed. Therefore farmers traditionally remove all pods (reproductive pruning). The objecives of the present study are to evaluate the agronomical potential of yam bean under West African conditions and the effect of reproductive pruning. Thirty four accessions of yam bean from three species (*Pachyrhizus tuberosus*, *P. erosus* and *P. ahipa*) and ecologically diverse origins, were tested in a field trial at two locations in Benin during 2001/2002. At both locations, 31 agronomical traits were recorded. Significant differences were observed among locations, accessions and species for most of the characters. Without reproductive pruning, the mean tuber fresh matter yield ranged from 12.4 in *P. ahipa* to 23.4 t ha^{-1} in *P. erosus*. Reproductive pruning resulted in an increase of 48, 91 and 61% of tuber fresh matter yield in *Pachyrhizus tuberosus*, *P. erosus* and *P. ahipa*, respectively. With pruning, the tuber dry matter yield increased by 58, 100 and 66% in *Pachyrhizus tuberosus*, *P. erosus* and *P. ahipa*, respectively. Most of the traits presented their higher value at the location Songhai (where irrigation was done) than at Niaouli (where no irrigation was done). Reproductive pruning had a positive effect on tuber yield in all accessions. Accessions with genes for high tuber yield as well as high seed yield have been identified mainly within *P. erosus*. *P. tuberosus* and particularly the Chuin cultivar group shows a high tuber dry matter content (about 30%). The best Chuin type of *P. tuberosus* had a tuber dry matter content of 37%. TC118 (Ashipa cultivar group of *P. tuberosus*) had a lower tuber dry matter content (lower than 25%). Accessions with potential genes of interest to improve earliness have been identified within *P. ahipa*. The study shows the potential of *Pachyrhizus* Rich. ex DC. for its introduction into farming systems of Benin and of West Africa in general.

3. Genetic diversity in yam bean (*Pachyrhizus spp.*) revealed by multivariate analyses of morphological and agronomic traits

3.1. Introduction

In *Pachyrhizus*, Døygaard and Sørensen (1998) reported the genetic variation in the genus using morphological traits and material from herbarium. The Andean yam bean (*Pachyrhizus ahipa*) is morphologically distinct from the Mexican yam bean (*P. erosus*) and the Amazonian yam bean (*P. tuberosus*). Heredia-Zepada and Heredia-Garcia (1994) studied the genetic variation in some accessions of *Pachyrhizus erosus*. Tapia and Sørensen (2003) reported the genetic variation in *P. tuberosus* using morphological characters. *P. tuberosus* is the species with the largest plants, reaching stem lengths of up to 10 m. *P. ahipa* has much smaller plants of both bushy-erect as well as twining growth habit (Tapia and Sørensen, 2003). *P. tuberosus* consist essentially of three cultivar groups: the Chuin, Ashipa and Jíquima cultivar groups. An important morphological difference between these three cultivar groups is that the Chuins and the Jíquimas have a uniform, vertical tap-root (Sørensen et al., 1997). Moreover, the Chuins have a high dry matter content, comparable to that of cassava, while the Jíquimas and Ashipas have a low dry matter content like the other yam bean species. Ashipas and Jíquimas have entire to deeply lobed heart-shaped terminal leaflets with a low L/W (Length / Width) ratio, whereas Chuins have entire to somewhat lobed leaflets with a high L/W ratio.

In addition to the studies of Døygaard and Sørensen (1998) on herbarium material and that of Heredia-Zepada and Heredia-Garcia (1994), Márquez (1992) provides a morphological characterization of the CATIE (Costa Rica) collection of *P. erosus* using living plants and both quantitative and qualitative characters. Márquez reported that when classifying the different accessions belonging to the Mexican yam bean*, P. erosus,* the flower and vegetative growth traits are the most important factors. Márquez (1992) identified three groups within *P. erosus,* while Døygaard

and Sørensen (1998) reported that the wild specimens von *P. erosus* were clearly separated from the cultivated ones. According to Márquez (1992), number of nodes of the main stem, length of stem, number of leaves and growth velocity were closely associated with the flower characters, i.e. inflorescences per stem, date of flower initiation and duration of flowering. This fact indicated that the genotypes with enhanced vegetative growth have intense flowering and consequently high seed production.

Different species can be differentiated with few morphological characters, But for the analysis of genetic variation within species and the estimation of genetic distance between species, there is the need to use as far as possible many quantitative traits.

The present study has the objectives to investigate the genetic variation in yam bean using morphological and agronomic traits measured under field conditions in Benin, West Africa. The study was conducted to differentiate the three cultivated species, to determine which traits contribute most to the differentiation of the three species and to examine the amount of diversity within the three species.

3.2 Materials and methods

3.2.1 Plant material

A total of 34 accessions representing better agronomic types from diverse ecogeographical backgrounds were used for the present study. The accessions consist of 14 *Pachyrhizus ahipa* lines, 14 *P. erosus* accessions and 6 *P. tuberosus* accessions. The *P. ahipa* material was selected from single plant progenies out of 13 accessions and genotypes were designated by accession and progeny line number respectively. At least one genotype was selected out of each accession. From AC214, two lines (AC214-109 and AC214-110) were selected. No selection was carried out in the *P. erosus* and the *P. tuberosus* material. An overview of the accessions is given in Table 2.1 (see page 20).

3.2.2 Field experiments

Seed multiplication was done from June 2000 to January 2001 at the “Centre Songhai“ in Porto-Novo (Benin) with 4 to 8 plants of each accession.

The germplasm was grown at the “Centre Songhai“ station in Porto-Novo and at the experimental station of INRAB (Institut National des Recherches Agricoles du Bénin) in Niaouli. The soil were well drained at both stations and is sandy loam red. The experiments were carried out between June 2001 and January 2002. The characteristics of the two experimental sites are presented in Table 2.2 (see page 22).

Field experiments and morpho-agronomical measurements

Experimental design was a complete randomised block with two replications at both locations for the factor accessions and each complete block was conducted by the factor levels species (*P. tuberosus, P. erosus, P. ahipa*). Each plot consisted of 4 rows of 6 plants each and a plot measured 1.25 m by 2.25 m. The distance between plots was 1 m . Two rows were spaced 0.75 m apart and the distance between plants within a row was 0.25 m. Two seeds were sown per hole at a depth of about 2 cm. Irrigation was done at the station „Centre Songhai“ in Porto-Novo, during a period comprising the dry month August. At Niaouli, no irrigation was applied. Weeds were removed every two weeks. Thining of the plants to one per hole was done five weeks after sowing. Two pickets were erected to maintain the plant upright as vegetative growth became aboundant. No fertiliser or pesticide was applied.

In total, 71 morpho-agronomical characters were measured which are listed in Tables 3.1. These tables present the traits, codes and procedures of recording. The IPGRI descriptors lists for *Phaseolus spp.*, *Vigna spp.* and *Ipomoea batatas* (sweet potato) were used with small modifications.The traits recorded are listed in Table 3.1. Data on single plant basis were recorded on six randomly selected competitive individuals within the two center rows. A pachymeter was used to measure most of the morphological characters.

Table 3.1. Morpho-agronomic characters evaluated, code and procedure of measurement

Characters	Code	Procedure and time of recording
Tuber fresh yield	TUBY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows, fresh weight
Tuber dry matter yield	TDMY	TDMY = TUBY x DM
Vines and Leaves Weight	VLW	t ha^{-1} – at physiological maturity- 24 plants from 4 rows, sun dried
Seed yield	SEEY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Pod yield	PODY	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Total biomass	BIOM	BIOM = TDMY + VLW + PODY
Harvest-index for tuber and seed yield	HIT HIS	HIT = (TDMY / BIOM) x 100 HIS = (SEEY / BIOM) x 100
Total harvest-index	HITOT	HITOT = ((TDMY + SEEY) / BIOM) x 100
Shell weight	SHEL	t ha^{-1} – at physiological maturity- 24 plants from 4 rows
Begin of flowering	BF	Days from sowing to begin of flowering
Time of flowering	TF	days from sowing to 50% of plants flowering within center rows
Time of emergence	TE	days from sowing to 50% of plants emergence within center rows
Thousand seed weight	TSW	in g – at physiological maturity – measured on two samples of 100 seeds
Tuber dry matter content	DM	In % - measured on sun dried samples
Time of maturity	TM	days from sowing to physiological maturity – 80% dry pods within 2 center rows
Damage of stem and Leaves by Insects	DSLI	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of stem and Leaves by Fungi	DSLF	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of tubers by Nematodes	DTN	Scores from 0 to 6; 0 = no damage. 6 = high damage
Damage of tubers by Insects	DTI	Scores from 0 to 6; 0 = no damage. 6 = high damage
Early vigor (width of first leaf)	EV	in mm – at time of development of third leaf– 6 plants within plot center and two measurements per plant
Period of flowering	PF	Days from begin of flowering to end of flowering - 6 plants within plot center
Start of climbing	SC	days from sowing to begin of climbing – 6 plants within plot center
Plant height	PH	in cm – at time of full flowering – 6 plants within plot center
Plant type	PT	Scores from 3 to 9 (3=erect; 5=semi-erect, 7=spreading, 9=extremely spreading)
Number of pods per plant	PN	counted – at harvest – 6 plants within plot center
Number of tubers per plant	NTP	Counted – at harvest – 6 plants within plot center
Seed number per pod	SNP	counted – at harvest - 6 plants within plot center (6 pods per plant)
Protein content	PRO	In % TDMY

Table 3.1. Continued

Characters	Code	Procedure and time of recording
Leaf green colour	LC	very light to very dark (5 scores) – at time of full flowering – 6 plants within plot center
Termial leaflet length, width and length to maximum width	TLL, TLW, TLMW	in cm – at time of full flowering – 6 plants, within plot center (6 leafs per plant)
Lateral leaflet length, width and length to maxium width	LLL, LLW, LLMW	in cm – at time of full flowering – 6 plants within plot center (6 leafs per plant)
Number of leaves	LN	Counted – at time of full flowering – 6 plants within plot center
Terminal leaflet lobe type	TLLT	Entire to very deep (6 scores from 0 to 9: 0=entire, 1=very slight lobes, 3=slight, 5=moderate, 7=deep, 9=very deep) – at time of full flowering- 6 plants within plot center (6 leaflets per plant).
Lateral leaflet lobe type	LLLT	Entire to very deep (6 scores from 0 to 9: 0=entire, 1=very slight lobes, 3=slight, 5=moderate, 7=deep, 9=very deep) – at time of full flowering- 6 plants within plot center (6 leaflets per plant).
Shape of central terminal leaflet lobe	SCTLL	Absent to linear (narrow) (10 scores from 0 to 9) – at time of full flowering- 6 plants within plot center and 6 leaflets per plant.
Shape of central lateral leaflet lobe	SCLLL	Absent to linear (narrow) (10 scores from 0 to 9) – at time of full flowering- 6 plants within plot center and 6 leaflets per plant.
Terminal leaflet lobe number	TLLN	At time of full flowering. 6 plants within plot center and 6 leaflets per plant.
Lateral leaflet lobe number	LLLN	At time of full flowering. 6 plants within plot center and 6 leaflets per plant.
Inflorescence length	IL	in cm – at time of full flowering – 6 plants within plot center (6 inflorescences per plant)
Flower colour of sepales	CS	Green, a little purple, purple – 6 plants within plot center (12 flowers per plant)
Flower colour of standard and wing	FCS, FCW	white, pink, violet – 6 plants within plot center (12 flowers per plant)
Pod length (including beak), width and height	PL, PW, POH	in mm – at harvest after 6 days sun drying –6 plants within plot center (6 pods per plant)
Pod green colour	PC	very light to very dark (5 scores) – after 7 weeks of full flowering – 6 plants within plot center

Table 3.1. Continued

Stem Colour	SCO	At time of full flowering. 6 plants within plot center. 9 scores from 1 to 9: 1= green, 3= green with few purple spots, 4= green with many purple spots, 5= green with many dark purple spots, 6= mostly purple, 7= mostly dark purple, 8= totally purple, 9= totally dark purple.
Pod degree and shape of curvature	PDS PC	angle – at harvest after 6 days sun drying – 6 plants within plot center (6 pods per plant)
Pod beak length and curvature	PBL, PBC	in mm and angle – at harvest after 6 days sun drying – 6 plants within plot center
Dehiscence of pods	DP	At harvest – 6 plants within plot center - . 3 scores from 3 to 7: 3= absent, 5= a little dehiscent, 7= dehiscent.
Colour of mature pods	CMP	At harvest – 6 plants within plot center - . 3 scores from 1 to 3: 1= yellow, 2= brown, 3= dark brown.
Seed length, width and height	SL, SW, SH	in mm – at harvest after 6 days sun drying – 6 plants within plot center (5 pods per plant and 3 seeds per pod)
Colour of seeds	CSE	At harvest. 9 scores from 1 to 9: 1= olive, 2= brown, 3= orange red, 4= dark red, 5= pink, 6= purple, 7= purple or black with white mottled, 8= black, 9= others.
Tuber Shape	TS	- at harvest – 6 plants within plot center. 9 scores from 1 to 9: 1=round, 2=round elliptic, 3= elliptic, 4=ovate, 5=obovate, 6=oblong, 7=long oblong, 8=long elliptic, 9=long irregular or curved.
Tuber Color	CT	- at harvest – 6 plants within plot center. 5 scores from 1 to 5: 1=white, 2=yellow, 3=brown, 4=purple-red 5=dark purple.
Tuber surface defects	TSD	- at harvest – 6 plants within plot center. 9 scores from 0 to 8: 0=absent, 1=alligator-like skin, 2=veins, 3=shallow horizontal constrictions, 4=deep horizontal constrictions, 5=shallow longitudinal grooves, 6=deep longitudinal grooves, 7=deep constrictions and deep grooves.
Secondary flesh colour and distribution of secondary flesh colour	SFC DSFC	- at harvest – 6 plants within plot center - . 10 scores from 0 to 9: 0=absent, 1=white, 2=cream, 3=yellow, 4=orange, 5=pink, 6=red, 7=purple-red, 8= purple, 9=dark purple. - at harvest – 6 plants within plot center - . 10 scores from 0 to 9: 0=absent, 1=narrow ring in cortex, 2=broad ring in cortex, 3=scattered spots in flesh, 4=narrow ring in flesh, 5=broad ring in flesh, 6=ring and other areas in flesh, 7=in longitudinal sections, 8=covering most of the flesh, 9=covering all flesh.
Tuber Length	TL	In cm - at harvest – 6 plants within plot center -
Tuber Width	TW	In cm - at harvest – 6 plants within plot center -

Table 3.1. Continued

Tuber Lenght to Maximum Width	TMW	In cm - at harvest – 6 plants within plot center -
Tuber stalk	TST	- at harvest – 6 plants within plot center - . 6 scores from 0 to 9: 0=sessile or absent, 1= very short (<2 cm), 3=short (2-5 cm), 5=intermediate (6-8 cm), 7=long (9-12 cm), 9=very long (>12 cm).
Tuber cracking	TC	- at harvest – 6 plants within plot center - . 4 scores from 0 to 7: 0=absent, 3=few cracks, 5=medium number of cracks, 7=many cracks.

3.2.3 Statistical analysis

The genetic diversity between accessions was determined by multivariate statistics. For those traits measured repeatedly on single plant basis plant mean values were calculated first and then all plant measurements were averaged to get one single plot value for each accession. In a second step for all 71 traits mean values across replications and location were calculated to arrange the data sets for principal component and cluster analysis. The SAS software version 6.12 (SAS, Cary, NC, USA 1997) was used.

The principal component analysis was carried out by SAS PROC PRINCOM to determine the principal components, corresponding eigenvalues and proportions of eigenvalues as well as the scores of the principal components. The spatial relationships of accessions were presented by plotting the scores of the first, second and third principal components. Moreover, the correlations of all traits and principal components 1 to 5 were calculated by SAS PROC CORR using the Pearson correlation coefficient.

The cluster analysis was carried out by SAS PROC CLUSTER for hierarchically formed clusters. Therefore the 71 traits were standardized by their mean value and standard deviation ($z = (x - \bar{x}) / s$) using the STD option. Distances between objects and clusters, respectively were calculated by the Euclidian Distance and agregated by the unweighted average linkage method using the option AVE. A cluster dendrogram was plotted using the SAS-Macro DENDRO (Nicholson, 1995).

3.3 Results

In order to assess the patterns of variation, principal component analysis was done by considering all the 71 morpho-agronomic characters simultaneously. The first ten principal components accounted for more than 91% of the total variation (Table 3.2). The first principal component concentrated 41.2% of total variance, the second 20,9%, the third 8.5%, the fourth 6% and the fifth 4% for the morpho-agronomic data (Table 3.2). The first component is primarily correlated with seed yield, yield components, maturity, begining of flowering, time of flowering, plant height, total biomass and protein content of the tuber (Table3.3). The second component is mainly related with variation in tuber dry matter content and 1000-seeds weight. The third component is highly correlated with tuber dry matter yield, harvest index for tuber and total harvest index (tubers and seeds). The fourth component described the patterns of variation in the tuber fresh matter yield and the tuber dry matter yield. The fifth principal component is only significantly correlated with the variation in the terminal and lateral leaflet lobe type (Table 3.3).

The pattern of divergence between the 34 accessions for the first two principal components is given in Figure 3.1. It can be discerned from the figure that the genotypic diversity between species was large. Nearly all accessions of *P. ahipa* have negative values of both principal components. Accessions of *P. tuberosus* had positive values for both components, whereas accessions of *P. erosus* showed positive values for the first component, but negative values for the second.

Table 3.2. Results of Principal Component Analysis, Eigenvalues of the Correlation Matrix

	Eigenvalues	Proportion	Cumulative
PC1	29.287	0.412	0.412
PC2	14.850	0.209	0.621
PC3	6.092	0.085	0.707
PC4	4.272	0.060	0.767
PC5	2.878	0.040	0.808
PC6	2.238	0.031	0.839
PC7	1.646	0.023	0.862
PC8	1.323	0.018	0.881
PC9	1.192	0.016	0.898
PC10	1.027	0.014	0.912

Table 3.3. Pearson Correlation Coefficients for principal components

	PC1	PC2	PC3	PC4	PC5
TUBY	0.464*	-0.201	0.506*	0.630**	0.172
TDMY	0.368	0.135	0.615**	0.603**	0.128
VLW	0.623**	0.337	-0.587**	0.109	0.218
SEEY	0.795**	-0.413	-0.078	-0.119	-0.041
PODY	0.793**	-0.452*	-0.080	-0.081	-0.038
BIOM	0.883**	-0.010	-0.226	0.160	0.134
HIT	-0.421	0.168	0.696**	0.503*	0.105
HIS	-0.084	-0.672**	-0.071	-0.488*	-0.302
HITOT	-0.510*	-0.137	0.740**	0.324	-0.029
SHEL	0.784**	-0.473*	-0.081	-0.056	-0.036
BF	0.739**	0.516**	-0.239	-0.179	0.107
TF	0.829**	0.418	-0.216	-0.116	0.124
TE	-0.646**	0.054	-0.124	-0.082	0.256
TSW	-0.568**	0.726**	-0.157	0.230	0.071
DM	-0.086	0.826**	0.232	-0.223	-0.157
TM	0.842**	0.395	-0.271	0.126	-0.028
DSLI	0.365	-0.457**	0.467**	-0.165	-0.183
DSLF	-0.678**	-0.224	-0.095	-0.228	0.063
DTN	-0.861**	0.273	-0.010	-0.088	0.065
DTI	-0.862**	-0.254	0.109	0.111	0.071
EV	-0.318	-0.255	-0.669**	0.329	0.056
PF	0.092	0.429*	-0.657**	0.413	0.262
SC	0.331	-0.055	0.033	-0.026	0.207
PH	0.919**	0.300	-0.169	-0.013	0.008
PT	0.875**	0.401	-0.032	-0.088	0.149
PN	0.752**	-0.454*	0.061	-0.234	0.064
NTP	-0.488*	-0.077	0.063	0.292	0.429
SNP	0.882**	0.017	0.045	-0.014	-0.122
PRO	0.746**	0.015	0.050	-0.252	-0.155
LC	0.763**	0.416	0.092	0.110	-0.040
TLL	0.606**	0.760**	0.130	-0.063	0.012
TLW	0.843**	-0.423	-0.144	0.130	0.134
TLMW	0.584**	0.794**	0.001	-0.011	-0.021
LLL	0.488*	0.842**	0.111	-0.059	-0.026
LLW	0.910**	-0.117	-0.189	0.178	0.103
LLMW	0.675**	0.708**	0.065	-0.035	-0.087
LN	0.354	-0.387	-0.332	-0.086	0.219
TLLT	0.505*	-0.428	0.132	-0.101	0.616**
LLLT	0.423	-0.485*	0.060	-0.200	0.593**
SCLTL	0.559**	0.562**	0.421	-0.249	0.227
SCLLL	0.514**	0.683**	0.431*	-0.200	0.070
TLLN	0.607**	-0.641**	-0.088	-0.089	0.059
LLLN	0.668**	-0.581**	-0.047	-0.119	0.060
IL	0.953**	-0.109	0.056	-0.021	0.063
CS	-0.304	-0.722**	-0.088	0.271	-0.286
FCS	-0.479*	-0.633**	-0.129	0.153	-0.368
FCW	-0.479*	-0.633**	-0.129	0.153	-0.368
PL	-0.184	0.840**	-0.221	0.186	-0.120
PW	-0.812**	0.337	-0.148	0.112	-0.043
POH	-0.892**	0.034	-0.185	0.186	0.275
PC	0.635**	0.063	-0.344	-0.030	-0.186
SCO	0.301	0.828**	0.057	-0.063	0.011
PDSPC	-0.939**	-0.256	-0.045	-0.042	0.128
PBL	-0.958**	0.164	0.072	0.039	0.039
PBC	0.053	-0.223	0.686**	-0.464*	-0.027
DP	0.688**	-0.212	0.298	0.287	-0.393
CMP	0.817**	0.357	0.080	0.125	-0.369
SL	-0.552**	0.703**	-0.155	0.219	-0.138

Table 3.3. Continued

	PC1	PC2	PC3	PC4	PC5
SW	0.265	0.675**	-0.044	0.427	-0.355
SH	-0.912**	-0.086	-0.136	0.139	0.284
CSE	-0.841**	0.331	0.148	-0.185	0.211
TS	0.049	0.400	0.283	-0.332	0.151
CT	-0.573**	0.555**	0.233	-0.331	-0.006
TSD	0.601**	-0.180	0.307	0.235	-0.131
SFC	-0.789**	0.417	0.228	-0.188	0.072
DSFC	-0.579**	0.568**	0.322	0.013	0.035
TL	0.807**	-0.037	0.249	0.319	0.098
TW	0.451*	-0.382	0.461*	0.579**	0.118
TMW	0.244	-0.043	-0.245	0.488*	-0.033
TST	-0.033	0.533**	-0.536**	0.125	0.024
TC	0.579**	-0.307	0.202	0.143	0.313

(**) significant at the level 0.001
(*) significant at the level 0.01

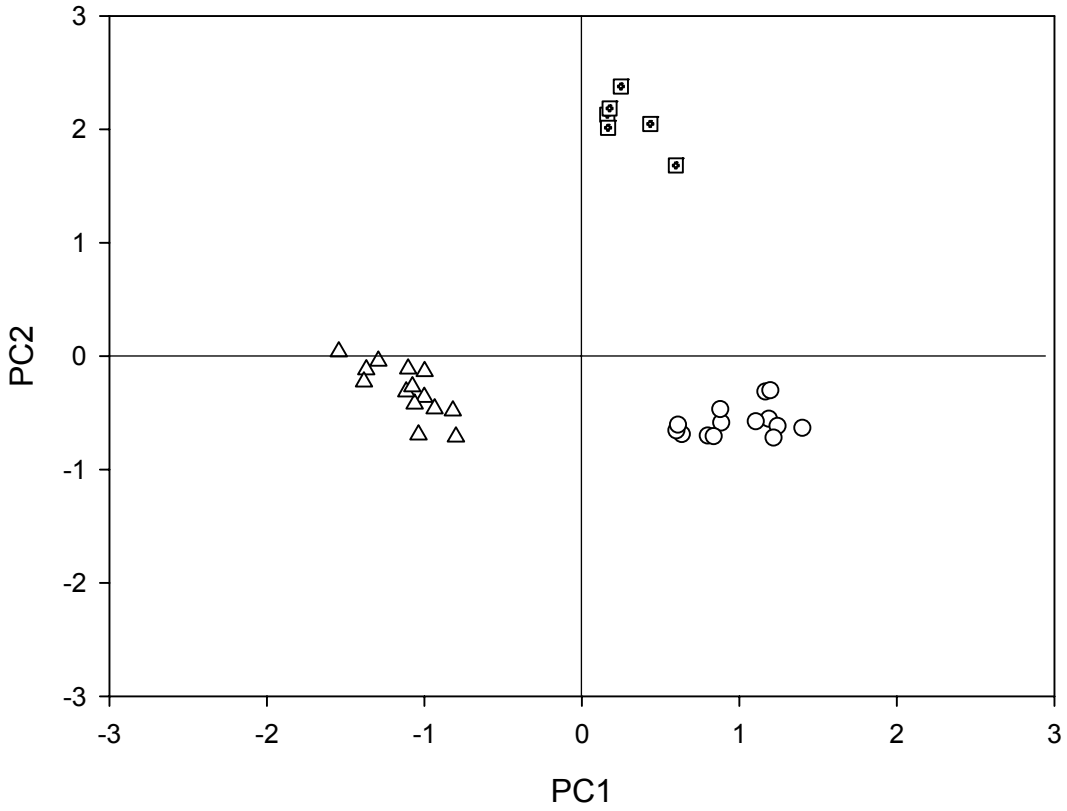

Figure 3.1. Plot of the first and second component scores for the 34 accessions of yam bean, *Pachyrhizus spp*.

⊡ *P. tuberosus* △ *P. ahipa* ○ *P. erosus*

To have a clear idea of the patterns of variation in the traits correlated with the third principal component (tuber dry matter yield, tuber harvest index and total harvest index) in relation to the differences in the morpho-agronomic traits correlated with the first principal component, the distribution of the accessions was plotted along the axes of the first and the third principal components (Figure 3.2, Table 3.3). TC118 of *P. tuberosus* (Ashipa cultivar group) showed large negative value for the third principal component which can be explained by very low tuber dry matter yield, tuber harvest index and low total harvest index. The 5 remaining accessions of *P. tuberosus* and some accessions of *P. erosus* contributed positively most to the third component. All the accessions of *P. ahipa* showed negative values for the first component.

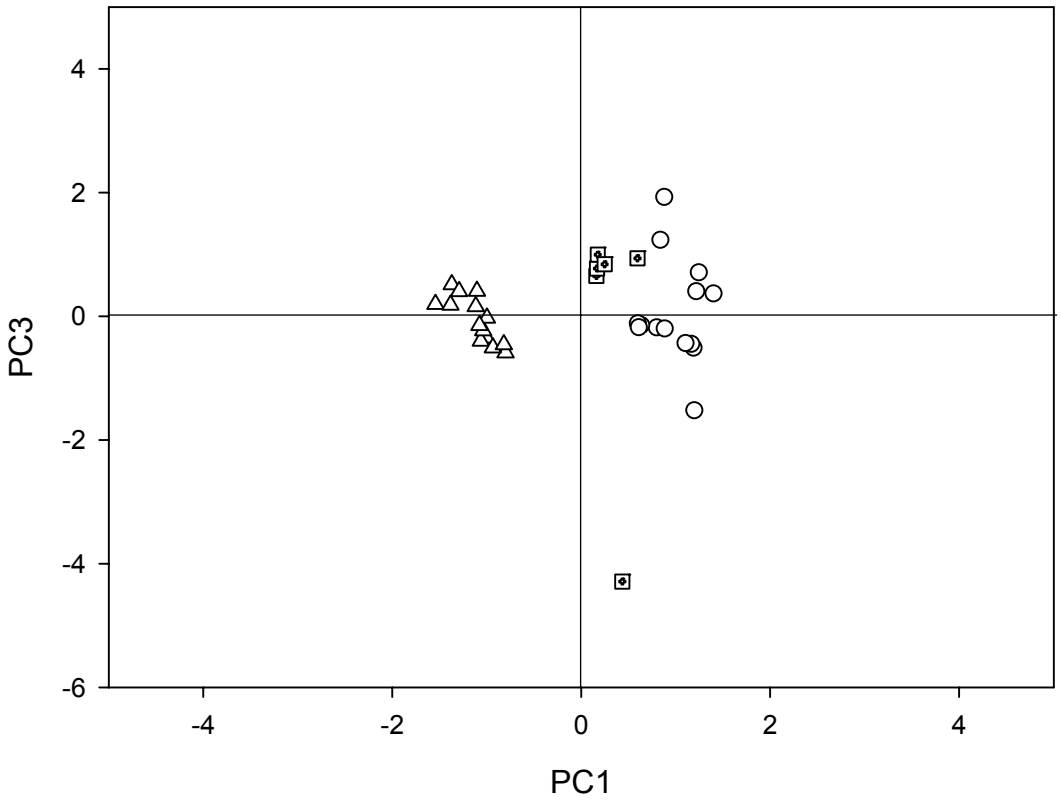

Figure 3.2. Plot of the first and third component scores for the 34 accessions of yam bean, *Pachyrhizus spp*.

⊡ *P. tuberosus* △ *P.ahipa* ○ *P. erosus*

The average linkage technique of clustering produced a more portrayal of the 34 accessions by grouping them into three principal parts (Figure 3.3). The three species were well separated in these clusters due to the agronomic and some morphological differences. In each of the groups, many clusters can be distinguished.The group of *P. tuberosus* included three subgroups, namely: TC118 of the Ashipa cultivar group from Haiti, CC354 of the Chuin cultivar group, (CC362, CC353, CC361, CC355) also of the Chuin cultivar group. In the group of *P. erosus*, accessions ECKEW and EC533 from Mexico and Maccau (Asia) respectively formed one subgroup. The remaining subgroups in *P. erosus* comprised one (EC104), 3 (EC041, EC042, EC040), 4 (EC253, EC033, EC114, EC032), 4 (EC557, EC204, EC550, EC006) accessions each. In the group of *P. ahipa*, the subgroups comprised 5 (AC214-110, AC214-109, AC209-73, AC215-129 and AC202-27), one (AC213-92), six (AC205-68h, AC525-170, AC208-72h, AC203-43, AC216-139 and AC201-19) and two (AC524-164 , AC102-153) accessions, respectively.

The results obtained for the cluster analysis are in general consistent with those of principal component analysis. Hence, there is a clear separation between the three species used in the present study. *P. ahipa*, *P. erosus* and *P. tuberosus* (Chuin types) could be separated in three different groups. But the *P. tuberosus* accession TC118 appeared not together with the other *P. tuberosus* accessions, but in a separate group. This accession is from Haiti, a different geographical region in comparison with the 5 remaining Chuin types from Peru. Within the Chuin cultivar group of *P. tuberosus*, CC354 with an average genetic distance of 0.66 seemed to be isolated from the other accessions. Within *P. erosus*, the genotypes (ECKEW and EC533 from Mexico and Maccau respectively) with high tuber fresh matter yield (see Chapter 2) formed together one group with an average distance of 0.47. EC104 with a genetic distance of 0.75 was in a distinct group. The remaining accessions of *P. erosus* appeared in diverse groups independantly of the geographical origins. The same observation was made for the accessions of *P. ahipa*. Here it can be mentioned, that the bush types (AC102-153 and AC524-164) formed together one group.

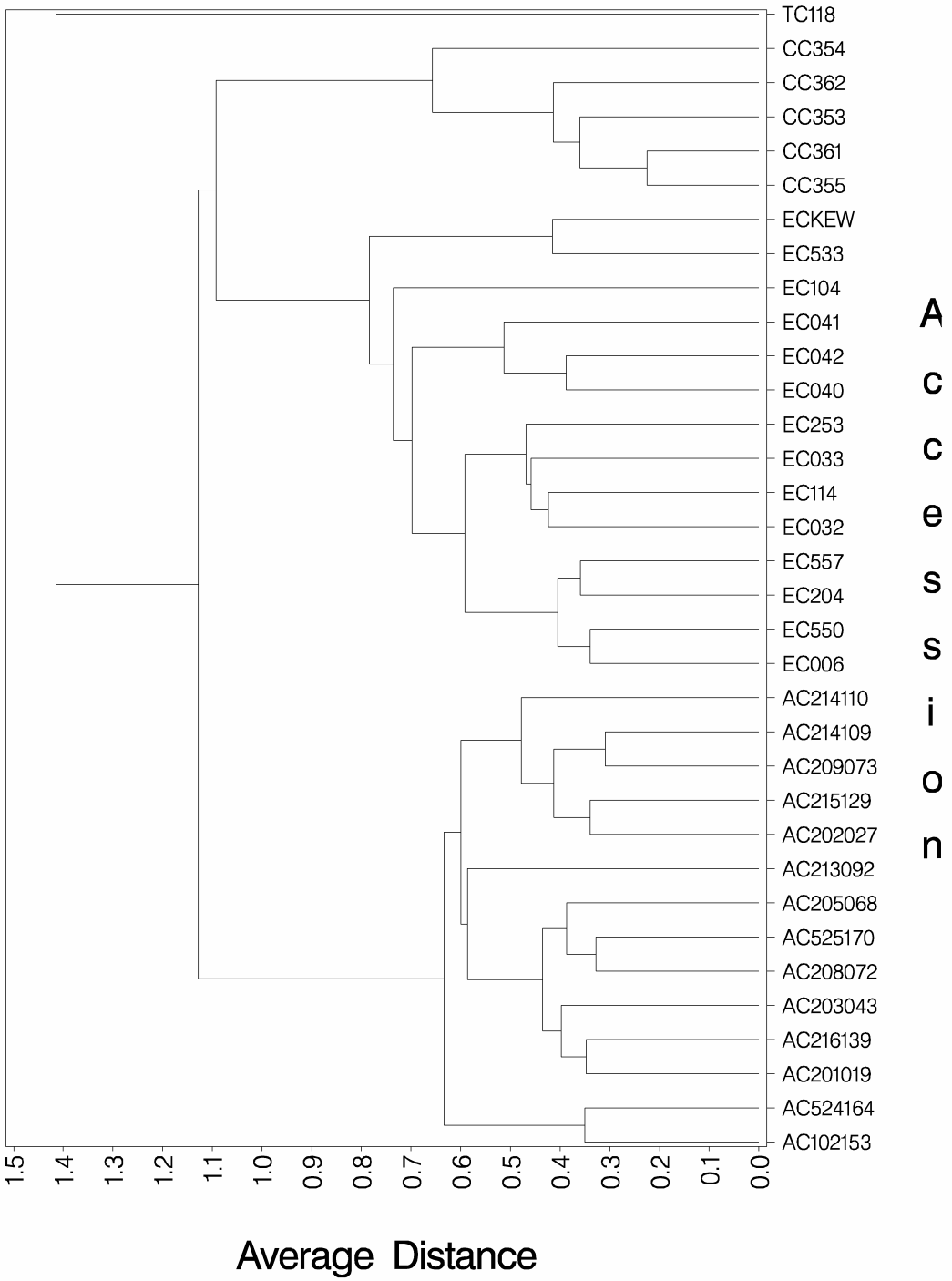

Figure 3.3. Cluster analysis on basis of 71 agronomic and morphological traits from a two locations field trial in Benin / West Africa; TC and CC = the Amazonian yam bean (*Pachyrhizus tuberosus*) CC = high dry matter Chuin types, AC = the Andean yam bean (*P. ahipa*) and EC = the Mexican yam bean (*P. erosus*).

3.4 Discussion

The cluster and principal component analyses with morphological and agronomic data of *Pachyrhizus spp*. revealed the existence of genetic variability among accessions, as well as differences between species. The principal component analysis has shown that ten of the principal component accounted for 91% of the total variation encountered among the accessions taking into account all the 71 morpho-agronomic traits simultaneously. Ordinations of the accessions along the axes of the first and second principal components have revealed that all the accessions of *P. ahipa* from diverse ecogeographical regions of Bolivia could be considered as a potential source of earliness in maturity but are not suitable for increasing the level of traits such as seed and tuber yields and their components, plant height and leaves characters. The diversity among the accessions, however, doesn´t suggest a simultaneous improvement of earliness and yield components since accessions concurrently having the lowest value of the first and the highest value of the second component were not discovered. All the accessions of *P. tuberosus* showed positive value for the first two components. That means, that only in this species a combination of characters such as tuber dry matter content, 1000-seeds weight, yield and its components and leaves traits could be found. Accessions of *P. erosus* showed positive value for the first component, but negative value for the second. This means, that it will not be possible to have simultaneously a combination of traits such as tuber dry matter content, 1000-seeds weight and characters such as earliness, yield and its components and leaves characters.

Divergence studies using the techniques of principal component and cluster analyses have been made in several crops such as wheat (Bhatt, 1970; Lee and Kaltsikes, 1973; Yadav and Murty, 1982), barley (Tolbert et al., 1979; Verma and Gulati, 1982; Prasad and Singh, 1990; Abebe and Bjornstad, 1996), maize (Prasad and Singh, 1990; Alika et al., 1993), rice (Sinha et al., 1991), millet (Dhagat and Singh, 1983), oats (Rezai and Frey, 1990), triticale (Kamboj and Mani, 1983), faba bean (Katiyar and Singh, 1990), pigeon pea (Murty and Dorairaj, 1990),

lablab (Kumari and Chandrasekharan, 1991) and mustard (Anand and Rawat, 1984; Alemayehu and Becker, 2002). The findings are in support of the present study that principal component and cluster analyses can disclose complex relationships between populations of diverse origin in a more understandable way. These multivariate analyses might also be effective in indicating high yielding accessions in different clusters which could then be usefully intercrossed.

The intraspecific variability was evident for all three species. Within species, accessions differed in relation to various characters, which is the reason why they were classified in distinct groups in the cluster analysis with morphological and agronomic data. Differences between species was evidenced principally in relation to agronomic characters, with the differentiation of groups occuring mostly due to tuber and seed production, which was much greater for accessions of *P. erosus* than for those of the remaining two species.

Investigations of the genetic diversity in germplasm are frequently done by analysing morphological and agronomic traits with principal component and cluster analyses. The feasibility of these methods to describe comprehensively the diversity in germplasm has been demonstrated for many crops (Gaur et al., 1978; Vanderborght and Depiereux, 1987; Sauza and Sorells, 1991). Both the principal component analysis and the cluster dendrogram show a large diversity of the accessions. There is no clear relationship between genetic diversity and geographic origin.

In total 71 morpho-agronomic characters were used in the present study. This number of traits used is high and the recording is time consuming and demands much labor. In order to reduce the number of characters to be evaluated in studies of this nature, a character discard can be performed considering the large quantity of characters significantly correlated among themselves, both for morphological and agronomic data. For character discard, a criterion was proposed by Jolliffe (1972, 1973) and was also applied by Santos et al. (1995), Strapasson (1997), Daher et al. (1997) and Veasey et al. (2001). The discard of redundant characters

reduces manual work, time and cost (Cruz and Regazzi, 1994). Santos et al. (1995) reduced the list of characters initially evaluated in pigeon pea (*Cajanus cajan*) from 20 to only seven characters. Daher et al. (1997) selected eight characters from a total of 22, as the most important for determining genetic divergence between accessions of elephant grass. Heering et al. (1996) reduced from 18 to 14 the morphological characters evaluated in *Sesbania sesban*, based on significant correlations between characters. Veasey et al. (2001) reduced from 26 to 8 the characters used for the discrimination of *Sesbania spp* accessions, using the criterion of Jolliffe (1972, 1973). There are different methods that might be used for discarding characters, and character correlations should not be considered solely, but in conjunction with other methodologies. In the present study, no direct character discard was done. But traits with no correlation with the first five principal components could be discarded. It is the case of traits like start of climbing (SC), number of leaves (LN) and tuber shape (TS). However the character discard might be helpful in describing the inter- and intraspecific genetic variability in yam bean.

In conclusion, the results have generally established that there exists a large amount of genetic diversity among the 34 accessions in all traits considered. The species were clearly separated. The Chuin types were clearly separate from TC118. The variation within the species was in general the same for the three species (*P. ahipa*, *P. erosus* and *P. tuberosus*). Interestingly, the variation within *P. erosus* is not greater than within *P. ahipa* and the Chuin types of *P. tuberosus*.

3.5 Summary

Thirty four accessions of *Pachyrhizus spp.* were investigated, belonging to three species (*P. ahipa*, *P. erosus* and *P. tuberosus*), to characterize the accessions based on morphological and agronomic data using multivariate methods. The accessions from diverse ecological regions were tested at two locations in Benin. In total 71 morpho-agronomic characters were measured. Principal component analysis indicated that variance accumulated by the first two components for morphological and agronomic data accounted for 62,1% of the total variation. Three large groups of accessions were formed in the principal component analysis as well as in the cluster analysis. The groups consist of *P. ahipa*, *P. erosus* and *P. tuberosus* accessions respectively. Accession TC118 of *P. tuberosus* (Ashipa cultivar group) was classified in an isolated group (cluster analysis) due probably to its low tuber fresh matter yield, tuber dry matter content and seed yield. Geographic isolation of genes was not observed. Accessions of *P. ahipa* were found to be earlier in maturity than all other ones. Many accessions of *P. erosus* have been detected as high yielding types and *P. tuberosus*, especially accessions from the Chuin cultivar group showed a high dry matter content.

4. Genetic diversity in yam bean (*Pachyrhizus spp.*) germplasm revealed by Near Infrared Reflectance Spectroscopy

4.1. Introduction

Gene banks were initially established to meet the demands of plant breeders. The classical gene bank, the ex situ collection, is a seed collection kept under physical conditions that secure long-term storage, without loss of viability (Hill et al., 1998). For most species there is a large number of accessions, which are kept in gene banks, e.g. for wheat there are more than 400000 accessions in gene banks. But only a small part of this gene bank material is sufficiently evaluated, and the breeder can not find what he urgently needs for his breeding goals.

Moreover, in a large breeding program it is necessary to screen thousands of early generation material to determine which lines have the necessary agronomic, yield and quality traits to make successful new varieties. While it is possible to screen lines for some agronomic traits quickly in the field, quality analysis is often time consuming and expensive (Oatway and Helm, 1998). In addition, traditional methods of determining quality involve the use of hazardous chemicals, and destruction of the seed. NIRS can provide rapid, non-destructive analysis of whole seed samples, using a relatively small sample size. This allows the plant breeders to screen thousands of early generation lines for quality characteristics in a short time period.

Until now, NIRS has been used to determine the chemical composition of materials. The NIR spectral region, in various spectroscopic forms (diffuse reflectance, transmission, etc.) over the last decade has been used increasingly to determine the composition and quality of many products and to monitor the progress of various biological or chemical processes (Kemeny, 1992). With solids, NIRS in the reflectance mode has been used extensively to determine the composition and / or quality of materials such as hays (Marten et al., 1989), silages

(Reeves et al., 1991), grains (Tkachuk, 1987) and food products (Osborne and Fearn, 1986). In the pharmaceutical industry, NIRS has also being used to monitor the identity of the raw materials at loading docks (Ciurczak, 1992). Transmission NIRS has been used extensively to monitor biological processes, such as fermentations and chemical reactions (Kemeny, 1992). The successful application of NIRS to problems in such diverse areas is due to the nature of the absorptions in the spectral region, and the variety of instrumentation available (Kemeny, 1992). No use of NIRS for studying the genetic diversity of plant germplasm has been reported, except the study on the classification and comparison of *Gliricidia* provenances (Lister et al., 2000).

The main objective of the investigations presented in this chapter was to determine whether NIRS-spectra could be used to correctly assess the genetic diversity of yam bean (*Pachyrhizus* Rich. ex DC.) germplasm.

4.2 Material and Methods

Plant material and field plan

The seeds used for NIRS scanning were obtained from the accessions shown in Table 2.1 (see page 20). A total of 34 accessions representing better agronomic types from diverse ecogeographical backgrounds were used for the present study. The accessions consist of 14 *Pachyrhizus ahipa* lines, 14 *P. erosus* accessions and 6 *P. tuberosus* accessions. The *P. ahipa* material was selected from single plant progenies out of 13 accessions. At least one genotype was selected out of each accession. In *P. ahipa*, genotypes were designated by accession and progeny line number respectively. From AC214, two lines (AC214-109 and AC214-110) were selected. No selection was carried out for the *P. erosus* and the *P. tuberosus* material. The material analysed was identical with the material used in Chapter 2 (Table 2.1, page 20).

The germplasm was grown in 2001/2002 at the “Centre Songhai“ station in Porto-Novo and at the experimental station of INRAB (Institut National des Recherches Agricoles du Bénin) in Niaouli. The soil was well drained at both stations and is sandy red loam. The experiments were carried out between June 2001 and January 2002.

Experimental design was a completely randomised block with two replications at both locations. Each plot consisted of 4 rows of 6 plants each and a plot measured 1.25 m by 2.25 m. The distance between plots was 1 m . Two rows were spaced 0.75 m apart and the distance between plants within a row was 0.25 m. Irrigation was done at the station „Centre Songhai“ in Porto-Novo, during a period comprising the dry month August. At Niaouli, no irrigation was applied. Weeds were removed every two weeks. No fertiliser or pesticide was applied.

NIRS Scanning

The seed samples were scanned using a monochromator NIRSystems model 6500, equipped with a transport module. Samples were scanned in a large natural product cell with a removable back. Six seed samples from the first replication of each location were used, so that 12 seeds of each accession and the two sides of each seed were scanned. The reflectance spectrum of 400 – 2500 nm with a 2 nm resolution was recorded. The spectrum for each sample was collected and stored on a PC interfaced to the NIRS instrument using the software ISI, version 3.10 (Infrasofi International, Port Matilda, PA, USA). The NIRS instrument utilizes two different detectors for measuring the reflectance of the samples, one for the region from 400 to 1100 nm and another one for the region from 1100 to 2500 nm. However, the absorption bands for the region below 1100 nm are very weak and the inclusion of this region for quantitative measurements is not generally recommended (Velasco and Grüneberg, pers. comm.). In consequence, for the analyses of the results, only the spectral data from 1100 to 2500 nm were used.

The original spectra, expressed as the log(1/R) (R=reflectance), were transformed using the utilities of ISI software into their corresponding second derivative spectra. Second derivative was calculated from the log(1/R) spectra at gaps of 5 data points (10 nm) and a smoothing over segments of 5 data points. In ISI software, this calculation is expressed as (2,5,5,1). Additionally, a combination of two spectral corrections, SNV+De-trend (Barnes et al., 1989), was applied to the spectra.

Statistical Analysis

Multivariate statistical techniques, namely PCA and cluster analysis, were used to examine the inter- and intraspecific differences between the species and accessions. Principal components analysis and cluster analysis of the data from the spectra were done using SAS software version 6.12 (SAS, Cary, NC, USA 1997).

All the transformed NIRS spectra from each of the 34 accessions were averaged using ISI software and exported from this software into an ASCII format file. This file contained 34 average spectra, each spectrum consisting of a total of 700 variables, i.e. transformed spectral information from 1100 to 2500 nm measured every 2 nm. The ASCII format file was used for statistical analysis using the package SAS.

One of the main features of NIRS is the multicolinearity, i.e. the different variables (spectral information at each wavelength) are highly intercorrelated (Velasco and Grüneberg, pers. comm.). The principal component analysis was carried out by SAS PROC PRINCOM to determine the principal components, corresponding eigenvalues and proportions of eigenvalues as well as the scores of the principal components. The spatial relationships of accessions were presented by plotting the scores of the first and second principal components.

The cluster analysis was carried out by SAS PROC CLUSTER for hierarchically formed clusters. Therefore the variables were standardized by their mean value

and standard deviation (z = (x - $\bar{x}$) / s) using the STD option. Distances between objects and clusters, respectively were calculated by the Euclidian Distance and agregated by the unweighted average linkage method using the option AVE. A cluster dendrogram was plotted using the SAS-Macro DENDRO (Nicholson, 1995).

4.3. Results

On the scale of the log(1/R) spectra (R is the reflexion of the light), NIR spectra are very difficult to interpret and chemical differences difficult to detect, largely due to particle size and pathlength. Application of transformation of the spectra results in reducing these effects and enhances underlying chemical differences.

For the principal components analysis, the first 10 principal components accounted for almost 94% variability in the sample population, with 36.78, 22.43, 13.39, 7.67, 3.95, 3.50 and 2.57% of the variation associated with the first seven components, respectively (Table 4.1). The pair-wise plot of the first two components is presented in Figure 4.1. No clear groupings were observed, but some accessions were found at extreme positions: 4 accessions of *P. erosus* in the lower quadrants and 3 accessions of *P. erosus* and one of *P. ahipa* show separation in the upper quadrants. All accessions of *P. ahipa* occupied a position in the figure, which designates positive values of the first principal component. Apart from TC118, all accessions of *P. tuberosus* showed negative values of the first principal component. No grouping according to the geographical origin were observed.

Table 4.1. Results of Principal Component Analysis from the spectral data, Eigenvalues of the Correlation Matrix

	Eigenvalue	Proportion	Cumulative
PC1	375.848	0.367	0.367
PC2	229.220	0.224	0.592
PC3	136.878	0.133	0.726
PC4	78.378	0.076	0.802
PC5	40.378	0.039	0.842
PC6	35.788	0.035	0.877
PC7	26.242	0.025	0.902
PC8	16.489	0.016	0.919
PC9	12.553	0.012	0.931
PC10	8.682	0.008	0.939

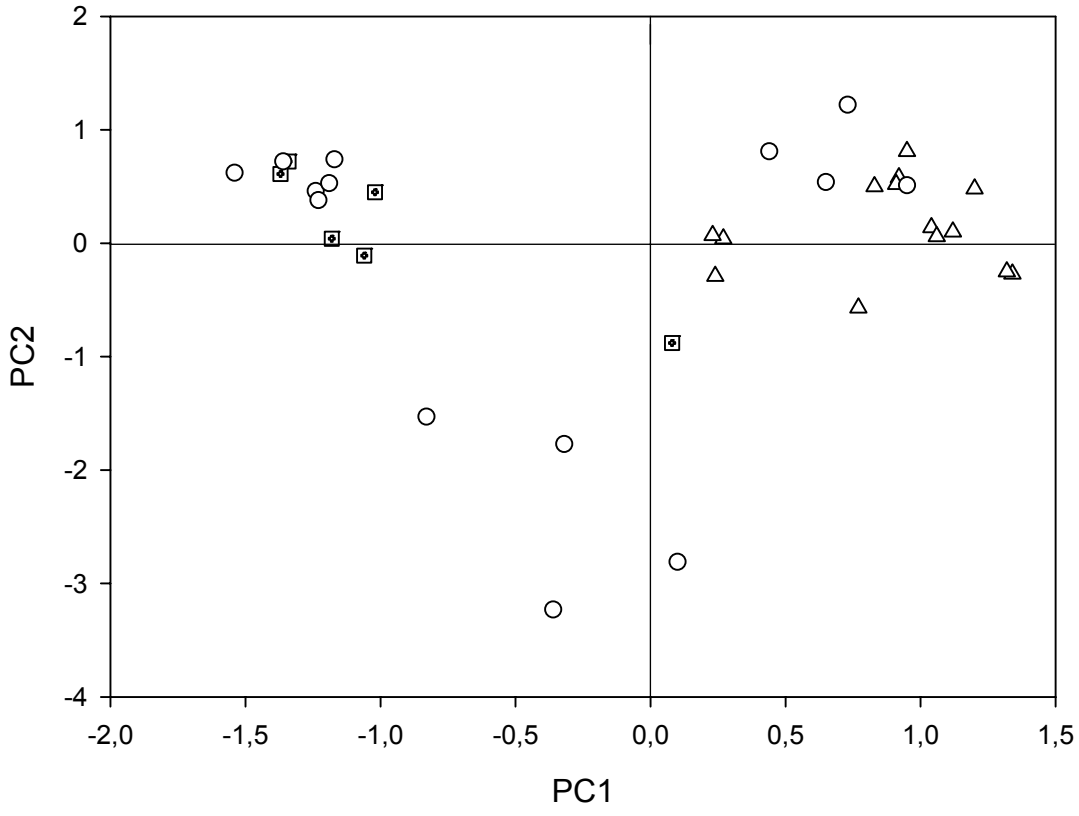

Figure 4.1. Plot of the first and second component scores for the spectral data from 34 accessions of yam bean (*Pachyrhizus spp.*). AC = *Pachyrhizus ahipa*, EC = *Pachyrhizus erosus*, TC = *Pachyrhizus tuberosus*.

TC AC EC

Cluster analysis groups samples with multidimensional information into disjoint set which may correspond to defining features of the samples. Diagramatical output from cluster analysis for the 34 accessions is presented as a dendrogram which indicates the distance at which the various groups are formed and join together (Figure 4.2). Three main groups were evident as follows: (EC042, EC040, EC033, CC354), (EC104, EC041, EC032, ECKEW, EC533, EC557, EC550, EC114, EC204, EC253, EC006) and (CC355, CC353, AC213-92, AC202-27, CC362, CC361, AC205-68h, AC208-72h, AC214-110, AC215-129, AC209-73, AC216-139, AC214-109, AC203-43, AC525-170, AC201-19, AC524-164,AC102-153). TC118 (Ashipa cultivar group of *P. tuberosus*) is isolated from the previous groups. In each group, many subgroups can be observed.

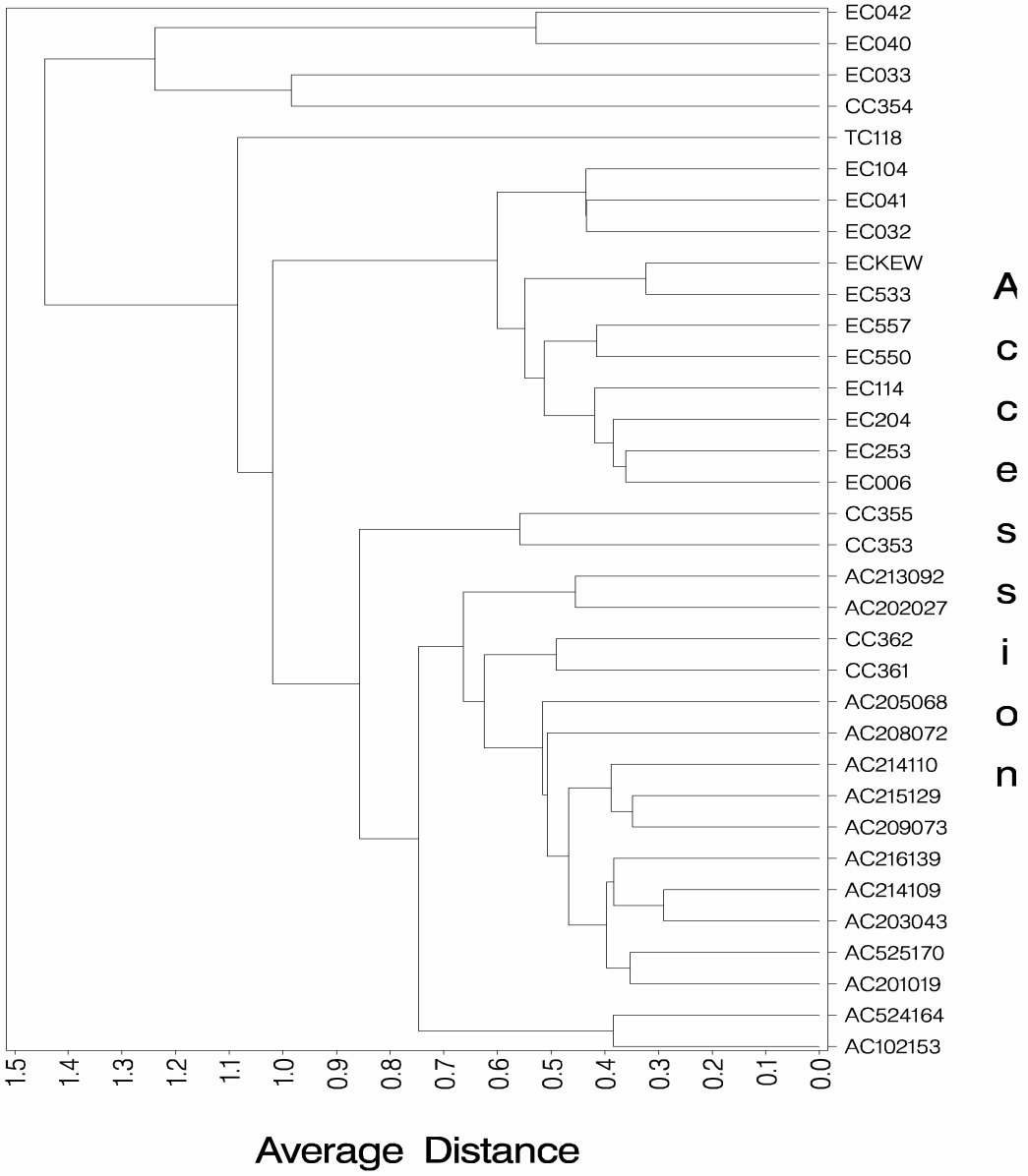

Figure 4.2. Cluster analysis on basis of Near Infrared Reflectance Spectroscopy (NIRS) spectra of seeds from a two locations field trial in Benin / West Africa; TC and CC = the Amazonian yam bean (*Pachyrhizus tuberosus*) CC = high dry matter Chuin types, AC = the Andean yam bean (*P. ahipa*) and EC = the Mexican yam bean (*P. erosus*).

4.4. Discussion

The present report describes a study to compare and classify 34 accessions of *Pachyrhizus spp.* on the basis of the NIRS. PCA and cluster analysis of the spectral data were used to obtain a general overview and provide graphical presentation of the populations and to show the interrelationships between accessions. PCA was used to simplify the data by reducing the number of variables into a smaller number of orthogonal variables which are linear combinations of the original wavelength variables and maximise the variation within them, thereby displaying most of the original variability in a smaller number of dimensions. Graphical presentation of the pair-wise components allows the natural grouping of the samples to be observed, indicating the similarity between accessions and allowing different groups of accessions to be identified. In principal components derived from log(1/R) data (R is the reflexion of the light), the first component accounts for a large amount of variation which is mainly due to physical effects. The application of spectral transformations reduced these effects and the first principal component will no longer be associated with particle size effects, but with chemical information (Barnes et al., 1989; Lister et al., 2000). There is no clear grouping of accessions according to the species. TC118 emerged in the cluster analysis between the accessions of *P. erosus* and CC361 and CC362 emerged between the accessions of *P. ahipa*. This remark is in the first point of view misunderstanding, but can be explained by the fact, that NIR spectra depend upon chemical composition of the samples and it is possible that seeds of accessions from different species have similar composition.

Results from field trials have shown that a large amount of genetic variation exists for P. ahipa, P. erosus and P. tuberosus in agronomic and morphological traits (Ørting et al., 1996; Nielsen et al., 1999, 2000). Significant differences between accessions have been observed for many traits including tuber yield, quality and seed traits (Nielsen et al., 1999, 2000). In addition, evidence might be supportive of the genetic variability in the content of anti-nutritive factors (rotenone) in the seeds of the species (Santos et al., 1996). To determine the level, structure and origin of

genetic variation within and between species, some molecular approaches have been used (Estrella et al., 1998). For example, random amplified polymorphic DNA (RAPD) markers were used to investigate genetic variation between and within populations of *P. erosus*, *P. tuberosus*, *P. ferrugineus* and *P. panamensis* (Estrella et al., 1998). Extensive genetic variability was detected between species and the above technique allowed the genetic variation within single species to be partitioned and facilitated greater discrimination. But the problem with morphological as well as molecular markers is that they are laborious and demand more time. NIRS has the potential to be used as an effective screening tool on the basis of spectral information, to classify samples and to identify accessions with specific traits which then can be related to agronomic information. The more traditional use of NIRS is to determine the concentration of chemical or quality components which requires the development of appropriate calibration models. This approach with qualitative analysis using multivariate techniques would provide a valuable, rapid and non-destructive characterisation of many crops (Lister et al., 2000) such as yam bean (*Pachyrhizus spp.*). However, in the present study the results were not consistent with those obtained from morpho-agronomic characters and classical taxonomy.

According to the results obtained in the present investigations, it can be concluded that other plant materials (ground seeds, tubers and leaves) could be helpful in assessing the genetic diversity in yam bean by NIRS.

4.5. Summary

There is an ever-increasing need to identify new rapid methods to assess the genetic diversity of many crops. Near infrared reflectance spectroscopy (NIRS) has the potential to aid the evaluation of crops and in this study was employed to compare and analyse the patterns of variation of three different species of yam bean (*Pachyrhizus spp.*). Multivariate statistical techniques, including PCA and cluster analysis, were used to compare the seed samples of 34 accessions of yam bean, which were grown at two sites in Benin. Cluster analysis showed

approximately clear groups within the species. Also no clear separation between the species was observed. NIRS combined with multivariate techniques could have the potential to analyse the genetic diversity within and between species.

5. Conclusion

The results from the evaluation of yam bean (*Pachyrhizus spp.*) under West African conditions showed highly significant differences in all the characters among the accessions, the species, the locations and the interactions genotype x location. Genotypes within *P. erosus* contain the genes for high yields and yield components. However, some genotypes within the other two species are also high yielding. Two genotypes (EC533 and ECKEW) have been demonstrated as relatively stable over locations for yield and its components. Pruning practices resulted in an increase of tuber yield in all genotypes. These results show the natural competition between tuber production and pod filling process. Genotypes of *P. tuberosus*, particularly within the Chuin cultivar group, contain genes for high tuber dry matter content, while within *P. ahipa* genes for early maturity are present.

The results from the analyse of the genetic diversity within and between yam bean species have generally established that there exists a large amount of genetic diversity among the 34 accessions in all traits considered. The species were clearly separated. The Chuin types of *P. tuberosus* were clearly separated from TC118 (Ashipa cultivar group of *P. tuberosus*). The variation within the species was in general similar for the three species (*P. ahipa*, *P. erosus* and *P. tuberosus*). Interestingly, the variation within *P. erosus* is not greater than within *P. ahipa* and the Chuin types of *P. tuberosus*.

Near Infrared Reflectance Spectroscopy (NIRS) combined with multivariate techniques could provide a valuable, rapid and non-destructive characterisation of many crops such as yam bean (*Pachyrhizus spp.*). However, in the present study the results were not consistent with those obtained from morpho-agronomic characters and classical taxonomy. According to the results obtained in the present investigations, it can be supposed that other plant materials (ground seeds, tubers and leaves) could be helpful in assessing the genetic diversity in yam bean by NIRS.

6. Summary

The yam bean (*Pachyrhizus spp.*) is a legume root crop usually known as a vegetable crop. Three cultivated species are distinguished: Amazonian yam bean (*P. tuberosus*), Mexican yam bean (*P. erosus*) and Andean yam bean (*P. ahipa*). Within *P. tuberosus* there are three distinct cultivar groups: the Ashipa and the Jíquima cultivar groups with low dry matter content of the tubers (below 20%) and the Chuin cultivar group with high dry matter content (above 30%) of the tubers. The Chuin cultivar group is used like cassava. The yam bean is nowadays cultivated in Central and South America as well as in South East Asia. Cultivation on large scale for selling is only known in Mexico, Philippines and in Indonesia. The crop is established by seeds, but only the tubers are consumed due to the high rotenone content of the seeds (about 1% of seed weight). Therefore, often the flowers are removed to increase the tuber yield. Until recently very little breeding work had been carried out on this genus, and levels of genetic diversity and interspecific relationships within the genus are not well understood. This study was conducted in Benin (West Africa) to investigate the possibility to grow the yam bean in West Africa for its introduction into the farming systems of this region as a new tuber crop rich in protein. Thirty four accessions from the three cultivated species (*P. tuberosus*, *P. erosus* and *P. ahipa*) and ecologically diverse origins were tested in a field trial at two locations in Benin during 2001/2002 and 31 agronomic and 40 morphological traits were recorded. The objectives were: (i) to evaluate the agronomic potential of the accessions and to investigate the effect of reproductive pruning (removing all flowers of the plants) on yield, (ii) to assess the genetic diversity within and between the yam bean species using multivariate statistics of morpho-agronomic traits, (iii) to examine the possibility to assess the genetic diversity with the help of Near Infrared Reflectance Spectroscopy (NIRS).

For the evaluation of the agronomic potential, 31 agronomic traits were recorded at both locations. Significant differences were observed among locations, accessions and species for most of the characters. Without reproductive pruning, the mean of tuber fresh matter yield ranged from 12.4 t ha^{-1} in *P. ahipa* to 23.4 t ha^{-1} in *P.*

erosus. Seed yield ranged from 1.5 to 2.9 t ha^{-1}, 3.5 to 4.6 t ha^{-1} and 2.6 to 2.7 t ha^{-1} for *P. tuberosus*, *P. erosus* and *P. ahipa*, respectively. Reproductive pruning resulted in an increase of 48, 91 and 61% of the tuber fresh matter yield in *P. tuberosus*, *P. erosus* and *P. ahipa*, respectively. The tuber dry matter yield increased at the same time by 58, 100 and 66% in *P. tuberosus*, *P. erosus* and *P. ahipa*, respectively. The Chuin cultivar group of *P. tuberosus* showed a high tuber dry matter content (about 30%). Accessions with genes of interest to improve earliness (early maturity) have been identified within *P. ahipa*. Accessions with high tuber yield as well as high seed yield have been identified in *P. erosus*. In this species, two accessions were found to have a high tuber yield under both environmental conditions. In all three species high genetic variation was observed for tuber fresh matter and tuber dry matter yields.

Multivariate analyses (Principal component analysis and cluster analysis) of 71 morpho-agronomic characters were used to assess the genetic diversity in the yam bean germplasm. Principal component analysis indicated that the variance accumulated by the first two components for morphological and agronomic data was 62.1%. Three large groups of accessions were formed in the principal component analysis as well as in the cluster analysis. The groups consist of *P. ahipa*, *P. erosus* and *P. tuberosus* accessions, respectively. Accession TC118 of *P. tuberosus* (Ashipa cultivar group) was classified as an isolated group due mainly to its low tuber fresh matter yield, tuber dry matter content and seed yield. No relationship between geographic origin and pattern of diversity was observed.

Near Infrared Reflectance Spectroscopy (NIRS) has the potential to analyse the physical and chemical composition of seeds and in this study was applied to compare and analyse the patterns of variation of the three different species of yam bean. Multivariate statistical techniques, including PCA and cluster analysis, were used to compare the spectral data from the seed samples of the accessions of yam bean. Cluster analysis showed often similarity between accessions within the species, but no complete separation between the species was observed.

The study showed the potential of *Pachyrhizus spp*. for its introduction into the farming systems of Benin and of West Africa in general. Accessions with high tuber yield were observed within *P. erosus* and accessions with high dry matter content of tubers and earliness in maturity were found in *P. tuberosus* and *P. ahipa*, respectively. Interspecific hybridizations are possible between these species. Multivariate analyses have shown that there is a great amount of genetic variability between the three cultivated species and also within *P. tuberosus* because accessions belonging to the Chuin cultivar group differed significantly from the accession of the Ashipa cultivar group.

6. Zusammenfassung

Die Yambohne (*Pachyrhizus spp.*) ist eine Knollenleguminose, die fast ausschließlich als Gemüsekultur genutzt wird. In der Gattung werden drei Kulturarten unterschieden: Amazonas-Yambohne (*P. tuberosus*), Mexikanische Yambohne (*P. erosus*) und die Anden-Yambohne (*P. ahipa*). Innerhalb von *P. tuberosus* werden weiterhin drei Gruppen unterschieden: die Ashipa und Jíquima Gruppen mit niedrigem Trockensubstanzgehalt der Knollen (unter 20 %) und die Chuin Gruppe mit hohem Trockensubstanzgehalt (über 30%) der Knollen. Im Gegensatz zu allen anderen Formenkreisen der Yambohne, die aufgrund ihres hohen Wassergehaltes roh konsumiert werden, werden Chuin-Typen wie Maniok genutzt. Die Yambohne wird heutzutage in Mittel- (*P. erosus*) und Südamerika (*P. tuberosus, P. ahipa*) sowie in nahezu allen Ländern Südostasien (*P. erosus*) angebaut, allerdings meist lokal auf kleinen Flächen. Ein kommerzieller Anbau auf großen Flächen ist aus Mexiko, den Philippinen und Indonesien bekannt. Diese Studie wurde in Benin / Westafrika durchgeführt um die Möglichkeit für die Einführung der Yambohne als proteinreiche Knollenfrucht zu prüfen.

Bis vor kurzem wurde die Yambohne züchterisch kaum bearbeitet. Die weite öko-geographische Verbreitung, Stickstofffixierung, leichte Kreuzbarkeit der Arten, Vermehrung über Samen und die Nutzbarkeit als proteinreiche Knollenfrucht lassen den Schluss zu, dass eine Weiterentwicklung dieser vernachlässigten Kulturpflanze sehr erfolgreich sein könnte. Für diese Hypothese sind jedoch genauere und vor allem vergleichbare agronomische Studien des Yambohnen-Genpools erforderlich mit dem Ziel das Ertragspotential, die Variation der wichtigsten agronomischen Merkmale und die genetische Diversität im Yambohnen-Genpool zu schätzen. Hierzu wurden in der vorliegenden Arbeit 34 Akzessionen der Arten *P. tuberosus*, *P. erosus* und *P. ahipa* aus den wichtigsten Anbaugebieten der Yam-Bohne an zwei Standorten in Benin / Westafrika im Jahr 2001/2002 angebaut und 31 agronomische Merkmale und 40 morphologische Merkmale wurden erfasst. In der Anbaupraxis erfolgt ein Entfernen der Blütenstände zur Steigerung der Knollenerträge, da die Samen der Yambohne

aufgrund des hohen Rotenongehalts (ca. 1% des Samengewichts) nicht genutzt werden. Aus diesem Grund erfolgte die Erfassung der wichtigsten agronomischen Merkmale: Knollenfrischmasseertrag, Knollentrockenmasseertrag, Biomasse, Trockenmasse der Knolle, Harvest-Index für den Knollenertrag sowie die Schädigung der Knolle durch Nematoden, Insekten und Pilzen in den zwei Behandlungsstufen mit ("pruning") und ohne Entfernen der Blütenstände ("no-pruning"). Ziele der vorliegenden Arbeit waren: (i) die Evaluierung des agronomischen Potentials der verschiedenen Akzessionen und die Analyse der Auswirkung des "Pruning" auf den Ertrag, (ii) die Diversitätsschätzung innerhalb und zwischen den drei Arten aufgrund agronomischer und morphologischer Merkmale mit Hilfe multivariater Statistik, (iii) die Untersuchung der Möglichkeit der Anwendung der Nahinfrarotspektroskopie (NIRS) zur Diversitätsschätzung.

Signifikante Unterschiede zwischen Standorten, Akzessionen und Arten wurden für die meisten Merkmale beobachtet. Bei "no pruning" variierte das Populationsmittel der Landrassen für den Knollenfrischmasseertrag zwischen 12,4 t ha^{-1} bei *P. ahipa* und 23,4 t ha^{-1} bei *P. erosus.* Der Samenertrag variierte zwischen 1,5 und 2,9 t ha^{-1} bei *P. tuberosus,* 3,5 und 4,6 t ha^{-1} bei *P. erosus* und zwischen 2,6 und 2,7 t ha^{-1} bei *P. ahipa.* Mit "Pruning" wurde der Knollenfrischmasseertrag um 48 % bei *P. tuberosus*, 91 % bei *P. erosus* und 61 % bei *P. ahipa* gesteigert, wobei gleichzeitig der Knollentrocken-masseertrag um 58 % bei *P. tuberosus*, 100 % bei *P. erosus* und 66 % bei *P. ahipa* zunahm. Die Chuin-Typen zeigten einen hohen Knollentrocken-massegehalt (ungefähr 30 % der Knollenfrischmasse). Akzessionen mit Frühreife wurden innerhalb von *P. ahipa* identifiziert und Akzessionen mit hohem Knollenertrag und hohem Samenertrag bei *P. erosus*, wobei in dieser Art zwei Akzessionen auffielen, die am Standort mit Trockenstress nur eine geringe Reduktion des Knollen- und Samenertrags zeigten. In allen drei Arten wurde eine hoch signifikante genetische Variation für Knollenfrischmasse-ertrag und Knollentrockenmasseertrag festgestellt.
Die Schätzung der Diversität im Yambohnen Genpool erfolgte mit multivariater Statistik (Hauptkomponenten und Clusteranalysen) anhand von 71 morpho-agronomischen Merkmalen. Die Hauptkomponenanalyse zeigte, dass mit den

ersten beiden Hauptkomponenten 62% der Gesamtvariation aller Merkmale erklärt werden konnten. Drei klar getrennte Gruppen konnten mit der Hauptkomponentenanalyse sowie der Clusteranalyse festgestellt werden, die den drei Arten *P. ahipa*, *P. erosus* und *P. tuberosus* entsprechen. Die *P. tuberosus* Akzession TC118 wurde aufgrund des niedrigen Knollenfrischmasseertrags, Knollentrockenmassegehalts und Samenertrags in einer isolierten Gruppe eingestuft. Keine Beziehung wurde zwischen geographischem Ursprung und Diversität beobachtet.

Die Nahinfrarotspektroskopie (NIRS) ist heute ein wichtiges Hilfsmittel für die Qualitätsevaluierung von Kulturarten geworden. In der vorliegenden Studie wurde NIRS verwendet um die Diversität im Genpool einer Kulturpflanzengruppe zu schätzen. Die spektralen Daten von intakten (nicht zermahlenen) Samenproben der Akzessionen wurden hierzu als Merkmale betrachtet und mit einer Hauptkomponenten- und Clusteranalysen verrechnet. Die Clusteranalyse zeigte klare Gruppen innerhalb der Arten und häufig eine Fusionierung von Akzessionen entsprechend denen der Clusteranalyse auf Basis morphologisch-agronomischer Daten. Allerdings konnte auf höheren Fusionierungsstufen lediglich *P.ersous* und *P. ahipa* von einander getrennt werden. Eine klare Trennung aller drei Arten war nicht möglich.

Die Arbeit zeigt das Potential der Yam-Bohne für ihre Einführung in Benin und West Afrika. Akzessionen mit dem höchsten Knollenfrischmasseertrag wurden innerhalb von *P. erosus* gefunden. Bei allen drei Arten traten Akzessionen mit hohem Knollentrockenmasseertrag auf und Akzessionen mit Frühreife wurden bei *P. ahipa* gefunden. Multivariate Analysen konnten zeigen, dass es eine große genetische Variabilität zwischen und innerhalb der drei Arten gibt. Die Variabilität innerhalb von *P. tuberosus* ist auch groß, da Akzessionen der Chuin Gruppe sich von der Akzession der Ashipa Gruppe unterscheiden. Da interspezifische Hybridisierungen zwischen allen drei Arten möglich sind, kann die gesamte genetische Variabilität innerhalb von *Pachyrhizus* züchterisch genutzt werden. Einige Landrassen der Yam-Bohne sind bereits jetzt für die Einführung in Benin

und in Westafrika im allgemein attraktiv. Eine züchterische Bearbeitung der Yambohne in kleinem Maßstab sollte zu deutlichen Zuchtfortschritten führen – die erforderlichen Kreuzungseltern für ein solches Programm konnten in dieser Arbeit identifiziert werden.

6. Résumé

Le dolique tubéreux (*Pachyrhizus spp.*) est une légumineuse à tubercules communément connue comme légume. Trois espèces cultivées sont distinguées dans le genre: le dolique tubéreux de l´Amazonie (*P. tuberosus*), le dolique tubéreux Mexicain (*P. erosus*) et le dolique tubéreux des Andes (*P. ahipa*). *P. tuberosus* renferme trois différent groupes de cultivar: les groupes de cultivar Ashipa et Jíquima avec une faible teneur en matière sèche des tubercules et le groupe de cultivar Chuin avec une forte contenance de matière sèche (près de 30%) des tubercules. Le groupe de cultivar dénommé Chuin est utilisé comme le manioc. De nos jours, le dolique tubéreux est cultivé aussi bien en Amérique Centrale et du Sud qu´en Asie du Sud-Est. La plante se reproduit par les graines, mais seul les tubercules sont consommés en raison de la teneur élevée des graines en roténone (1% du poids des graines). Jusqu´à une période récente, très peu de travaux ont été réalisés sur l´amélioration variétale au sein de ce genre *Pachyrhizus*. Pour espérer des résultats encourageants, il est cependant nécessaire que la diversité génétique et les relations interspécifiques soient déterminées. Il est souvent discuté la possibilité de cultiver les espèces sous-exploitées en dehors de leur milieu d´origine. La présente étude a été réalisée au Bénin (Afrique de l´Ouest) afin d´analyser la possibilité de cultiver le dolique tubéreux en Afrique de l´Ouest pour son introduction dans le paysage agraire de cette région. 34 accessions provenant des trois espèces cultivées (*P. tuberosus*, *P. erosus* et *P. ahipa*) et de diverses origines écologiques ont été testées sur deux sites expérimentaux au Bénin pendant 2001/2002. Les objectifs de la présente étude étaient: (i) d´évaluer le potentiel agronomique des accessions et d´analyser l´effet de l´ablation florale sur le rendement en tubercules, (ii) d´estimer la diversité génétique au sein des espèces et entre les espèces du dolique tubéreux en utilisant les analyses en composantes principales et de clusters des caractères morpho-agronomiques, (iii) d´examiner la possibilité d´estimer la diversité génétique sur la base de NIRS (“Near Infrared Reflectance Spectroscopy“).

Pour l´évaluation du potentiel agronomique, 31 caractères agronomiques ont été mesurés au niveau des deux sites experimentaux. Des différences significatives ont été observées entre les sites expérimentaux, accessions et espèces pour la plupart des caractères. Sans ablation florale, le rendement moyen de tubercules frais a varié entre 12.4 t ha^{-1} (*P. ahipa*) et 23.4 t ha^{-1} (*P. erosus*). Le rendement en graines a varié de 1.5 à 2.9 t ha^{-1}, 3.5 à 4.6 t ha^{-1} et de 2.6 à 2.7 t ha^{-1} respectivement pour *P. tuberosus*, *P. erosus* et *P. ahipa*. L´ablation florale a résulté en un accroissement de 48, 91 et 61% du rendement de tubercules frais respectivement pour *P. tuberosus*, *P. erosus* et *P. ahipa*. Le rendement de tubercules en matière sèche a connu dans le même temps une augmentation de 58, 100 et 66% respectivement pour *P. tuberosus*, *P. erosus* et *P. ahipa*. Le groupe de cultivar Chuin au sein de *P. tuberosus* a montré une teneur élevée en matière sèche des tubercules (environ 30% du poids frais des tubercules). Des accessions avec des gènes potentiels pour améliorer la précocité (en maturité) ont été identifiées au sein de *P. ahipa*. Des accessions avec des gènes pour un rendement élevé aussi bien en tubercules qu´en semences ont été aussi identifiées.

Les analyses en composantes principales et de clusters de 71 caractères morpho-agronomiques ont été utilisées pour estimer la diversité génétique du dolique tubéreux. L´analyse en composantes principales a indiqué que la variance accumulée par les deux premières composantes principales pour les données morphologiques et agronomiques était de 62.1%. Trois larges groupes d´accessions ont été formés aussi bien par l´analyse en composantes principales que par celle de clusters. Les groupes renferment respectivement les accessions de *P. ahipa*, *P. erosus* et de *P. tuberosus*. L´accession TC118 de *P. tuberosus* (groupe de cultivars Ashipa) a été classée dans un groupe isolé lié principalement à son faible rendement en tubercules frais, à sa faible teneur des tubercules en matière sèche ainsi qu´à son faible rendement en graines. Aucune relation entre l´origine géographique des accessions et les motifs de diversité n´a été observée.

NIRS (“Near Infrared Reflectance Spectroscopy“) a le potentiel d´aider dans l´évaluation des plantes cultivées et a été utilisé dans le présent travail pour

comparer et analyser les motifs de variation de trois différentes espèces du dolique tubéreux. L´analyse en composantes principales et l´analyse de clusters ont été utilisées pour comparer les données spectrales issues d´échantillons de semences des diverses accessions du dolique tubéreux. L´analyse de clusters a permis de mettre en évidence des groupes approximativement claires au sein des espèces. Mais aucune séparation claire entre les espèces n´a été observée.

La présente étude a montré le potentiel de *Pachyrhizus spp*. Rich. ex DC. pour son introduction dans le paysage agraire du Bénin et de l´Afrique de l´Ouest en général. Des accessions avec un rendement élevé en tubercules ont été observées au sein de *P. erosus* et des accessions avec une teneur élevée en matière sèche des tubercules et la précocité (en maturité) ont été trouvées respectivement au sein de *P. tuberosus* et de *P. ahipa*. L´hybridisation interspécifique est possible entre ces espèces. L´analyse en composantes principales et l´analyse de clusters ont montré que la variabilité génétique est large entre les espèces cultivées du dolique tubéreux et au sein de celles-ci. Au sein de *P. tuberosus*, les accessions appartenant au groupe de cultivars Chuin ont été significativement différentes de la seule accession (incluse dans l´étude) du groupe de cultivars dénommé Ashipa.

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Appendix

Table A 1. Field plan at Centre Songhai, Porto-Novo, for the treatment without and with pruning

Unpruned

AC 525-170	AC 201-19	AC 215-129	AC 203-43	AC 205-68h	AC 214-110	AC 102-153	AC 208-72h	AC 213-92	AC 524-164	AC 216-139d	AC 209-73	AC 214-109
AC 102-153	AC 524-164	AC 214-109	AC 209-73	AC 525-170	AC 213-92	AC 216-139d	AC 215-129	AC 205-68h	AC 201-19	AC 214-110	AC 208-72h	AC 203-43

EC KEW	EC 114	EC 033	EC 006	EC 104	EC 040	EC 557	EC 533	EC 204	EC 253	EC 032	EC 550	EC 041	EC 042
EC 040	EC 253	EC 533	EC 041	EC 032	EC 006	EC KEW	EC 550	EC 042	EC 033	EC 114	EC 104	EC 557	EC 204

TC 118	TC 362	TC 353	TC 354	TC 361	TC 355
TC 354	TC 355	TC 362	TC 118	TC 353	TC 361

Pruned

AC 102-153	AC 201-19	AC 203-43	AC 205-68h	AC 208-72h	AC 209-73	AC 213-92	AC 214-109	AC 214-110	AC 215-129	AC 216-139d	AC 524-164	AC 525-170
AC 216-139d	AC 209-73	AC 213-92	AC 525-170	AC 102-153	AC 214-110	AC 524-164	AC 205-68h	AC 201-19	AC 209-73	AC 214-109	AC 203-43	AC 215-129

EC 006	EC 032	EC 033	EC 040	EC 041	EC 042	EC KEW	EC 104	EC 114	EC 204	EC 253	EC 533	EC 550	EC 557
EC 104	EC KEW	EC 114	EC 550	EC 006	EC 557	EC 032	EC 040	EC 533	EC 032	EC 041	EC 253	EC 033	EC 204

TC 353	TC 354	TC 355	TC 361	TC 362	TC 118
TC 361	TC 355	TC 354	TC 118	TC353	TC 362

Table A 2. Field plan at Niaouli for the treatment without and with pruning

Unpruned

AC 102-153	AC 201-19	AC 203-43	AC 205-68h	AC 208-72h	AC 209-73	AC 213-92	AC 214-109	AC 214-110	AC 215-129	AC 216-139d	AC 524-164	AC 525-170
AC 216-139d	AC 209-73	AC 213-92	AC 525-170	AC 102-153	AC 214-110	AC 524-164	AC 205-68h	AC 201-19	AC 209-73	AC 214-109	AC 203-43	AC 215-129

EC 006	EC 032	EC 033	EC 040	EC 041	EC 042	EC KEW	EC 104	EC 114	EC 204	EC 253	EC 533	EC 550	EC 557
EC 104	EC KEW	EC 114	EC 550	EC 006	EC 557	EC 032	EC 040	EC 533	EC 032	EC 041	EC 253	EC 033	EC 204

TC 353	TC 354	TC 355	TC 361	TC 362	TC 118
TC 361	TC 355	TC 354	TC 118	TC353	TC 362

Pruned

AC 525-170	AC 201-19	AC 215-129	AC 203-43	AC 205-68h	AC 214-110	AC 102-153	AC 208-72h	AC 213-92	AC 524-164	AC 216-139d	AC 209-73	AC 214-109
AC 102-153	AC 524-164	AC 214-109	AC 209-73	AC 525-170	AC 213-92	AC 216-139d	AC 215-129	AC 205-68h	AC 201-19	AC 214-110	AC 208-72h	AC 203-43

EC KEW	EC 114	EC 033	EC 006	EC 104	EC 040	EC 557	EC 533	EC 204	EC 253	EC 032	EC 550	EC 041	EC 042
EC 040	EC 253	EC 533	EC 041	EC 032	EC 006	EC KEW	EC 550	EC 042	EC 033	EC 114	EC 104	EC 557	EC 204

TC 118	TC 362	TC 353	TC 354	TC 361	TC 355
TC 354	TC 355	TC 362	TC 118	TC 353	TC 361

Table A.3 Coefficients of Correlation of different traits in *P. ahipa* without pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12	13
2	VLW I	0.583*												
3	DM I	-0.359	-0.500											
4	SEEY I	-0.044	0.474	-0.393										
5	PODY I	0.176	0.455	-0.424	0.926**									
6	TSW I	0.766**	0.179	-0.084	-0.356	-0.126								
7	TM I	0.207	0.549*	-0.693**	0.580*	0.578*	-0.304							
8	BF I	-0.234	0.212	-0.161	0.303	0.195	-0.684**	0.616*						
9	TF I	-0.209	0.287	-0.259	0.447	0.308	-0.737**	0.601*	0.874**					
10	TE I	-0.143	-0.169	0.174	-0.476	-0.511	-0.209	-0.161	0.482	0.315				
11	DSLI I	-0.269	-0.352	0.019	0.114	0.106	-0.154	-0.127	-0.014	0.004	-0.152			
12	DSLF I	-0.342	0.098	-0.239	0.189	0.020	-0.443	0.047	0.298	0.462	0.027	0.442		
13	EV I	0.267	-0.180	0.074	-0.241	-0.177	0.337	-0.240	-0.462	-0.442	-0.181	0.410	-0.157	
14	PF I	0.585*	0.257	-0.062	0.208	0.444	0.586*	-0.033	-0.287	-0.290	-0.373	-0.041	-0.169	0.173
15	SC I	0.166	0.393	-0.495	0.268	0.301	0.026	0.689**	0.252	0.140	-0.339	-0.129	-0.032	-0.249
16	PH I	-0.124	0.417	-0.302	0.735**	0.706**	-0.368	0.551*	0.476	0.578*	-0.163	0.114	0.199	-0.495
17	PT I	0.225	0.534*	-0.682**	0.536*	0.561*	-0.061	0.830**	0.389	0.363	-0.337	-0.105	0.101	-0.341
18	PN I	-0.192	0.301	-0.210	0.879**	0.824**	-0.549*	0.531	0.556*	0.686**	-0.176	0.240	0.260	-0.365
19	SNP I	-0.121	0.202	-0.272	0.705**	0.681**	-0.277	0.410	0.361	0.454	-0.342	0.336	0.355	-0.412
20	DTN I	-0.417	-0.280	0.446	-0.360	-0.540*	-0.298	-0.526	-0.067	-0.005	0.464	-0.256	0.082	-0.111
21	DTI I	0.492	0.144	0.137	-0.405	-0.314	0.454	0.012	-0.163	-0.343	0.015	-0.712**	-0.587*	0.079
22	NTP I	0.649*	0.449	-0.262	-0.325	-0.167	0.466	0.323	0.212	-0.015	0.320	-0.555*	-0.329	-0.169
23	%N I	-0.371	-0.370	0.678**	-0.466	-0.495	-0.006	-0.583*	-0.153	-0.381	0.286	0.083	-0.083	0.207
24	%C I	0.141	0.218	-0.593*	0.401	0.324	-0.028	0.243	-0.077	0.091	-0.114	0.101	0.114	0.130
25	TDMY I	0.971**	0.558*	-0.166	-0.116	0.095	0.749**	0.116	-0.204	-0.205	-0.110	-0.301	-0.345	0.242
26	BIOM I	0.813**	0.831**	-0.442	0.503	0.651*	0.425	0.499	0.037	0.112	-0.349	-0.230	-0.159	0.002
27	HIT I	0.732**	0.102	0.163	-0.642*	-0.456	0.705**	-0.261	-0.342	-0.367	0.211	-0.382	-0.483	0.312
28	HIS I	-0.735**	-0.302	0.027	0.620*	0.441	-0.692**	0.137	0.250	0.307	-0.286	0.481	0.353	-0.127
29	HITOT I	0.608*	-0.118	0.329	-0.558*	-0.397	0.602*	-0.345	-0.379	-0.368	0.101	-0.220	-0.535*	0.449
30	SHE I	0.358	0.376	-0.396	0.733**	0.936**	0.104	0.500	0.068	0.138	-0.476	0.084	-0.140	-0.094
31	PRO I	-0.371	-0.370	0.678**	-0.466	-0.495	-0.006	-0.583*	-0.153	-0.381	0.286	0.083	-0.083	0.207
32	C/N I	0.391	0.316	-0.669**	0.403	0.429	0.053	0.474	0.058	0.312	-0.251	-0.072	0.093	-0.131
		TUBY	VLW	DM	SEEY	PODY	TSW	TM	BF	TF	TE	DSLI	DSLF	EV

Table A.3. Continued

		14	15	16	17	18	19	20	21	22	23	24	25	26
15 SC	I	0.145												
16 PH	I	0.108	0.467											
17 PT	I	0.237	0.920**	0.641*										
18 PN	I	0.090	0.144	0.851**	0.420									
19 SNP	I	0.224	0.219	0.726**	0.460	0.798**								
20 DTN	I	-0.584*	-0.622*	-0.450	-0.680**	-0.319	-0.569*							
21 DTI	I	0.212	0.114	-0.551*	-0.030	-0.570*	-0.481	0.025						
22 NTP	I	0.252	0.384	-0.107	0.330	-0.325	-0.193	-0.300	0.675**					
23 %N	I	-0.028	-0.132	-0.257	-0.398	-0.393	-0.512	0.337	0.025	-0.112				
24 %C	I	-0.201	-0.141	-0.025	0.070	0.174	0.002	0.174	-0.247	-0.271	-0.542*			
25 TDMY	I	0.617*	0.124	-0.156	0.149	-0.219	-0.133	-0.379	0.567*	0.674**	-0.250	-0.051		
26 BIOM	I	0.635*	0.328	0.362	0.505	0.344	0.298	-0.550*	0.224	0.445	-0.483	0.186	0.784**	
27 HIT	I	0.256	-0.163	-0.558*	-0.283	-0.640*	-0.569*	0.055	0.686**	0.615*	0.023	-0.187	0.790**	0.273
28 HIS	I	-0.197	-0.012	0.429	0.112	0.638*	0.566*	-0.045	-0.634*	-0.768**	-0.037	0.177	-0.767**	-0.328
29 HITOT	I	0.274	-0.313	-0.597*	-0.409	-0.537*	-0.479	0.056	0.626*	0.359	0.005	-0.165	0.683**	0.173
30 SHE	I	0.604*	0.291	0.584*	0.509	0.663**	0.569*	-0.635*	-0.188	0.002	-0.457	0.210	0.277	0.702**
31 PRO	I	-0.028	-0.132	-0.257	-0.398	-0.393	-0.512	0.337	0.025	-0.112	1.000**	-0.542*	-0.250	-0.483
32 C/N	I	0.012	0.024	0.124	0.293	0.297	0.373	-0.195	-0.026	0.045	-0.971**	0.677**	0.254	0.438
		PF	SC	PH	PT	PN	SNP	DTN	DTI	NTP	%N	%C	TDMY	BIOM

Table A.3. Continued

		27	28	29	30	31
28 HIS	I	-0.920**				
29 HITOT	I	0.917**	-0.688**			
30 SHE	I	-0.223	0.216	-0.194		
31 PRO	I	0.023	-0.037	0.005	-0.457	
32 C/N	I	0.036	-0.002	0.065	0.395	-0.971**
		HIT	HIS	HITOT	SHE	PRO

Table A.4. Coefficients of Correlation of different traits in *P. erosus* without pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12	13
2	VLW I	-0.330												
3	DM I	-0.240	-0.111											
4	SEEY I	-0.245	0.610*	-0.009										
5	PODY I	-0.302	0.582*	0.000	0.985**									
6	TSW I	0.260	-0.391	-0.212	0.237	0.239								
7	TM I	-0.394	0.909**	0.064	0.421	0.379	-0.472							
8	BF I	-0.386	0.889**	0.037	0.650*	0.625*	-0.377	0.867**						
9	TF I	-0.269	0.797**	0.092	0.571*	0.571*	-0.506	0.768**	0.933**					
10	TE I	-0.150	0.563*	-0.286	0.156	0.139	-0.497	0.499	0.453	0.467				
11	DSLI I	0.179	-0.711**	0.084	-0.844**	-0.798**	-0.199	-0.575*	-0.678**	-0.566*	-0.282			
12	DSLF I	-0.159	0.532	-0.014	0.504	0.427	0.125	0.413	0.403	0.158	0.038	-0.631*		
13	EV I	-0.159	0.235	-0.221	0.572*	0.529	0.500	0.046	0.132	-0.057	-0.047	-0.670**	0.611*	
14	PF I	-0.042	0.794**	0.030	0.113	0.053	-0.568*	0.819**	0.647*	0.572*	0.546*	-0.351	0.429	-0.094
15	SC I	0.248	-0.548*	-0.013	-0.618*	-0.589*	0.028	-0.456	-0.623*	-0.560*	-0.007	0.654*	-0.529	-0.526
16	PH I	-0.404	0.680**	0.195	0.654*	0.648*	-0.441	0.592*	0.767**	0.832**	0.201	-0.558*	0.239	0.168
17	PT I	-0.115	0.650*	-0.098	0.584*	0.577*	-0.386	0.484	0.695**	0.748**	0.122	-0.399	0.309	0.104
18	PN I	-0.148	0.596*	-0.123	0.841**	0.848**	-0.039	0.379	0.605*	0.606*	0.307	-0.562*	0.316	0.368
19	SNP I	0.700**	-0.307	-0.340	-0.156	-0.260	0.196	-0.413	-0.324	-0.291	-0.092	0.073	-0.087	0.206
20	DTN I	0.030	-0.253	0.281	0.052	0.050	-0.077	-0.267	-0.183	-0.033	-0.356	0.109	-0.013	-0.219
21	DTI I	0.344	-0.713**	0.232	-0.564*	-0.538*	0.207	-0.579*	-0.715**	-0.584*	-0.546*	0.470	-0.220	-0.294
22	NTP I	0.077	0.552*	-0.165	0.076	-0.017	-0.439	0.525	0.294	0.204	0.604*	-0.266	0.566*	0.137
23	%N I	-0.529	0.516	-0.183	0.588*	0.609*	0.103	0.373	0.567*	0.413	0.134	-0.534*	0.328	0.312
24	%C I	-0.068	0.046	0.405	-0.196	-0.228	-0.475	0.227	0.206	0.286	-0.148	0.265	-0.403	-0.435
25	TDMY I	0.983**	-0.359	-0.081	-0.286	-0.348	0.212	-0.385	-0.417	-0.289	-0.191	0.204	-0.160	-0.200
26	BIOM I	-0.041	0.882**	-0.108	0.827**	0.792**	-0.088	0.707**	0.803**	0.755**	0.407	-0.836**	0.539*	0.361
27	HIT I	0.915**	-0.556*	-0.111	-0.565*	-0.619*	0.198	-0.520	-0.622*	-0.518	-0.264	0.432	-0.223	-0.260
28	HIS I	-0.542*	-0.316	0.187	0.331	0.363	0.426	-0.308	-0.129	-0.208	-0.287	-0.078	-0.017	0.359
29	HITOT I	0.860**	-0.711**	-0.069	-0.532	-0.583*	0.344	-0.669**	-0.730**	-0.637*	-0.378	0.458	-0.253	-0.185
30	SHE I	-0.339	0.549*	0.006	0.954**	0.992**	0.236	0.341	0.594*	0.560*	0.124	-0.749**	0.362	0.486
31	PRO I	-0.529	0.516	-0.183	0.588*	0.609*	0.103	0.373	0.567*	0.413	0.134	-0.534*	0.328	0.312
32	C/N I	0.652*	-0.542*	0.151	-0.598*	-0.636*	-0.033	-0.420	-0.611*	-0.470	-0.233	0.539*	-0.315	-0.301
		TUBY	VLW	DM	SEEY	PODY	TSW	TM	BF	TF	TE	DSLI	DSLF	EV

Table A.4. Continued

		14	15	16	17	18	19	20	21	22	23	24	25	26
15 SC	I	-0.323												
16 PH	I	0.359	-0.728**											
17 PT	I	0.366	-0.644*	0.832**										
18 PN	I	0.147	-0.478	0.626*	0.748**									
19 SNP	I	-0.172	0.128	-0.212	-0.063	-0.117								
20 DTN	I	-0.290	-0.139	0.294	0.194	-0.069	-0.027							
21 DTI	I	-0.454	0.369	-0.510	-0.497	-0.629*	0.032	0.487						
22 NTP	I	0.715**	-0.166	0.116	0.218	0.215	0.054	-0.152	-0.234					
23 %N	I	0.075	-0.188	0.393	0.336	0.396	-0.247	-0.168	-0.589*	-0.166				
24 %C	I	0.206	0.006	0.348	0.211	-0.146	0.125	0.051	-0.220	-0.223	-0.035			
25 TDMY	I	-0.019	0.260	-0.391	-0.165	-0.218	0.668**	0.101	0.420	0.090	-0.607*	0.000		
26 BIOM	I	0.591*	-0.595*	0.673**	0.696**	0.773**	-0.114	-0.121	-0.632*	0.420	0.460	-0.080	-0.079	
27 HIT	I	-0.117	0.396	-0.607*	-0.376	-0.479	0.641*	0.076	0.593*	0.063	-0.721**	-0.043	0.937**	-0.378
28 HIS	I	-0.686**	-0.111	0.075	-0.164	0.132	-0.218	0.274	0.002	-0.492	0.289	-0.161	-0.539*	-0.237
29 HITOT	I	-0.331	0.408	-0.654*	-0.466	-0.495	0.649*	0.165	0.661*	-0.073	-0.718**	-0.095	0.885**	-0.489
30 SHE	I	0.008	-0.556*	0.631*	0.560*	0.836**	-0.332	0.048	-0.508	-0.086	0.613*	-0.247	-0.388	0.751**
31 PRO	I	0.075	-0.188	0.393	0.336	0.396	-0.247	-0.168	-0.589*	-0.166	1.000**	-0.035	-0.607*	0.460
32 C/N	I	-0.074	0.220	-0.420	-0.330	-0.441	0.395	0.185	0.584*	0.128	-0.972**	0.117	0.728**	-0.449
		PF	SC	PH	PT	PN	SNP	DTN	DTI	NTP	%N	%C	TDMY	BIOM

Table A.4. Continued

		27	28	29	30	31
28 HIS	I	-0.498				
29 HITOT	I	0.967**	-0.262			
30 SHE	I	-0.647*	0.380	-0.609*		
31 PRO	I	-0.721**	0.289	-0.718**	0.613*	
32 C/N	I	0.823**	-0.353	0.812**	-0.652*	-0.972**
		HIT	HIS	HITOT	SHE	PRO

Table A.5. Coefficients of Correlation of different traits in *P. tuberosus* without pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12	13
2	VLW I	-0.364												
3	DM I	0.062	-0.619											
4	SEEY I	0.908*	-0.528	0.124										
5	PODY I	0.958**	-0.317	-0.065	0.962**									
6	TSW I	-0.534	0.936**	-0.600	-0.558	-0.413								
7	TM I	-0.509	0.973**	-0.674	-0.647	-0.456	0.950**							
8	BF I	-0.934**	0.418	-0.323	-0.907*	-0.899*	0.528	0.585						
9	TF I	-0.802	0.790	-0.300	-0.856*	-0.774	0.867*	0.862*	0.739					
10	TE I	0.711	0.010	-0.288	0.589	0.723	-0.206	-0.134	-0.520	-0.583				
11	DSLI I	0.350	-0.877*	0.584	0.461	0.296	-0.896*	-0.898*	-0.353	-0.814*	0.294			
12	DSLF I	-0.180	-0.522	0.117	0.090	-0.098	-0.253	-0.364	0.107	-0.094	-0.625	0.118		
13	EV I	-0.547	0.935**	-0.705	-0.614	-0.449	0.975**	0.982**	0.604	0.870*	-0.213	-0.916*	-0.209	
14	PF I	-0.371	0.979**	-0.690	-0.539	-0.328	0.931**	0.985**	0.449	0.807	-0.075	-0.938**	-0.380	0.962**
15	SC I	-0.514	-0.077	0.756	-0.534	-0.650	-0.047	-0.071	0.264	0.368	-0.623	0.086	-0.024	-0.121
16	PH I	0.093	0.821*	-0.680	-0.125	0.109	0.708	0.775	0.004	0.485	0.140	-0.878*	-0.348	0.749
17	PT I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
18	PN I	0.586	-0.852*	0.371	0.817*	0.652	-0.775	-0.892*	-0.617	-0.900*	0.347	0.815*	0.351	-0.828*
19	SNP I	-0.007	-0.442	0.360	0.240	0.057	-0.194	-0.377	-0.209	-0.065	-0.636	0.001	0.853*	-0.237
20	DTN I	0.100	-0.293	-0.541	0.187	0.181	-0.243	-0.158	0.175	-0.355	0.213	0.234	0.426	-0.090
21	DTI I	-0.277	-0.066	-0.241	-0.159	-0.180	-0.035	-0.013	0.439	-0.123	0.345	0.410	-0.181	-0.015
22	NTP I	0.233	-0.159	0.365	-0.099	-0.050	-0.401	-0.176	-0.203	-0.108	-0.081	0.085	-0.162	-0.307
23	%N I	0.119	-0.769	0.442	0.156	0.022	-0.819*	-0.726	-0.038	-0.636	0.191	0.919**	0.106	-0.768
24	%C I	-0.007	-0.593	0.104	0.112	-0.029	-0.449	-0.432	0.029	-0.233	-0.499	0.196	0.903*	-0.334
25	TDMY I	0.965**	-0.453	0.306	0.899*	0.904*	-0.605	-0.617	-0.985**	-0.805	0.607	0.435	-0.172	-0.658
26	BIOM I	0.185	0.841*	-0.557	-0.012	0.225	0.694	0.714	-0.126	0.374	0.416	-0.706	-0.670	0.658
27	HIT I	0.697	-0.870*	0.600	0.731	0.598	-0.911*	-0.920**	-0.759	-0.845*	0.151	0.698	0.334	-0.918**
28	HIS I	0.303	-0.715	0.281	0.649	0.458	-0.530	-0.725	-0.375	-0.677	0.195	0.692	0.432	-0.616
29	HITOT I	0.679	-0.909*	0.588	0.772	0.618	-0.912*	-0.955**	-0.748	-0.879*	0.171	0.751	0.377	-0.934**
30	SHE I	0.941**	-0.108	-0.229	0.868*	0.970**	-0.257	-0.255	-0.835*	-0.652	0.796	0.129	-0.260	-0.274
31	PRO I	0.119	-0.769	0.442	0.156	0.022	-0.819*	-0.726	-0.038	-0.636	0.191	0.919**	0.106	-0.768
32	C/N I	-0.206	0.814*	-0.576	-0.233	-0.089	0.879*	0.802	0.171	0.687	-0.196	-0.953**	-0.072	0.854*
		TUBY	VLW	DM	SEEY	PODY	TSW	TM	BF	TF	TE	DSLI	DSLF	EV

Table A.5. Continued

		14	15	16	17	18	19	20	21	22	23	24	25	26
15	SC	I -0.140												
16	PH	I 0.872*	-0.382											
17	PT	I 0.000	0.000	0.000										
18	PN	I -0.873*	-0.281	-0.652	0.000									
19	SNP	I -0.341	0.161	-0.208	0.000	0.328								
20	DTN	I -0.181	-0.702	-0.131	0.000	0.337	-0.039							
21	DTI	I -0.143	-0.202	-0.433	0.000	0.192	-0.561	0.479						
22	NTP	I -0.112	0.361	0.064	0.000	-0.221	-0.077	-0.225	-0.500					
23	%N	I -0.799	0.129	-0.842*	0.000	0.578	-0.167	0.381	0.540	0.214				
24	%C	I -0.422	-0.075	-0.296	0.000	0.312	0.698	0.536	-0.249	0.208	0.267			
25	TDMY	I -0.486	-0.292	-0.043	0.000	0.629	0.088	-0.098	-0.350	0.277	0.159	-0.035		
26	BIOM	I 0.796	-0.332	0.883*	0.000	-0.529	-0.445	-0.329	-0.225	-0.097	-0.761	-0.689	0.094	
27	HIT	I -0.842*	-0.011	-0.488	0.000	0.773	0.443	0.088	-0.322	0.406	0.512	0.489	0.781	-0.518
28	HIS	I -0.750	-0.242	-0.674	0.000	0.922**	0.377	0.327	0.349	-0.548	0.456	0.240	0.352	-0.522
29	HITOT	I -0.891*	-0.055	-0.559	0.000	0.860*	0.465	0.139	-0.224	0.263	0.542	0.482	0.763	-0.559
30	SHE	I -0.118	-0.712	0.312	0.000	0.464	-0.110	0.164	-0.188	-0.002	-0.100	-0.153	0.851*	0.423
31	PRO	I -0.799	0.129	-0.842*	0.000	0.578	-0.167	0.381	0.540	0.214	1.000**	0.267	0.159	-0.761
32	C/N	I 0.859*	-0.210	0.858*	0.000	-0.621	0.111	-0.242	-0.423	-0.284	-0.983**	-0.233	-0.282	0.748
		PF	SC	PH	PT	PN	SNP	DTN	DTI	NTP	%N	%C	TDMY	BIOM

Table A.5. Continued

		27	28	29	30	31
28	HIS	I 0.509				
29	HITOT	I 0.987**	0.638			
30	SHE	I 0.440	0.259	0.441		
31	PRO	I 0.512	0.456	0.542	-0.100	
32	C/N	I -0.615	-0.469	-0.635	0.046	-0.983**
		HIT	HIS	HITOT	SHE	PRO

Table A.6. Coefficients of Correlation of different traits in *P. ahipa* with pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12
2	VLW	0.590*											
3	DM	-0.127	-0.265										
4	DTN	-0.401	-0.269	0.371									
5	DTI	0.631*	0.245	0.318	0.011								
6	NTP	0.640*	0.425	0.022	-0.261	0.570*							
7	%N	-0.395	-0.321	0.492	0.487	-0.124	-0.184						
8	%C	-0.146	0.112	-0.485	0.300	-0.275	-0.300	-0.332					
9	TDMY	0.984**	0.574*	0.031	-0.368	0.681**	0.679**	-0.320	-0.256				
10	BIOM	0.960**	0.762**	-0.061	-0.373	0.613*	0.670**	-0.353	-0.163	0.968**			
11	HIT	0.581*	-0.267	0.251	-0.073	0.548*	0.338	-0.006	-0.371	0.598*	0.390		
12	C/N	0.389	0.275	-0.479	-0.338	0.152	0.123	-0.978**	0.456	0.306	0.328	0.045	
13	PROT	-0.395	-0.321	0.492	0.487	-0.124	-0.184	1.000**	-0.332	-0.320	-0.353	-0.006	-0.978**
		TUBY	VLW	DM	DTN	DTI	NTP	%N	%C	TDMY	BIOM	HIT	C/N

Table A.7. Coefficients of Correlation of different traits in *P. erosus* with pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12
2	VLW	-0.239											
3	DM	-0.269	0.013										
4	DTN	0.043	-0.168	0.254									
5	DTI	0.195	-0.680**	0.145	0.492								
6	NTP	0.102	0.511	-0.002	-0.044	-0.193							
7	%N	-0.529	0.514	-0.081	-0.147	-0.587*	-0.239						
8	%C	-0.260	0.206	0.340	0.032	-0.337	-0.238	0.245					
9	TDMY	0.975**	-0.255	-0.079	0.097	0.282	0.140	-0.610*	-0.196				
10	BIOM	0.383	0.796**	-0.037	-0.099	-0.473	0.576*	0.109	0.074	0.383			
11	HIT	0.763**	-0.735**	-0.134	0.186	0.586*	-0.047	-0.784**	-0.284	0.783**	-0.212		
12	C/N	0.630*	-0.543*	0.036	0.159	0.584*	0.198	-0.979**	-0.204	0.703**	-0.079	0.866**	
13	PROT	-0.529	0.514	-0.081	-0.147	-0.587*	-0.239	1.000**	0.245	-0.610*	0.109	-0.784**	-0.979**
		TUBY	VLW	DM	DTN	DTI	NTP	%N	%C	TDMY	BIOM	HIT	C/N

Table A.8. Coefficients of Correlation of different traits in *P. tuberosus* with pruning of reproductive parts

		1	2	3	4	5	6	7	8	9	10	11	12
2	VLW	I -0.383											
3	DM	I -0.168	-0.779										
4	DTN	I 0.129	-0.291	-0.133									
5	DTI	I -0.300	-0.277	0.211	0.645								
6	NTP	I 0.278	-0.075	-0.111	-0.031	-0.369							
7	%N	I 0.319	-0.965**	0.761	0.340	0.466	-0.127						
8	%C	I 0.047	-0.610	0.380	0.653	0.252	0.406	0.488					
9	TDMY	I 0.947**	-0.565	0.107	0.037	-0.330	0.152	0.492	0.104				
10	BIOM	I 0.089	0.878*	-0.877*	-0.330	-0.525	0.000	-0.878*	-0.676	-0.100			
11	HIT	I 0.686	-0.916*	0.545	0.251	-0.019	0.190	0.831*	0.543	0.828*	-0.624		
12	C/N	I -0.407	0.964**	-0.770	-0.188	-0.323	0.094	-0.983**	-0.389	-0.592	0.818*	-0.870*	
13	PROT	I 0.319	-0.965**	0.761	0.340	0.466	-0.127	1.000**	0.488	0.492	-0.878*	0.831*	-0.983**
		TUBY	VLW	DM	DTN	DTI	NTP	%N	%C	TDMY	BIOM	HIT	C/N

Acknowledgements

I am deeply indebted to Prof. Dr. H.C. Becker for his unlimited help, enthusiastic support and valuable advice during this study. His contribution in formulating the research work, critically reading the manuscript and many unforgotable suggestions are most gratefully acknowledged.

Acknowledgements are also due to Dr. Wolfgang J. Grüneberg for the suggestion of the study and his decisions at very difficult stages of the study.

I would like to expess my sincere gratitude to Prof. Dr. E. Pawelzik for her interest and willingness to take the role of a co-referee and for her further comments and advice. Also I wish to express my thanks to Prof. Dr. B. Märländer for his interest to be a member of the examination committee.

I would like to express my gratitude to Prof. Marten Sørensen for providing me with the *P. erosus* and *P. tuberosus* material.

I am thankful to the German Academic Exchange Service (DAAD) for the generous financial support during the entire period of my stay in Göttingen, Germany.

I owe Mr S. Ako, Mr R. Ahinon, Mr A. Koukè, Mr L. Sedagondji and his family, Prof. S. Ahouangonou and his family, Mr T. Dovonou and J. Sowanou a debt of gratitude for their helps during the implementation of the field research in Benin.

Recognition is also due to my colleagues and the research team members at the Institute of Agronomy and Plant Breeding (Georg August Universität, Göttingen) for their co-operation and interesting discussions and various help. I am particularly grateful to Prof. Dr. W. Link, Dr. Sabine von Witzke Ebrecht, Dr. Ulrike Bellin, Dr. Johannes Hippe, Dr. Antje Schierholt, Uwe Ammermann,

John Nyikako, Dr. Mahmoud Zeid, Rubens Marshalek, Agung Karuniawan, Andy Wijaya, Joe N. Dwi Sasongo, Tesfaye Baye, Dr. Bernd Horneburg, Thomas zum Felde, Stefan Abel and Adefris Teklewold.

My deepest and genuine gratitude is extended to my family in Benin who provided me with strength and for their patience and understanding during the execution of the present work.

My thanks are also extended to those who contributed in any way to this work but are not mentioned here.

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