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Genetic diversity and farmers' selection of cassava (*Manihot esculenta* Crantz) varieties on small-scale farms in Northern and Central Vietnam

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Abstract

Cassava (*Manihot esculenta* Crantz) is a tropical root crop grown for its starchy tuberous roots. In Vietnam, cassava's use as a raw product for the starch and ethanol industry led to its increasing importance. Also, Vietnam is one of the countries predicted to be most affected by climate change and cassava may be one important crop for securing agricultural production. These diverse needs highlight the necessity for a diverse gene pool for different breeding goals. The genetic diversity of cassava on farmers' fields in Northern and Central Vietnam was explored by using an interdisciplinary approach combining farmer interviews and genetic marker analysis. The study also included eight Vietnamese accessions from the cassava Germplasm Bank at CIAT, Colombia.

Cassava varieties on fifteen farms in Northern and Central Vietnam showed high genetic diversity among varieties. Vietnamese farmers cultivated clones of different varieties to a great extent, which they were able to distinguish based on different morphological traits. However, genetic variation was also detected in some cassava varieties grown in a single field. Farmers were able to conserve their varieties as clones over long periods of time. Local and improved varieties were grown in both regions. These two groups of cassava were genetically differentiated, showing the necessity of preserving local varieties for increasing the gene pool for breeding. Genetic diversity in the farmers' fields was not covered by the CIAT accessions, suggesting that local varieties should be incorporated into the germplasm bank.

This interdisciplinary study made it possible to detect the actual genetic structure and diversity within farmers' fields and it enhances the importance of understanding the farmers' influence on the genetic composition of cassava.

Keywords: cassava, genetic diversity, breeding, farmers' selection, Vietnam

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Abbreviations

CIAT	The Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture)
CIAT/Thai.....	The Centro Internacional de Agricultura Tropical/ Thailand
HARC.....	Hung Loc Agricultural Research Center
IAS.....	Institute of Agricultural Science for Southern Vietnam
IV.....	Improved variety
IITA	International Institute of Tropical Agriculture
LV.....	Local variety
NLU.....	Nong Lam University
PCR	Polymerase chain reaction
PPB.....	Participatory plant breeding
SSR.....	Simple Sequence Repeat
VAAS.....	Vietnam Academy of Agricultural Sciences
VNCP.....	Vietnam Cassava Program
VNM varieties.....	Vietnamese Accessions from germplasm collection at CIAT

Foreword

I strongly believe that issues concerning biodiversity are of multinational concern and can only be solved through knowledge across disciplines and sustainable and adapted solutions. This opinion evolved from the choice of studying *Agricultural Sciences* for my Bachelor in Vienna and the joint Master program *Environmental Sciences in Europe*, in Copenhagen and Uppsala, where I could study the natural and social demands concerning biodiversity issues. Consequently I wanted to write my thesis about a topic that follows an interdisciplinary approach and gives me the opportunity of studying farmers' management, genetic diversity, breeding and conservation in a diverse, international environment. I found exactly this in the project of genetic diversity and farmers' selection of cassava varieties on small-scale farms in Northern and Central Vietnam.



1 Introduction

1.1 General aim

The overall aim of this thesis was to investigate the genetic diversity of cassava varieties in farmers' fields combined with information provided by farmers in Northern and Central Vietnam. The hypothesis was that genetic diversity is available in farmers' fields and can provide new potential breeding material and information to breeders.

The outcome of this thesis will also give insights to conservation practitioners and specialists in future conservation of cassava. One aspect of conservation investigated in this thesis is whether the genetic diversity in farmers' fields is represented among the current Vietnamese cassava accessions in the Germplasm Bank at the International Center for Tropical Agriculture (CIAT), Cali, Colombia. Considerations in cultivation practices made by farmers potentially affecting the composition of cassava varieties were also explored. Knowledge about the above aspects will play an important role in adaptation for the increased cultivation of cassava projected in view of global climate change.

The aims were addressed by combining genetic marker analysis for genetic identification of cassava varieties grown in farmers' fields, and farmers' interviews for their classification and preference of varieties. Similar interdisciplinary approaches have been previously used in studies of the genetic diversity of cassava on small-scale farms in Peru (Salick *et al.*, 1997), Brazil (Sambatti *et al.*, 2001), Guyana (Elias *et al.*, 2000, 2001), Malawi (Mkumbira *et al.*, 2003), Uganda (Balyejusa Kizito *et al.*, 2007) and on the South Pacific Islands (Sardos *et al.*, 2008). However, the current project is the first genetic diversity study of cassava in Vietnam. In addition, we have added analyses of how the genetic diversity is structured on different geographical levels including the genetic diversity within individual farmer's fields, among farms and villages and between Northern and Central Vietnam.

1.2 Project background

This interdisciplinary project is an important contribution to a larger study about the genetic diversity in Vietnam, which was initiated by Associate Professor Anna Westerbergh and Associate Professor Per-Olof Lundquist, Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden. For Northern Vietnam the leaf samples were collected and the interviews conducted in November and December 2011 in cooperation with the Plant Resource Center in Hanoi, Vietnam. The interviews and collections in Central Vietnam were conducted in July 2013, in cooperation with the Vietnamese Academy of Forest Sciences, Hanoi, Vietnam. I then compiled and analyzed the interviews, extracted DNA from the collected leaf samples and analyzed the genotype data in collaboration with my supervisors at SLU. Collection of leaf material and farmers' interviews will also be conducted in villages in Southern Vietnam and together with the studies in Northern and Central Vietnam presented in this thesis it will improve the picture of cassavas' diversity composition for the whole country.

1.3 Cassava

Cassava is not well known outside the tropics, even though it sustains the lives of estimated 500 million people in Africa, Asia and Latin America (Cornell University, 2013), which ranks it the fifth most important food crop worldwide after maize, rice, wheat and potato (Kim *et al.*, 2010). In 2013, cassava reached a world production of 278 million tones, 57% of it in Africa, 32% in Asia and 11% in the Americas (FAO, 2013). Cassava is a starchy, tropical root and a multipurpose crop. Its tuberous roots are attractive for human nutrition, animal feed, the starch industry and as raw material for the production of ethanol for biofuel. Its leaves are also used for animal feed. Cassava is grown along the

tropics, between 30° north and 30° south of the equator, with numerous names as tapioca, mandioca in Brazil, yuca in South America and mihogo in Kenya.

Cassava uses soil nutrients and water highly efficiently. Hence, farmers using low or no inputs can still expect reasonable harvests, where most other crops would not be productive. This explains why cassava is widely grown by low-income, small-scale farmers, who are often faced with poor nutrient soils and no means of irrigation. In the face of climate change these features, in addition to its ability to grow on slopes, will enhance cassavas' importance, especially in areas with arable land shortage. Combined with cassavas' range of uses for food, in livestock feed and industrial applications as e.g. starch and dried chips, it is considered to be one of the most food secure crops of the 21st century by the FAO (Howeler *et al.*, 2013). Cassava consequently has a huge potential to improve food security and foster rural development.

1.3.1 Origin of Cassava and its distribution across the tropics

Cassava belongs to the *Euphorbiaceae* family and the genus *Manihot*, which also includes 97 wild species. Cassavas' wild relatives can be found in northern South America, Central America and Mexico. Evidence based on molecular genetic studies showed that cassava was domesticated in the southern Amazon region, Brazil (Olsen, 2004), about 9000 years ago, which ranks it one of the oldest crops in agriculture (Howeler *et al.*, 2013). European traders introduced cassava in West Africa already in the 16th century, whereas it got introduced on the East African coast 200 years later (Jones, 1959). Cassava was first promoted by the colonial powers against famine (Hillocks *et al.*, 2002). Today cassava is still produced for local consumption and its production for industrial and commercial purposes is increasing. Major cassava-producing countries in Africa today are Nigeria, Democratic Republic of Congo, Ghana, Angola Tanzania and Mozambique.

European explorers introduced cassava, from Latin America, in the late 18th and early 19th century to Asia (Hillocks *et al.*, 2002). India, Java and the Philippines were the first countries cassava arrived at. Cassava was first produced for local consumption and predominantly grown by marginal, low-input farmers as an emergency crop. It has been a cash crop for export in Asia since the second half of 20th century (Hillocks *et al.*, 2002). Major cassava-producing countries in Asia today are Indonesia, Thailand, India, China, Philippines and Vietnam.

1.3.2 Biology

Cassava is a woody perennial shrub, which can reach 1-5 m in height. Its biology is very adapted to low and no-input systems due to its efficient nutrient and water management. To take up micronutrients and phosphorus very efficiently the plant forms a symbiotic association with mycorrhiza. This enables it to grow on acidic and low-P soils and consequently increases the yield (Howeler & Sieverding, 1983). Cassava is considered to be tolerant to drought due to its mechanisms of stomata sensitivity towards atmospheric humidity, reduced leaf production and capacity of deep-water extraction. During a period of low air humidity, it rapidly closes its stomata to decrease water loss (El-Sharkawy, 1993). Under prolonged midseason water stress it also reduces leaf production until the next precipitation, while remaining reasonable photosynthesis rates. Additionally, the plant extracts water from deep-reservoirs through well-developed roots systems down to 2 m, which is another underlying potential physiological mechanism to drought tolerance (El-Sharkawy, 1993, 2006).

Cassava contains cyanogenic glycosides, linamarin and lotaustralin in all its organs except the seeds (Hillocks *et al.*, 2002). Linamarin, which is most abundant, is produced in the leaves and transported to the roots. When linamarin is hydrolysed by the enzyme linamarase the volatile poison HCN is released. Linamarase is found in all cassava tissues and released upon disruption of the tissue. The root content of cyanogenic glycosides varies and cassava varieties with a high and low content of cyanogenic glycosides in the roots are called 'bitter' and 'sweet' or 'cool', respectively. To detoxify cyanogenic glycosides and avoid cyanide intoxication careful processing of the bitter

cassava roots is required before human consumption (Onabolu *et al.*, 2002). ‘Sweet’ varieties are safe to consume without previous processing. Possible ways of processing can be juice extraction as e.g. drying, heating, fermentation or a combination. Both cassava roots and leaves are also used in animal feeding since animals can detoxify cyanide via several pathways. Ruminants can be fed directly but for monogastric animals previous processing like drying is necessary (Ravindran, 1993).

1.3.3 Harvest

During harvest, in general about ten months after planting, cassava roots are taken when the entire plant is pulled out from the soil. Cassava roots are highly perishable. They become inedible within 24-72 hours after harvest due to rapid physiological deterioration processes (Hillocks *et al.*, 2002). These processes cause many problems for storage and transportation of the root.

Another way of harvesting used in subsistence farming is when a farmer just harvests some of the tuberous roots (‘milking’) and leaves the remaining ones for later harvest. This allows the farmer to harvest when needed between six to 18 months after planting.

1.3.4 Vegetative and sexual propagation

In agricultural systems cassava is predominantly propagated through stem cuttings, which results in clonal propagation. This means that the offspring is genetically identical to the mother plant. However, sexual reproduction through seeds generated mainly by outcrossing between plants, also occurs in cassava. Bees are thought to be the major insect pollinators of cassava. Cassava is monoecious, which means that it produces separate male and female flowers on the same plant. The male flowers are on the upper part of the inflorescence, while the female flowers are located on the lower part (Alves, 2002). The male and female flowers mature at different times on the same plant, which makes selfing rare (Hillocks *et al.*, 2002). Seed crops have the benefit of genetic recombination to create new variability (Acquaah, 2007). Sexually produced cassava seeds are stored in soil seed banks and will possibly germinate in the following year (Sambatti *et al.*, 2001). The sexually reproduced seedlings may be a source of genetic variation within cassava fields if farmers incorporate seedlings into the planting population.

1.4 Agriculture in Vietnam

Vietnam is the most eastern country on the Indochina Peninsula in Southeast Asia and in 2012 it had 89 million inhabitants. About 70% of the population is considered to live in rural areas, which accounted for 15.34 million rural households in 2011 (Statistics Documentation Centre- General Statistics Office of Vietnam, 2014a). Of the total country area, 75% (26.21 million ha) is considered to be agricultural land and provides income for 57.9% of the population (Statistics Documentation Centre- General Statistics Office of Vietnam, 2014a; United Nations Statistics Division, 2014a; b; c). In 2011, 69% of farms were under a scale of 0.5 ha and 34.7% of those were smaller than 0.2 ha. However, trends are pointing towards an increase in land accumulation (General Statistics Office, 2012). As small-scale farms still dominate the agricultural sector in Vietnam, this thesis focuses on small-scale farms.

Modernization of Vietnamese agriculture started in the early 20th century in Southern Vietnam. Northern Vietnam’s agricultural modernization occurred later due to Vietnam’s separation from 1954-1975. In 1986, Vietnam launched political and economic reforms (Doi Moi) that facilitated the transition from a centrally planned economy to a socialist-oriented market economy. During this period the country showed high economic growth, in which the agricultural sector was a great part, and also the ability to rise from poverty. The increased production in agriculture depended on the use of high-yielding crops, intensified practices and increased land area for agriculture. In modern agricultural practices that use high-yielding monoculture crops, local varieties that may be more

adapted to local conditions are commonly displaced. This thesis investigates whether local varieties of cassava are still maintained and cultivated by Vietnamese farmers.

1.4.1 Cassava production in Vietnam

In Vietnam cassava experienced a decline in production in the 1980s and 90s as the economy improved and rice prices increased. This changed in the last 15 years as the utilization of cassava shifted and production increased. The utilization of cassava has shifted from an on-farm crop for food and feed for pigs and chicken towards a cash crop for the starch, chips and ethanol industries as well as towards an important export good (Kim *et al.*, 2013; Nguyen *et al.*, 2013). Vietnam was the eighth biggest producer of cassava worldwide in 2011 (Nguyen *et al.*, 2013) and produced 3.7 % of the world's production in 2012 (FAO, 2014). Since the year 2000 a significant boom in cassava production was observed due to a high market demand and improved varieties.

High market demand was driven by two major new factors, the export of cassava chips to China and cassava-based ethanol production (Kim *et al.*, 2010). Vietnam is the second largest exporter of cassava products after Thailand, while China remains the biggest importer of Vietnamese cassava (Kim *et al.*, 2010). Seventy per cent of the total cassava production in 2013 was exported, 85.6% to China for production of ethanol used for biofuel (Agency of Foreign Trade, 2014). In many provinces of Vietnam, the construction of large-scale cassava starch factories encouraged the increase of yields and production. This is a very recent development and since twenty years ago there were no such factories (Kim *et al.*, 2010). In 2013 there were 68 industrial starch processing factories with a total capacity of 8.8 million tonnes of fresh cassava roots per year (Kim *et al.*, 2013). There were 13 bio-ethanol factories encompassing a capacity of 1067.7 million litres of bio-ethanol per year (Nguyen *et al.*, 2013), supported by a new E10 policy, which requires the production of 1067 million litres of ethanol per year (Kim *et al.*, 2013). The E10 policy regulates that fuel encompasses 10% ethanol. This policy will be implemented in several regions in Vietnam in 2016 and nationwide in 2017 (TN news, 2012). These investments are predicted to create new jobs and initiate industrialization and modernization of rural areas in Vietnam.

The high increase in yields from 1.99 million tons in 2000 (Kim *et al.*, 2013) to 9.4 million tons in 2013 (Table 1) (Agency of Foreign Trade, 2014) was due to improved varieties through breeding and area expansion from 237,600 ha in 2000 (Kim *et al.*, 2013) to 560,000 ha in 2013 (Table 2) (Agency of Foreign Trade, 2014). The recent breeding successes of improved varieties might have built the main incentive for area expansion.

Table 1 Cassava Production in Vietnam and provinces of Northern Vietnam and Central Vietnam under investigation (data from *General Statistics Office Of Vietnam* (2014))

	Cassava Production (in thousand tonnes) from 1995 - 2013									
	1995	1997	1999	2001	2003	2005	2007	2009	2011	2013 Prel.
Vietnam	2211.5	2403.4	1800.5	3509.2	5308.9	6716.2	8192.8	8530.5	9897.9	9742.2
Hoà Bình province (Northern Vietnam)	-	-	-	-	-	102.1	109.9	117.2	146.6	146.3
Hà Nội province (Northern Vietnam)	-	-	-	-	-	1.8	1.8	44.2	38.1	39.0
Quảng Trị province (Central Vietnam)	31.3	37.2	29.0	27.4	67.6	121.8	170.5	163.8	171.9	176.4

Table 2 Area of planted Cassava in Vietnam and provinces of Northern Vietnam and Central Vietnam under investigation (data from *General Statistics Office Of Vietnam* (2014))

	Area of planted Cassava (in thousand hectares) from 1995 - 2013									
	1995	1997	1999	2001	2003	2005	2007	2009	2011	2013 Prel.
Vietnam	277.4	154.4	225.5	292.3	371.9	425.5	295.5	507.8	558.4	544.1
Hoà Bình province (Northern Vietnam)	-	-	-	-	-	10.7	11.4	11.0	12.6	12.2
Hà Nội province (Northern Vietnam)	-	-	-	-	-	0.2	0.2	2.5	2.1	2.1
Quảng Trị province (Central Vietnam)	4.1	4.0	3.8	3.2	5.4	7.8	9.9	9.9	10.7	11.0

1.5 Cassava breeding

In the past very little attention was given to cassava breeding worldwide in comparison to other major crops such as wheat, rice or maize. Cassava was considered inappropriate for the Green Revolution or intensification for several reasons: it is vegetatively propagated through stem cuttings, which are bulky and can easily carry and transmit pests and diseases, it has very low multiplication rate due to its vegetative propagation system rather than through seeds, very labor-intensive harvest, long growing cycles of 6-18 months and highly perishable roots (Fregene *et al.*, 1997; Howeler *et al.*, 2013). I personally believe that cassava was not attractive to private breeding companies due to its low-input system and that it is mainly grown by small-scale farmers, which would not result in high revenues.

In 1967 high priority to cassava breeding was given with the establishment of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and the International Center for Tropical Agriculture (CIAT), Cali, Colombia, which focus on cassava breeding worldwide (Hillocks *et al.*, 2002). These two institutes were major contributors to the global development of cassava breeding (Howeler, 2003). From 1975 until 2012 the area harvested increased by 58.8% and production by 137.9% (FAO, 2014) (Figure 1).

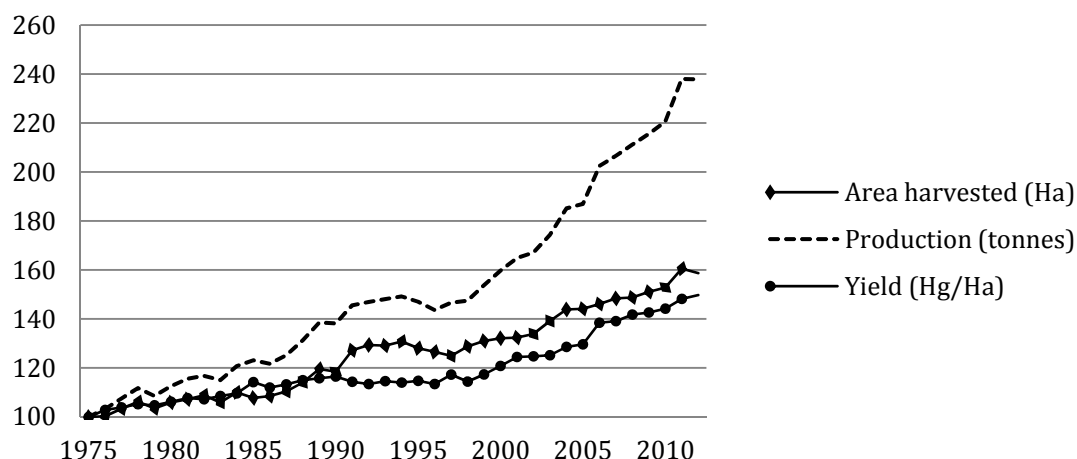


Figure 1 Growth of cassava worldwide from 1975-2012 (Index: 1975=100) (data from FAO (2014))

1.5.1 Cassava breeding in Asia

Early breeding of cassava in Asia took mainly place at the Rayong Field Crop Research Center in Thailand (Kawano, 2003). In the late 1970s the usage of large numbers of genotypes in the form of hybrid seeds from CIAT started. Before this, locally available material and only a small number of introduced clones from Java, the Virgin Island and CIAT were used. CIAT and the Department of Agriculture in Thailand started a collaborative cassava-breeding program (CIAT/Thai) in the 1980s, in which they disseminated advanced breeding material to many national programs in Asia targeting

marginal rural farmers. Major breeding goals were to increase harvest index, biomass and disease resistance. Many diseases are still undiscovered today in Asia due to those early disease resistance selections at CIAT/Thai (Kawano, 2003). Also in Vietnam, CIAT played a key role in cassava breeding.

1.5.2 Cassava breeding in Vietnam

In the 70s and 80s research on cassava in Vietnam concentrated on the maintenance and evaluation of local cultivars (Kawano, 2003). In 1989 the introduction of cassava hybrid clones from CIAT/Thai began, showing immediate improvement in yield levels (Kawano, 2003). During the 90s six high-yielding cassava varieties were introduced from Thai/CIAT and distributed in Vietnam (Table 3), namely *KM94*, *KM98-1*, *SM937-26*, *KM95*, *KM95-3* and *KM60* (Kim *et al.*, 2000, 2013; Bien *et al.*, 2007).

Table 3 Origin and outstanding characteristics of improved cassava varieties (Kim *et al.*, 2000)

Variety	Year released	Background and outstanding characteristics
KM60	1993	Originally named Rayong 60, was introduced from the Thai-CIAT program in 1989. High fresh yield. Recommended for early harvesting. Excellent agronomic traits. Good root shape, but flesh color is slightly yellow.
KM94	1995	Originally named MKUC 28-77-3 (Kasetsart 50), was introduced from the Thai-CIAT program in 1990. High yield and high starch content. Good root shape and white flesh. Good stake quality. Tolerant to major pests and diseases. Well adapted to unfavorable conditions.
SM937-26	1995	Originally named SM937-26, was introduced from the Thai-CIAT program in 1990; High fresh yield and high starch content. Good root shape and white flesh. Good plant type. Good stake quality.
KM95	1995	Selected from F ₁ hybrid seeds introduced from the Thai-CIAT program in 1991 (originally named OMR33-17-15). High fresh yield. Early harvestability. Multi-purpose use for direct human consumption, feed and processing. Good root shape and white flesh.
KM95-3	1998	Selected from F ₁ hybrid seeds introduced from the Thai-CIAT program in 1992 program (originally named SM1157-3). High fresh yield. Early harvestability. Multi-purpose use for direct human consumption, feed and processing. Good plant type. Good stake quality.
KM98-1	1999	Selected from F ₁ hybrid seeds introduced from the Thai-CIAT program in 1995 (pedigrees Rayong 1 x Rayong 5). High fresh yield. Early harvestability. Multi-purpose use for direct human consumption, feed and processing. Good root shape and white flesh. Good plant type.

Breeding progress in the last years in Vietnam by the breeding programs of VNPC (Vietnam cassava program) research partners and the CIAT collection, that is held in Vietnam, resulted in two new cassava varieties: *KM98-5* and *KM140* (Figure 2) (Kim *et al.*, 2010). *KM98-5* reaches a fresh yield of 34.5-37.8 ton/ha and a starch content of 27.2-29.8%, which makes it superior to *KM94* and *KM140*. It was distributed in Southern Vietnam in 2007 and in 2009, while *KM140* was distributed in Northern Vietnam in 2008

to a large number of households. Two other improved varieties, *KM98-7* and *KM21-12*, were also distributed in 2008 to a large number of households in the Northern mountainous areas of Vietnam (Kim *et al.*, 2010).

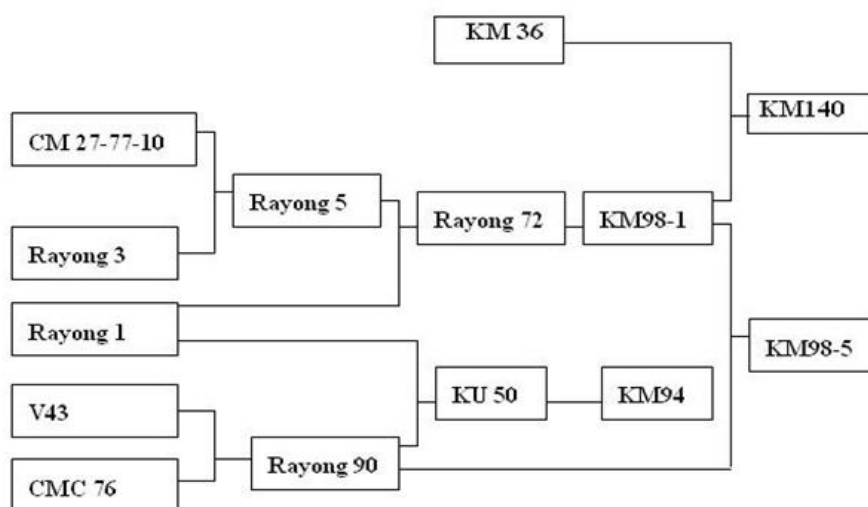


Figure 2 Ancestry of the improved cassava varieties (Kim *et al.*, 2010)

The success of the newly introduced improved varieties is illustrated in Figure 3. From 1975 to 2012 the production has increased by 727%, the yield by 138,4%, and the area harvested increased by 246,8% (FAO, 2014). Important to note is the increase in yield after the introduction of improved varieties from CIAT in 2000 (Kim *et al.*, 2010). For many small-scale farmers this resulted in an increase from 8.5 tons/ha in 2000 to 36 ton/ha in 2011 (Nguyen *et al.*, 2013). Including all Vietnamese cassava farmers, an average yield of 17.63 ton/ha (Nguyen *et al.*, 2013) was reached in 2011. In Vietnam this breeding success of increasing yields could be achieved due to many collaborations also including the Hung Loc Agricultural Research Center (HARC), Institute of Agricultural Science for Southern Vietnam (IAS), CIAT, Nong Liam University (NLU), Vietnam Academy of Agricultural Sciences (VAAS), Vedan Vietnam Enterprise Corp. Ltd. and other cassava processing factories (Kawano, 2003; Kim *et al.*, 2010). Most recently clone *KM419* showed very high performance and will be selected for release (Kim *et al.*, 2013).

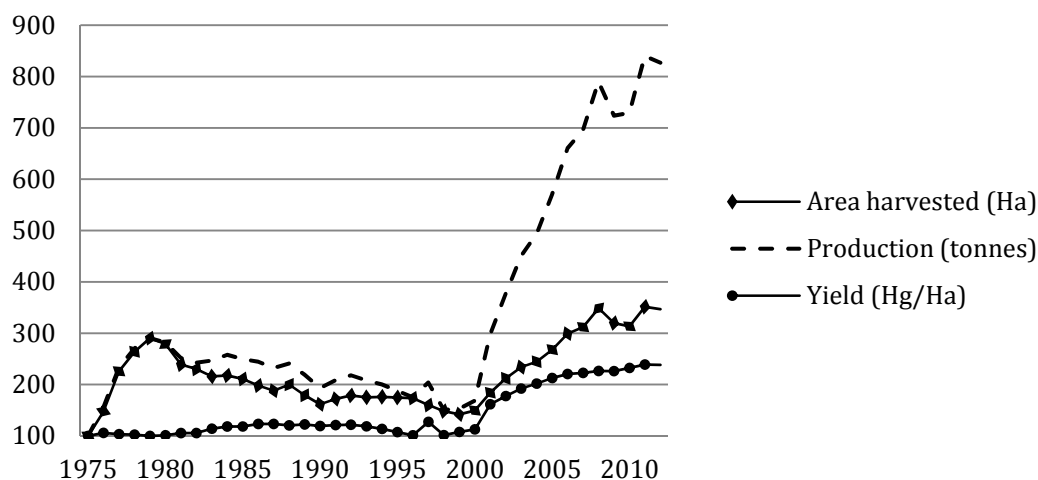


Figure 3 Growth of cassava in Vietnam from 1975-2012 (Index: 1975=100) (data from FAO (2014))

1.5.2.1 Breeding goals

From now on the East Asia Joint Research Program (e-ASIA JRP), whose members are NLU, VAAS, CIAT and the College of Agro-Forestry Thai Nguyen University (TUF) in Vietnam will focus on improving resistance to various pest and diseases in addition to increase in yield potential, starch content and nutritional value (Kim *et al.*, 2013). Additionally, research will focus on integrated cultivation techniques and transfer of those to small-scale farming systems. Small-scale farmers in major regions (South East, Central Highland, South Central Cost, and North Mountain regions) will be targeted in order to increase productivity and economic efficiency while meeting the various needs of rural communities (Kim *et al.*, 2013).

1.5.2.2 Cassava germplasm on the farmers' field

In 2009 new improved varieties, mainly *KM94*, *KM140*, *KM98-5*, *KM98-1*, *SM937-26*, *KM98-7*, were grown on more than 90% of the total cassava area of Vietnam (Kim *et al.*, 2010). Cultivation of a few newly improved varieties over a large area may have severely reduced the diversity in the cassava gene pool in Vietnam. To what extent farmers are still growing old varieties and how much these varieties contribute to the genetic diversity of Vietnamese cassava is therefore studied in this thesis.

1.6 Genetic resources and conservation of cassava

The identification of new genotypes and previously unknown diversity may be important as genetic resources in breeding to adapt cassava to future changes in climate and farmers' needs. Genetic diversity can therefore be seen as a resource used by breeders and farmers, which needs to be protected with help of conservation practitioners.

Knowledge about the extent of genetic diversity and structure of crops are crucial in order to select which genotypes and varieties to conserve and for developing effective gene conservation programs (Frison *et al.*, 2011). A variety is often referred to as the unit of conservation. However, a variety may consist of several clones with different genotypes. In this thesis the term variety refers to the unit of cassava plants identified by the farmer(s) under a single name irrespective of the genetic or phenotypic similarity of the plants.

Genetic diversity can be conserved in-situ (on-site) and ex-situ (off-site) (Commission on Genetic Resources for Food and Agriculture, 2010). One example for ex-situ conservation of cassava is the world's biggest in-vitro collection of cassava germplasm, which is maintained at the CIAT germplasm bank in Cali, Colombia. It was initiated in the beginning of the 70s and consists today of more than 6500 accessions from 28 countries. The term accession in this context refers to a stored unit assumed to be genetically identical, i.e. clones. The largest percentage of accessions is from Colombia (37.7%) and Brazil (24.1%) (International Center for Tropical Agriculture, 2014a). However, Asia is only represented by 7.1 % of the accessions and this cassava collection stores nine accessions from Vietnam, which are all landraces. Eight of them originate from Northern Vietnam and one is without any declaration of origin. These landraces were included into the germplasm bank in 1996 (International Center for Tropical Agriculture, 2014b). However, the genetic composition of these landraces is not known and they are not part of the core collection, which aims at representing the global genetic diversity of cassava. In Vietnam several ex-situ germplasm collections are maintained. The cassava collection held by Nong Lam University (NLU) and the Institute of Agriculture Science for Southern Vietnam (IAS) consists of 344 accessions, of which 31 are local Vietnamese varieties (Kim *et al.*, 2010).

1.7 The interaction of farmers' management and genetic diversity: calling for an interdisciplinary approach

Farmers are especially important as guardians and creators of genetic diversity of crops, as many processes of creation and maintenance of genetic diversity are conducted by them (Commission on Genetic Resources for Food and Agriculture, 2010). In order to fit

farmers' needs, these processes include decisions over e.g. which varieties to grow, their field's size, special cultivation practices e.g. inclusion of seedlings or naming (Balyejusa Kizito, 2006). Naming can create more diversity when farmers renamed their varieties as they differ after selection over long periods of time (Almekinders *et al.*, 2008) or naming of the same variety may differ between families, regions and communities. Other processes can also lead to high turnover of varieties e.g. the exchange of plant material or the purchase of plant material (Balyejusa Kizito, 2006). Farmers' selection over long periods of time about which cuttings to use for the next season also influence the specific genetic composition of a farmers' field. Their preferences regulate which varieties will stay on the field, adopt over time and will possibly be grown over generations (Balyejusa Kizito, 2006). As seen here, farmers can shape genetic diversity. However, genetic processes as mutation, recombination, genetic drift, gene flow and natural selection also play an important role in the creation of genetic diversity (Acquaah, 2007). Genetic drift and natural selection can be a factor in cassava populations, where some plants randomly die or are able to reproduce sexually. During genetic recombination of sexually reproduced plants mutations in the genome can spontaneously take place. Fixed somatic mutations can also occur in vegetative propagation and have been detected in cassava population before (Sardos *et al.*, 2008). The transfer from genes or alleles from one population of cassava to the other can be influenced by outbreeding and hybrids.

Coming back to farmers' influence, to understand the interaction of farmers' management and genetic diversity, an accurate identification of the genetic composition and organisation of cassava plants in the field is needed. Genetic analysis identifies each plants' own 'finger print' and can thereby explore the accurate genetic composition within one population or between populations. One example to identify a plant's 'finger print' are SSR markers, which are used here. For research on cassava SSR markers have been established as a valuable tool to characterize germplasm (Mba *et al.*, 2001) e.g. they have been used before to investigate the CIAT core collection (Fregene *et al.*, 2003), to locate the origin of cassava (Olsen, 2004) and to investigate its genetic diversity within and between varieties (Mkumbira *et al.*, 2003; Balyejusa Kizito *et al.*, 2007; Sardos *et al.*, 2008). SSR markers are polymorphic repetitive base pairs of di-nucleotide and compound repeats, which are abundant in all plant and animal genomes and the number of its repeats is enormously variable in a population (Excoffier & Heckel, 2006; Batley & David, 2009). SSR markers were previously called microsatellites (Litt & Luty, 1989) and are specifically suitable for investigating the diversity of cassava plants. SSR markers are able to display allelic bridges, if unique alleles in both parents are present which allows genetic mapping (Mba *et al.*, 2001).

1.8 Climate change in Vietnam

Vietnam is considered to be one of the countries in the world which will be most affected by climate change (Viet Nam Government Portal, 2011). Vietnam stretches over an area of 331 210 km² with over 3400 km of coastline. Geographically Vietnam shows two low, flat deltas, the Mekong delta in the south and the Red River delta in the north divided by the highlands in Central Vietnam. Due to its length of 1650 km the climate varies considerably within the country (Figure 4). However, North monsoon (October to April) and South monsoon (May to September) affect the whole country and annual rainfall exceeds 1000 mm almost everywhere.

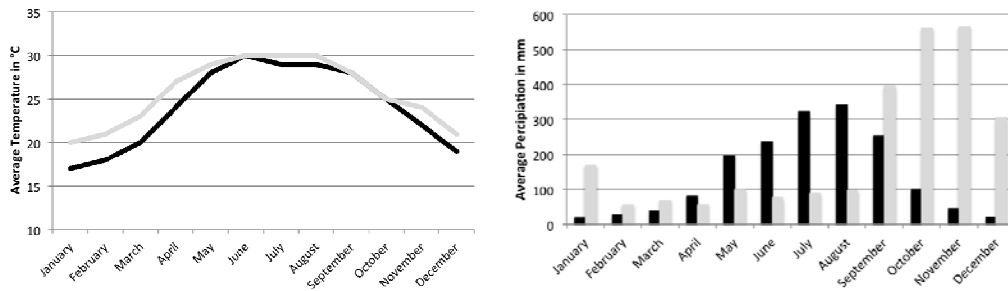


Figure 4 Climatic conditions in Northern (black) and Central Vietnam (grey) (data from climatemps.com, (2012))

The Vietnamese Ministry of Natural Resources and Environment (2009) predicts a temperature rise between 1.6°C to 2.3°C relative to the average temperature during the period 1980 to 1999 by the end of the 21st century. All over the country winter temperatures will rise faster than summer ones, especially in Northern Vietnam. Additionally, annual and rainy season's rainfall will increase by 5 % compared to the period 1980 to 1999. Compared to the same period the sea level is expected to rise 30 cm until the year 2050 and 75 to 100 cm until the year 2100, expecting severe floods of wide areas especially in the Mekong delta in Southern Vietnam. According to a research report by the World Bank a sea level rise of 1 m would impact 10.8% of Vietnam's population (Figure 5), which is the highest percentage of all 84 investigated countries in the report (Dasgupta *et al.*, 2007). However, in the Government Report (Ministry of Natural Resources and Environment, 2009) the effect of flooding, waterlogging and salt stress as well as biotic stresses such as pests and diseases on crop production due to climate changes are unmentioned .

The above describes future challenges for Vietnamese agriculture and crop cultivation. Firstly, the crops have to be adapted to climate changes, causing abiotic and biotic stresses. Secondly, Vietnam will have to cope with less available arable flat land along the coast and in the delta areas, the most fertile lands, which will be flooded due to rising sea levels. In a study in Southern Vietnam 36 farmers were interviewed and the results showed that farmers are already today conscious of local climate changes (Le Dang *et al.*, 2013).

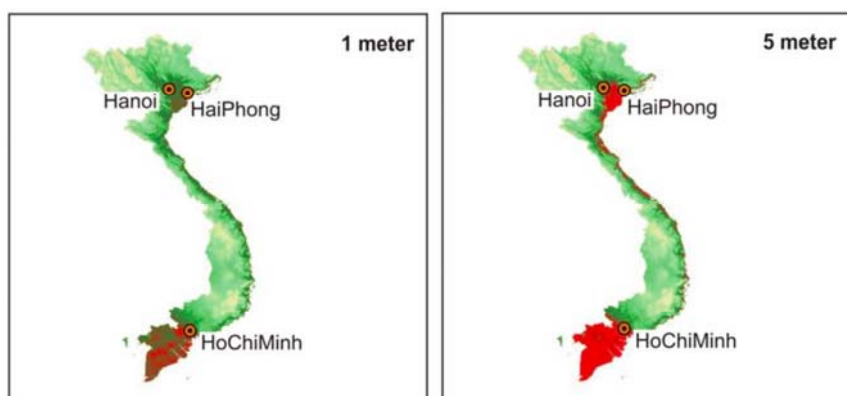


Figure 5 Flooded areas (red) at sea level rise of 1m and 5 m modified from Dasgupta *et al.*, (2007)

1.9 Specific Objectives

To sum it up, the genetic diversity of cassava varieties needs to be identified additionally with the small-scale farmer's preferences to be able to build strategies to maintain and create a high genetic diversity and assure that ex-situ conservations cover the genetic resources. Both can build the basis to be able to breed for a sustainable future. This enhances the importance of the following analysis, which aims to explore the following questions:

- (1) Which cassava varieties will be identified on small-scale farms in Northern and Central Vietnam based on farmers naming? How does this compare to varieties identified based on genetic analysis?
- (2) How is the genetic diversity structured on different geographical scales: within small-scale farms, among farms and villages and between Northern and Central Vietnam?
- (3) What are the farmers' preferences regarding traits of cassava and how do they select their varieties?
- (4) Which considerations are made by farmers that affect the selection of varieties?
- (5) Have small-scale farmers in Northern and Central Vietnam adopted new improved varieties or do they still cultivate or maintain old local varieties?
- (6) How much do the old varieties contribute to the genetic diversity of Vietnamese cassava?
- (7) Do the Vietnamese cassava accessions stored at the CIAT Germplasm Bank reflect the genetic diversity of cassava varieties in Northern and Central Vietnam?

2 Material and Methods

2.1 Study sites

The study sites are located in Northern and Central Vietnam (Figure 6). In total, 15 farms were visited. Six farms, three in each of two villages, one in the commune of Mường Chiềng (village 1) (20° 59' N, 104° 59' E), Hoà Bình province, and one in the commune of Khánh Thượng (village 2) (21° 01' N, 105° 20' E), Hà Nội province were located in Northern Vietnam. Cassava is mainly grown in hilly areas in Northern Vietnam, where the majority of cassava is grown on rocky soils (Kim *et al.*, 2010). In Central Vietnam nine farms, all in the commune of Hương Hóa (16° 38' N, 106° 40' E), Quảng Trị Province, from three different villages were visited.



Figure 6 Study area in Northern (black) and Central Vietnam (grey) (modified from d-maps.com (2007))

2.2 Interviewee selection

The villages were pre-selected by the partnering institutes the Plant Resources Center (PRC), Vietnam Academy of Agricultural Sciences, and the Institute of Forest Tree Improvement and Biotechnology, Vietnamese Academy of Forest Sciences (VAFS) in Hanoi, Vietnam. The participating farmers were then selected by the province officials with responsibility for agriculture and rural development in contact with the chiefs of the villages, who informed them beforehand about the purpose of the study.

2.3 Interviews

All farmers were interviewed using a structured interview questionnaire developed by the SLU researchers in the project in collaboration with the researcher Dr Vu Linh Chi at PRC. The interviews covered basic information about the farms such as farmer(s) name, village, commune, province, GPS location and cassava cultivated areal. Among other

questions the farmers were also asked: (1) to name the cassava varieties that they were currently cultivating in their fields, (2) to describe the characteristics of these varieties and how they were differentiating between them, (3) if they knew the origin of the different varieties (local, exchanged from other farmers or disseminated from government agency), (4) how long they had been growing each variety, (5) the reason why they were growing these particular varieties, (6) to describe cultivation practices (including planting/harvest, cultivation techniques and storage of plant material), and (7) if there were any obstacles for cultivation and production e.g. pest and diseases and climate.

The interviews were conducted in Vietnamese following the questionnaire and translated into English by the Vietnamese researchers. Previous experience by the collaborating Vietnamese researchers in interviewing and meeting farmers in the studied areas in connection with projects on other were highly beneficial for instructive interviews. In Northern Vietnam the researchers Dr Vu Linh Chi, PRC, MSc Hoang Thi Nga, PRC, Dr Per-Olof Lundquist, SLU and Dr Anna Westerbergh, SLU were present during the interviews. During the interviews in Central Vietnam, MSc Tran Duc Vuong, VAFS, Mr Le Cong, VAFS, Mr Ngô Quang Hung, associated with VAFS, and Dr Per-Olof Lundquist, SLU were present. The interviews took about 60 minutes followed by the collection of plant material samples.

2.4 Interview analysis

A comparative approach was chosen to conduct the interview analysis, which was done by counting answers, noting contrasts and comparisons between farmers and regions and noting patterns and repeating themes in the interview transcripts. The results are illustrated using quantitative and descriptive tools. Quantitative tools are used to classify and quantify farmer's statements. Descriptive tools are used to describe e.g. their cultivation practice or the utilization of cassava.

I am aware of the difficulties of analysing interviews I have not been involved in, but in order to compensate for this I have discussed the interviews thoroughly with my supervisor who conducted the interviews. Moreover, the interview analysis was most likely influenced by my background, gender and pre-knowledge. Farmers' background influenced their answers as well. The interviewed farmers had a history of cassava cultivation, which ensures that their answers are based on experiences with this crop (Table 4). Yet other factors like education in general, agricultural education, gender, power relations and previous experiences with scientists could not be taken into consideration here.

2.5 Plant material

2.5.1 Leaf collection in Vietnam

Following the interview the farmers were asked to come to the field to identify the different varieties grown by them in their cassava field. The interviewed farmers were asked to point out plants from each named variety. The accompanying researchers collected young leaves from 15 plants of each variety, which were randomly distributed in the field. The collected leaves from a single cassava plant was immediately transferred to a separate bag and labeled according to farmer's naming of the variety, location site (village and commune), farmer code number and plant individual number of the variety. The leaves were dried directly after collection and then stored at -20°C.

Table 4 Farmers' Background

Village	Farmer	Gender of the farmer interviewed	Duration of cassava cultivation	Size of cassava field (ha)
Northern Vietnam				
Village 1	M-1	couple: male and female	for generations in the family	2
	M-2	male	for generations in the family	0,8
	M-3	female	for generations in the family	4
Village 2	K-1	couple: male and female	for generations in the family	2
	K-2	couple: male and female	for generations in the family	1
	K-3	couple: male and female	5 years	2
Central Vietnam				
Village 3	AT-1	male	for generations in the family	4
	AT-2	male	for generations in the family	1,5
	AD-1	female	7 years	2
Village 4	Th-1	male	12 years	2
	Th-2	male	6 years	0,7
	Th-3	female	10 years	1
	Th-4	male	for generations in the family	2
Village 5	TL-1	male	7 years	1
	TL-2	male	7 years	1

2.5.2 Vietnamese accessions at CIAT

Nine Vietnamese accessions are held in tissue culture at the CIAT Germplasm Bank. In this study eight of these accessions were provided by the Germplasm Bank. The accessions are coded VNM1 through VNM5 and VNM7 through VNM9 (Figure 7). Variety VNM6 was not available at the time of order. All eight accessions are declared to be local cultivars. Seven of the accessions are from Northern Vietnam, six (VNM1 through VNM5, and VNM7 from the county Nam Ninh (20° 18' N, 106° 14' E). VNM 8 originates from county Phong Châu (21° 14' N, 105° 25' E) and for VNM 9 no county of origin is documented. A material transfer agreement (MTA) was signed declaring among other things that these accessions are used for research purposes only.

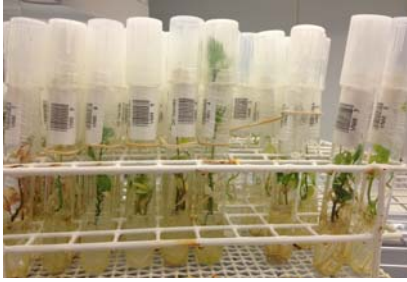


Figure 7 Tissue culture of VNM accessions from CIAT Germplasm Bank (photo Martina Lamprecht)

2.6 DNA extraction

Five to six leaf samples of each variety grown by a single farmer and the eight VNM accessions were grinded and extracted for DNA. DNA extraction was done using the DNeasy 96 plant kit following the quick-start protocol (QIAGEN, 2012). Additionally to the protocol, 0.2 g of DTT was added to the lysis solution to improve the purity and yield of the extracted DNA. Concentration and DNA purity was analyzed using NanoDrop spectrophotometer.

2.7 Genetic marker analysis

The DNA samples were sent to the Uppsala Genome Center, Uppsala University, Uppsala, Sweden, where the DNA samples were amplified by polymerase chain reaction (PCR) using fluorescently labeled primers for 23 polymorphic simple sequence repeat (SSR) markers. The 23 SSR markers used in this study (Table 5) were selected based on a previous analysis of a subset of the collected leaf samples from Vietnam. These SSR markers are spread across the length of the 18 chromosome pairs in the cassava genome (Mba *et al.*, 2001). The PCR followed the protocol described by Mba *et al.* (2001).

Table 5 SSRY markers: SSR locus, Base Repeat, Left and Right primer sequence (data from Mba *et al.*, 2001; Montero-Rojas *et al.*, 2011)

SSR locus	Base Repeat	Left primer sequence	Right primer sequence
SSRY 4	(GA) ₁₆ TA(GA) ₃	ATAGAGCAGAAAGTGCAGGCG	CTAACGCACACGACTACGGA
SSRY 9	(GT) ₁₅	ACAATTCATCATGAGTCATCAACT	CCGTTA TTGTTCTGGTCTCT
SSRY 12	(CA) ₁₉	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT
SSRY 19	(CT) ₈ (CA) ₁₈	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA
SSRY 20	(GT) ₁₄	CATGGACTTCTACAAATATGAAT	TGATGGAAAGTGGTTATGTCCTT
SSRY 21	(GA) ₂₆	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA
SSRY 34	(GGC) ₃ GGTGGC (GGT) ₂	TTCCAGACCTGTCCACCAT	ATTGCAGGGATTATTGCTCG
SSRY 47	(CA) ₁₇	GGAGCACCTTTTGTGAGTT	TTGGAACAAAGCAGCATCAC
SSRY 59	(CA) ₂₀	GCAATGCAGTGAACCATCTTT	CGTTTGTCTTTTCTGATGTTT
SSRY 63	(GA) ₁₆	TCCAGAATCATCTACTCTGGCA	AAGACAATCATTTTGTGTCTCCA
SSRY 64	(CT) ₁₅ CG(CT) ₆	CGACAAGTCGTATATGTAGTATTGAG	GCAGAGGTGGCTAACGAGAC
SSRY 68	(CT) ₁₂ CC(CT) ₁₇	GCTGCAGAATTTGAAAGATGG	CAGCTGGAGGACCAAAAATG
SSRY 69	(CT) ₁₈ ATT(AT) ₂ (N) ₇ (CTTT) ₂	AGATCTCAGTCGATACCCAAG	ACA TCCGTTGCAGGCA TTA
SSRY 82	(GA) ₂₄	TGTGACAATTTTTCAGATAGCTTCA	CACCATCGGCATTAATACTTG
SSRY 100	(CT) ₁₇ TT(CT) ₇	ATCCTTGCCTGACATTTTGC	TTCGAGAGTCCAATTGTTG
SSRY 102	(GT) ₁₁	TTGGCTGCTTCTACTAATGC	TTGAACACGTTGAACAACCA
SSRY 103	(GA) ₂₂	TGAGAAGGAAACTGCTTGCAC	CAGCAAGACCATCACCAGTTT
SSRY 105	(GT) ₆ GC(GT) ₂ (GA) ₁₆	CAAACATCTGCACTTTTGGC	TGAGTGGCTTCTGGTCTTC
SSRY 106	(CT) ₂₄	GGAAACTGCTTGCACCAAAGA	CAGGCAAGACCATCACCAGTTT
SSRY 108	(CT) ₂₄ CCT	ACCCTATGATGTCCAAAGGC	CA TGCCACA T AGTTCGTGCT
SSRY 148	-	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC
SSRY 171	(TA) ₃ CATA(GATA) ₈ GC(GA) ₂₃ (GTGA) ₂	ACTGTGCCAAAATGCCAAATAGT	TCATGAGTGTGGGATGTTTTATG
SSRY 181	(GA) ₂₂ (G) ₃ C(GA) ₃ GGAA(GA) ₄	GGTAGATCTGGATGGAGGAGG	CAATCGAAAACCGACGATACA

2.8 Data analyses

The genotype data from the 23 SSR markers was exported to Microsoft Excel and further coded for genetic analyses using software packages based on (Excoffier & Heckel (2006). PGD-Spider (Lischer & Excoffier, 2012) was used to convert input data files between the different software packages. Distribution of different alleles in Northern and Central Vietnam as well as in local and improved varieties, and observed heterozygosity within

varieties (average number of heterozygote individuals of the total number of studied plants within a variety) were analyzed by the software GENEPOP (Raymond & Rousset, 1995; Rousset, 2008) Pairwise genetic distances between varieties was calculated based on average F_{ST} over all SSR loci (Weir & Cockerham, 1984) using Arlequin ver. 3.5.2 (Excoffier & Lischer, 2010). Hierarchical cluster analysis was carried out in PC-ORD 6.0 using Euclidean distances and Ward's group linkage method (McCune & Mefford, 2011). The cluster distance scale is according to Wishart (1969).

2.9 Ethical Considerations

Genetic resources are of emerging importance in today's world (Commission on Genetic Resources for Food and Agriculture, 2010) and it needs to be assured that research working in this field includes ethical considerations (Engels *et al.*, 2011). As a balance between legal protection, access to genetic resources and benefit sharing is crucial for farmers, breeders, conservation professionals, researchers and the public in order to stimulate food production, innovation, equity and conservation (Commission on Genetic Resources for Food and Agriculture, 2010). In this thesis ethical considerations were made as the interviewed farmers will not be referred to by their name but by a code in the thesis (see Table 4), the samples taken will be coded as well, to ensure their confidentiality. Access to the interviews is limited to the researchers involved in this project. The interviewees gave prior oral consent and it was made clear in advance that the samples were taken for research purposes only. Consequently none of the participating researchers will have any financial benefits from the farmers' previous selections and work.

3 Results

3.1 Cassava varieties named by farmers

During the interviews, the farmers named in total 12 different varieties, which were grown in their fields (Table 6). Two of the varieties, *sắn cao sản* and *sắn lá tre*, were named to be grown in both Northern and Central Vietnam. Three of the varieties were given two names by individual farmers. *Sắn dù đỏ* was also called *sắn nông*, *sắn T.134* also named *sắn lõi vàng* and *sắn my* also named *can tua*.

In Northern Vietnam the average number of named varieties grown per farmer was higher than in Central Vietnam, 3 and 1.78 varieties, respectively. In Central Vietnam four out of the nine farmers only cultivated one variety each according to their naming. Two farmers explained that they only knew the variety they cultivated in their fields.

Table 6 Distribution of varieties in Northern and Central Vietnam, their categorisation of improved (IV) and local varieties (LV) and duration of cultivation by the farmers

Variety (code)	Northern Vietnam	Central Vietnam	IV /LV	Duration of cultivation
<i>Sắn xanh (X)</i>	✓		LV	grown for several generations
<i>Sắn Vedan (Ve)</i>	✓		IV	5 years
<i>Sắn dù trắng (T)</i>	✓		IV	7-10 years and grown for several generations
<i>Sắn vỏ đỏ (VD)</i>	✓		LV	grown for several generations
<i>Sắn dù đỏ / Sắn nông (D)</i>	✓		LV	grown for several generations
<i>Sắn T.134 / Sắn lõi vàng (LV)</i>	✓		IV	5 years
<i>Sắn cao sản (C)</i>	✓	✓	IV	NV: first season- 5 years CV: unknown
<i>Sắn lá tre (L)</i>	✓	✓	IV	grown for several generations
<i>KM94 (K4)</i>		✓	IV	between 4 and 12 years
<i>KM98 (K8)</i>		✓	IV	2 years
<i>Thailand, new (TN)</i>		✓	IV	2 years
<i>Sắn my / Can tua (M)</i>		✓	LV	grown for several generations

3.1.1 Cassava variety names

Cassava is called *sắn* in Vietnamese (Howeler *et al.*, 2013). The different cassava varieties with a Vietnamese name are also given an epithet describing an important characteristic of the cassava variety. Five of the varieties were named based on the color of stems or storage roots, one based on the shape of the leaves and one indicating high yield. According to the interviewed farmers, four of the varieties were named by breeding companies or institutions or the Ministry of Agriculture and Rural Development (Table 7).

Table 7 Local naming categories of cassava varieties and the origin of varieties

Variety	Naming of varieties	Category	Origin
<i>Sắn xanh</i>	xanh: green	colour	grown within the family
<i>Sắn cao sản</i>	cao: high (yielding)	yield	exchange with farmer within village and bought from agricultural department
<i>Sắn dù trắng</i>	trắng : white (storage roots, petioles and stems)	colour	exchange with farmer from other village and grown within the family
<i>Sắn lá tre</i>	lá tre: bamboo leaves (very narrow leaves)	shape	grown within the family
<i>Sắn dù đỏ / Sắn nông</i>	đỏ: red (outer surface of storage root cortex); nông: hot	colour	grown within the family
<i>Sắn T.134 / Sắn lõi vàng</i>	lõi vàng: gold core (yellow flesh storage root)	colour	exchange with farmer within village through information by the agricultural department
<i>Sắn vỏ đỏ</i>	vỏ đỏ: red skin	colour	grown within the family
<i>Sắn my / Can tua KM94</i>	-	-	grown within the family
		named by Ministry of Agriculture and Rural Development	disseminated from factory
<i>KM98</i>	-	-	grown within the family
		named by Ministry of Agriculture and Rural Development	disseminated from factory
<i>Thailand, new</i>	-	-	grown within the family
		named by company/institution	disseminated from factory
<i>Sắn Vedan</i>	-	-	grown within the family
		named by company	disseminated from factory through information by the agricultural department

3.2 Farmers' description of cassava varieties

3.2.1 Farmers ability to differentiate varieties

When asked to describe the varieties, farmers often compared one variety with their other varieties, which led to one conflicting description by two farmers, as the roots of *sắn dù đỏ / sắn nông* were once described as short roots and once as big and long ones (Table 8). In general, farmers' knowledge was highly variable. Three interviewed farmers found it very hard to describe their varieties while not being in the field. Two farmers were not able to name which varieties they were growing until they were in the field or after discussing it with their neighbour or husband. One farmer also first mixed up *sắn xanh* and *sắn cao sản*. It was the first season she cultivated *sắn cao sản*. Eight farmers were asked if they knew of any improved varieties. Six of them did not know any other improved varieties, than the ones they were growing themselves. Two farmers were aware about *sắn cao sản* being an improved variety even though they were not cultivating it themselves and one farmer knew about other improved varieties on the market than *sắn cao sản* and the improved variety grown by that farmer. For about half the varieties the yield could be quantified.

3.2.2 Differentiation of local and improved

Each named variety was clearly described as local or improved by all farmers (Table 6). All four local varieties were grown within the farmer's family for several generations. Most farmers grew both local and improved varieties (see Table 14), separated in different field plots. In Northern Vietnam 28% of the named varieties were described as local cultivars and 19% in Central Vietnam. In total 27 ha were cultivated by the interviewed farmers of which 25 ha were cultivated with improved varieties, which makes up to 93% of the cultivated area. The local varieties were typically used for local human consumption and not sold at the market or to the starch factories. They were therefore grown on small areas and according to some farmers only maintained on the farm.

Table 8 Characteristics of varieties identified by the farmers (*contrasting statements by farmers)

Characteristics identified by the farmers						
Variety	Outstanding characteristics	Pests and diseases	Sweet/Bitter	Utilization	Average cultivation size (ha)	Yield (t/ha)
<i>Sắn xanh</i>	straight green stem, long storage roots	resistant to pests and diseases, low temperature during the growing season is considered main limitation	sweet	human on farm consumption (only during harvest period)	0.55	-
<i>Sắn cao sắn</i>	straight stem, long and thin leaves, 7 leaflets, high quality yield	resistant to pests and diseases, low temperature during the growing season is considered main limitation, some spots on leaves, root disease	bitter / sweet*	sold at the market, fresh starch production, human consumption	0.65	20
<i>Sắn dù trắng</i>	narrow leave lobes, many branches (about three), light green stem and petiole, white storage roots	resistant to pests and diseases	sweet/ bitter*	ethanol production; sold at the market; animal feed (storage roots), fresh starch production	1.1	-
<i>Sắn lá tre</i>	long thin leaves, red storage roots	"dragon head" disease	sweet	human food, animal feed and starch	1	20
<i>Sắn dù đỏ / Sắn nông</i>	harvested every second year; leaves bigger and differently shaped than Sắn (dù) trắng, dark brown stem, line shaped leaves, red petiole, dark brown outside skin, short/big and long roots*	resistant to pests and diseases, low temperature during the growing season is considered main limitation for growth of young plants	sweet	human on farm consumption (only during harvest period); ethanol production	0.35	-
<i>Sắn T.134/ Sắn lõi vàng</i>	yellow flesh storage roots	resistant to pests and diseases, low temperature during the growing season is considered main limitation	bitter	fresh starch production on farm, sold to factories	1	-
<i>Sắn my/Can tua</i>	short leaves, 5 leaflet per petiole, red petiole on the upper side, brown stem nearby the leaf	black and white spots on leaves	sweet	human on farm consumption and animal feed	0.1	-
<i>Sắn vô đỏ</i>	red root cortex, best cooking quality	-	sweet	animal feed (storage roots), ethanol production, human consumption (one ore twice a week), sold to the market	0.3	-
<i>KM94</i>	long broad leaves, 7-9 leaflets per petiole, light green stem; when the plant grows bigger, the stem will be changed to white color; storage roots connected close to stem base, white bark; brown and big storage roots	black spots on leaves; brown plant hopper; "Dragon head" disease: The farmer explained that the dragon disease can make the leaves on top of plants roll together and the plants grow very slowly. This may lead to small roots or no storage roots; One farmer described a kind of disease that can make the leaves change its color into yellow and plants have only small roots, but no storage roots; fungi that is called "Sung" in Van Kieu language.	bitter	starch	1.6	range from 14-28, on average 22
<i>KM98</i>	thin and long leaves similar to bamboo cassava, white stem, red petiole in the upperside, brown storage root	black spots on leaves	sweet	human on farm consumption and animal feed	0.7	20
<i>Thailand, new</i>	broad and shorter leaves than KM94, good drought tolerance and no weed grown under the canopy	-	unknown	starch	0.5	30-35
<i>Sắn Vedan</i>	green stem, red petiole	-	bitter	fresh starch production	0.85	-

3.2.3 Differentiation of bitter and sweet

The majority of varieties were defined as bitter or sweet by the farmers (Table 8). The bitter varieties were used for the production of starch and the sweet ones for human consumption. All four local varieties were described as sweet, while the improved varieties were in general considered bitter. However, the varieties *sắn cao sản* and *sắn dù trắng* were considered either sweet or bitter by different farmers. Three farmers considered *sắn dù trắng* as bitter and only one farmer categorized it as ‘a little bit sweet’. Three farmers considered *sắn cao sản* as bitter, while one farmer considered it to be sweet. The improved variety Thailand, new was the only variety which could not be classified as sweet or bitter. It is only utilized for the production of starch.

3.2.4 Pests, diseases and abiotic stress

For 25% of the varieties no pests or diseases were mentioned and half of the varieties were described as resistant to pest and diseases by the farmers (Table 8). One farmer claimed that his cassava fields were free of pests and diseases, which was in contrast to the interviewers’ observation. However, *KM94* was described to display many pests and diseases by some farmers. For the varieties *sắn xanh*, *sắn dù đỏ / sắn nông*, *sắn cao sản* and *sắn T.134 / sắn lõi vàng* low temperature during the growing season was described to be the main limitation for cultivation and production.

3.2.5 Utilization of cassava roots

The majority of farmers used cassava roots for local human consumption and starch production. Additionally one third of all farmers used cassava for animal feed and one-fourth for ethanol production, fresh starch production or sold it straight to the market (Table 8). Many varieties, both sweet and bitter, were used for more than one purpose such as *sắn cao sản* and *sắn dù đỏ / sắn nông*.

For human consumption in Northern and Central Vietnam only sweet varieties and interestingly *sắn cao sản*, which was considered bitter by the majority of farmers, were used. For food preparation it was described that, first the skin of the storage roots is peeled off and then they are cooked in boiling water. One farmer immerses them first for 1 hour in water before cooking. Sometimes the roots are boiled together with rice and served as a dish.

Of the villages visited in this study, fresh starch production on farm was only conducted in the village 2 of commune Khánh Thượng, Hà Nội province in Northern Vietnam. These farmers utilized the improved varieties *sắn cao sản*, *sắn Vedan* and *sắn T.134 / sắn lõi vàng* for this purpose. The farmers’ own machine was used in order to produce fresh starch. According to the farmers the production for 100 kg storage roots could produce 50 kg fresh starch. The fresh starch processing encompasses several steps: (1) Cleaning the storage roots with water to remove the soil and outer skin. (2) Drying the roots. (3) Crushing the roots into two parts, liquids and residues, by adding some water. The residues are used as animal food for pigs and fish. The liquids are kept in the large tank. (4) Liquids precipitate after six to eight hours and then separate into two parts, water (surface of liquids) and starch (bottom of tank). Afterwards the water gets removed and the fresh starch is transferred to another tank. This fresh starch can be sold to the industry immediately or preserved for a longer time. (5) For storage, the fresh starch is put in a plastic bag and some water is added. The water level needs to be about 5 cm above the starch. The plastic bag is then tied up and covered with soil. The starch can be stored for two to three years this way.

3.2.6 Origin of varieties

All improved varieties, which are named by breeding programs and breeding companies, were disseminated from factories (Table 7). *Sắn Vedan* and *sắn T.134 / sắn lõi vàng* were promoted by the government, the Vietnamese Ministry of Agriculture and Rural Development, to the farmers. In Northern Vietnam the transfer within the family and exchange with other farmers were still very important ways of obtaining plant material.

One farmer explained a very special incidence of how she obtained her planting material. She bought *sắn cao sản* cuttings under the condition to sell her yield back to a price that was fixed already when buying the cuttings from the re-seller.

3.3 Farmers' variety preferences

Farmers were asked to rank their varieties according to their preferences using first, second and third choice. The local varieties *sắn xanh* and *sắn dù đỏ / sắn nông*, and the newly improved varieties *sắn T.134 / sắn lõi vàng* and *sắn Vedan*, were all ranked as farmers' most preferable varieties in Northern Vietnam (Figure 8). One farmer ranked *sắn xanh* as most preferable due to its utility in human consumption. However, she only grew it on 0.5 ha in comparison to her other two varieties which were grown on 1 and 1.5 ha each, so in her case there was no observed correlation between preference and size of plots.

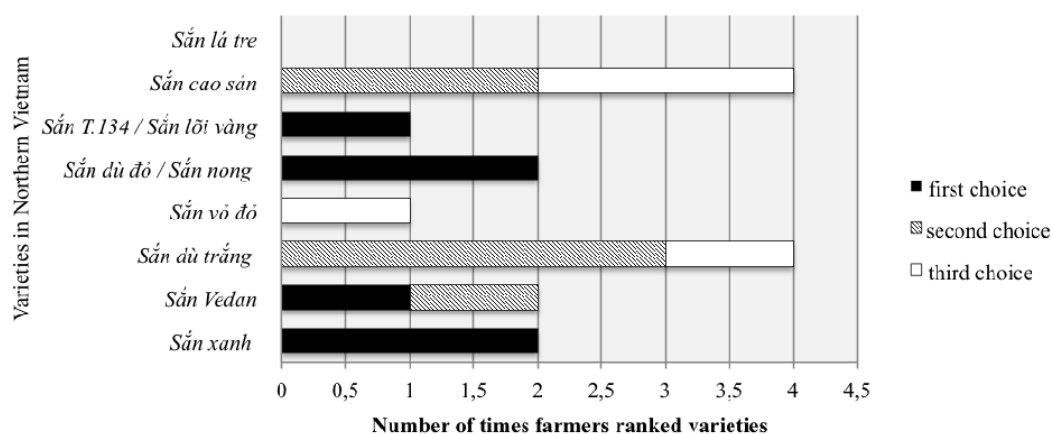


Figure 8 Farmers' preferences for cassava varieties in Northern Vietnam

When asked to rank varieties according to yield and preference, interestingly, only one farmer ranked his preferences according to the highest yield (Table 9). Four out of the six varieties preferred by the farmers in Northern Vietnam were sweet varieties used for human consumption. Only *sắn Vedan* and *sắn T.134 / sắn lõi vàng* are bitter varieties.

Table 9 Varieties ranked according to their yield versus their preference in Northern Vietnam

Farmer	Varieties ranked according to their yield	Varieties ranked according to the farmers' preference
M1	<i>Sắn dù trắng</i> > <i>Sắn cao sản</i> > <i>Sắn xanh</i>	<i>Sắn xanh</i> > <i>Sắn cao sản</i> > <i>Sắn dù trắng</i> > <i>Sắn lá tre</i>
M2	<i>Sắn dù trắng</i> > <i>Sắn vỏ đỏ</i>	<i>Sắn vỏ đỏ</i> > <i>Sắn dù trắng</i>
M3	<i>Sắn dù trắng</i> highest yield	<i>Sắn xanh</i> > <i>Sắn dù trắng</i> > <i>Sắn cao sản</i> > <i>Sắn lá tre</i>
K1	<i>Sắn Vedan</i> > <i>Sắn T.134 / Sắn lõi vàng</i> > <i>Sắn vỏ đỏ</i>	<i>Sắn T.134 / Sắn lõi vàng</i> > <i>Sắn Vedan</i> > <i>Sắn vỏ đỏ</i>
K2	<i>Sắn Vedan</i> > <i>Sắn cao sản</i>	<i>Sắn Vedan</i> > <i>Sắn cao sản</i>
K3	<i>Sắn cao sản</i> > <i>Sắn dù trắng</i> > <i>Sắn dù đỏ / Sắn nông</i>	<i>Sắn dù đỏ / Sắn nông</i> > <i>Sắn dù trắng</i> > <i>Sắn cao sản</i>

In contrast to Northern Vietnam's scattered preferences, eight out of nine farmers ranked the improved variety *KM94* as their most preferred variety in Central Vietnam (Figure 9). One farmer explained that he solely grew *KM94* due to its superiority in quality and productivity.

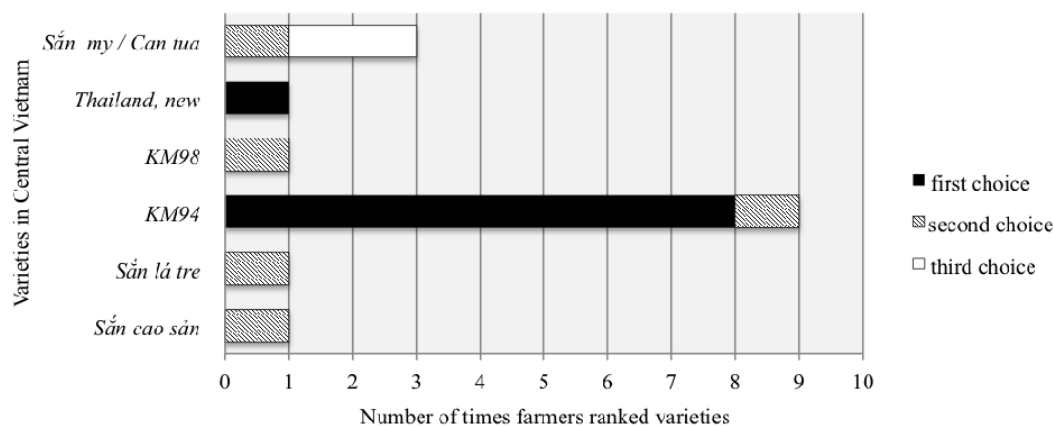


Figure 9 Farmers' preferences for cassava varieties in Central Vietnam

3.4 Farmers' cultivation practises

The size of a cassava field ranged from 0.7-4 ha with an average size of 1.8 ha. According to the interviews and the morphological similarity of plants observed in the field by the interviewers, farmers avoided to mix different varieties in the same field plot. Instead they were grown separately and the field plots were in some cases located in different parts of the farm. However, one farmer couple in the commune Khánh Thượng (village 2) in Northern Vietnam were growing both the local variety *sắn dù đỏ / sắn nông* and the improved variety *sắn dù trắng* in the same field. According to them they could differentiate between the two varieties based on the colour of the petioles and stems. Moreover, after the leaf sampling in the field of one of the farmers in the commune of Mường Chiềng (village 1) in Northern Vietnam, she told that she and her husband had replaced some plants of *sắn cao sắn*, which were killed by cold, with another variety not mentioned during the interview. She felt that she could differentiate them visually and only showed the plants of *sắn cao sắn* for the researchers for sampling of leaves.

All improved varieties were planted on significantly larger field plots than local varieties summing up to 93% of the overall cultivation area investigated here. The average cultivation area was highest for *KM94* with 1.6 ha (Table 8). All improved varieties were planted on significantly larger field plots than local varieties summing up to 93% of the overall cultivation area investigated here. The average number of cassava plants cultivated per farmer was 19 000 and on average 12 600 plants per ha were grown.

Cultivating practices based on interviews are summarized in Tables 10 and 11. Planting times in Northern Vietnam are from February to March as well as harvesting time from November to December. In contrast, in Central Vietnam planting and harvest times varied a lot as cassava was planted from October to May and harvested after eight to 24 months. Storage of stems (0.4 to 1.5 m) used for making stem cuttings for planting the following season was kept in bundles vertically in the field for most farmers, others kept them horizontally or buried them in the field. The stems were divided in two-node cuttings and planted either horizontally or slanted by different farmers in Central Vietnam, while all farmers in Northern Vietnam planted them horizontally in the sloping field. In contrast to the farmers in Central Vietnam, who prepared rows of ridges for planting, the farmers in Northern Vietnam planted the cuttings randomly in the soil.

Village 1 in Mường Chiềng is located in the highland and mountainous area in the north. In this area farmers used the land effectively. Typically steep slopes were characterized by cassava fields and on flat land, next to the village, rice was cultivated (Figure 10). In between, at the beginning of the slopes maize was grown. However, rice terraces were also seen in adjacent highland areas. Some of the cassava fields were located on high hills at long distances from the village. During harvest time the farmers walked this distance a couple of times per day to harvest and carried the roots in baskets down to the village. This shows the contrast with the more mechanized agriculture in Central Vietnam.

Table 10 Cultivation practise in Northern Vietnam

Northern Vietnam	
Planting time	February to March
Inclusion of seedlings	No
Land preparation	Cleaning of the field before new cuttings are planted
Planting	Each variety is planted on separate plots; cuttings planted horizontally, flat (no ridges) and randomly within the plot; 0.5-1 m distance between plants depending on the variety
Weed control	No
Fertilization	In Mường Chiềng there was no fertilization; In Khánh Thượng farmers use chicken manure and NPK fertilizer. Chicken manure is applied when planting 5-6 t/ha and after 2-3 months 400-500 kg or 600-700 kg of NPK fertilizer are applied
Crop system	Observed intercropping with maize, trees, litchis and sugar cane in the field.
Cross-pollination by hand	No
Collecting seeds	No
Crop rotation	4 farmers had no crop rotation; 1 farmer: grows cassava for max 5 years and then moves it due to its emerging need for fertilization; 1 farmer: grows cassava for 3-5 yrs and then rotates with rice or maize
Harvest time	(October) November or December
Storage of plant material	In Mường Chiềng: 1.- 1.5 stem cuttings are tied together in bundles and stored vertically in the field until next season, small cuttings (2-node cuttings) are planted horizontally. In Khánh Thượng: stem cuttings are buried in the soil (10-15 cm)

Table 11 Cultivation practise in Central Vietnam

Central Vietnam	
Planting time - harvest time	Planting and harvest times varied a lot in Central Vietnam. Cassava is planted from October until May and harvested after 8-24 months.
Inclusion of seedlings	No
Land preparation	2 farmers are using machines and are preparing the land by hand when there is a slope; 3 farmers are solely using machines and 4 farmers are solely preparing by hand
Planting	Stem cuttings are planted with a length of 10-15cm, 5 farmers plant them slanted and 4 horizontally, covered by the thin layer of soil; a distance of 0.6-1 m are kept between rows and 0.6-1 m is kept between the plants within the rows
Weed control	All farmers are weeding 1-4 times a year. 5 of the 9 farmers are using herbicides and 3 of them are using Roundup.
Fertilization	6 farmers are not using any fertilisers. 1 farmer uses it after the fourth year. 1 farmer uses 600 NPK/ha and one uses a local fertilizer mix (100-150 kg/ha)
Crop system	observed intercropping with e.g. maize
Cross-pollination by hand	No
Collecting seeds	No
Crop rotation	No
Storage of plant material	Plant material was stored vertically in the field putting 1-1.3m long stem cuttings tied together in bundles for the next planting season, in a shadow area. 1 farmer has an exceptional technique where he ties together 40-80cm long stem cuttings in a bundle and puts them down in the field.

Only one farmer in Northern Vietnam claimed to sometimes intercrop cassava with maize. On two additional farms in Northern Vietnam and three in Central Vietnam sugarcane and fruit trees such as mango and lychee were observed in cassava fields by the interviewers but not mentioned by the farmers. Crop rotation is uncommon but was sometimes practiced by farmers in Northern Vietnam where cassava was replaced with maize or rice after three to five years of cassava cultivation.

Farmers in Central and Northern Vietnam used fertilization to a similar extent. Inorganic (NPK) fertilizers were used by farmers in both regions, whereas chicken manure was only commonly used in Northern Vietnam. No weed control was used in Northern Vietnam in contrast to Central Vietnam where the herbicide Roundup was used by some farmers (Table 10 and Table 11).

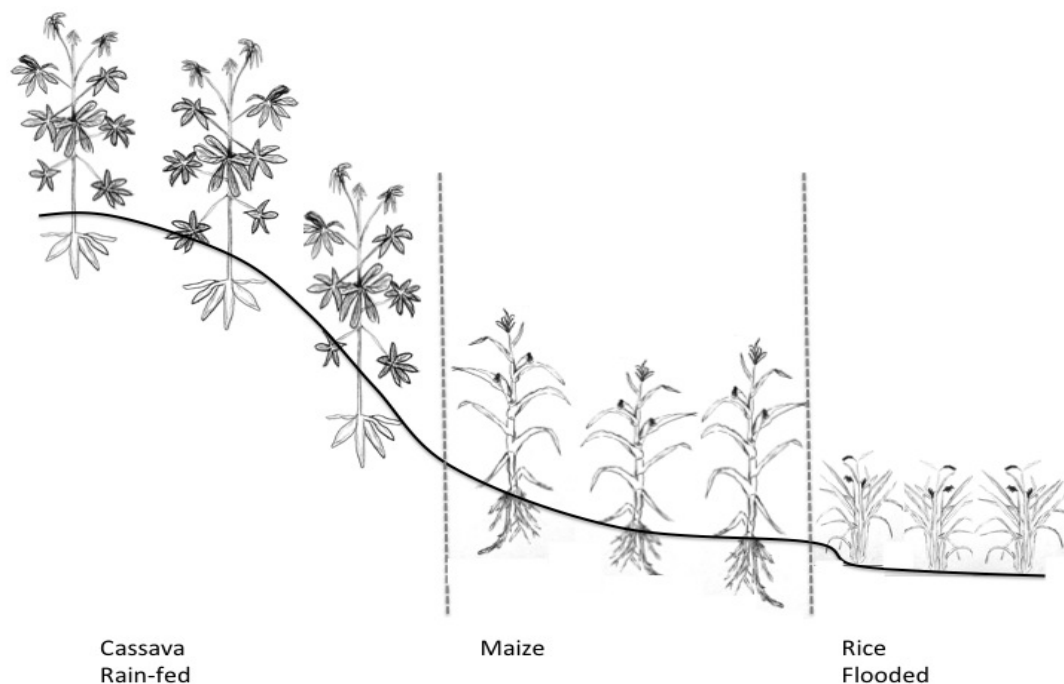


Figure 10 Scheme of cultivated land in Northern Vietnam showing rain-fed cassava cultivation (drawing by Martina Lamprecht)

3.4.1 Volunteer seedlings

In one farmer's field in Central Vietnam many seedlings were observed in which the variety *Thailand new* was currently cultivated. This field was the previous season used for that variety and another variety from Thailand. This farmer also currently and previously cultivated *KM94*. Leaf samples were collected from five seedlings for genetic marker analysis.

In Northern Vietnam two farmers had observed seedlings in their fields, but none of them had taken cuttings from them and included them in the next years planting material. One of the farmers explained that he removed the seedlings in the field due to the poor production of only one tuberous root per plant. In another farmer's field volunteer seedlings were observed in contrast to the farmer's statement. Moreover, one of the farmers in Northern Vietnam was convinced to grow hybrids between *sắn xanh* and *sắn than do* in their field as some plants showed traits from both varieties. When visiting the farmer's cassava field she pointed out the pink cortex of the storage roots, a trait shared with *sắn dù đỏ / sắn nong*, and the green petiol and stem colour also shown by *sắn xanh*. Leaves from these were collected for genetic markers analysis. *Sắn than do* was according to the farmer a variety grown by their neighbor, a farmer not interviewed and included in this study.

3.5 Analyses of genetic diversity

3.5.1 Distribution of alleles

In the whole sampled collection of Vietnamese varieties, including all plants from all varieties, the largest number of alleles was found in locus SSRY19, showing eight alleles, whereas the smallest number was found in SSRY4 and SSRY102, with two alleles, respectively (Table 12). In total 104 different alleles were identified for all 23 SSR loci in the sampled collection of cassava plants. All except two of these alleles were found in cassava varieties from Northern Vietnam, whereas the Central Vietnamese varieties showed only 88 of them. The larger number of alleles in the Northern varieties occurred in 10 of the studied SSR loci, with one to two alleles per locus compared to the varieties in Central Vietnam. When comparing local and improved varieties a larger number of alleles was found in the local ones. As three out of four local varieties were grown in Northern Vietnam, this could partly explain the higher number of alleles found in the North. In three SSR loci the improved varieties displayed one allele more than the local ones, while the local ones showed one to two alleles more in eight loci. Of the 104 alleles found in the sampled collection of cassava varieties, one allele in marker SSR171 had a lower frequency than 1% in the Vietnamese farmers' varieties. This rare allele is only found in some of the plants of the improved variety *sắn dù trắng* grown in Northern Vietnam. The eight cassava Vietnamese accessions in the CIAT Germplasm Bank, Cali, Colombia carried 75 of the 104 alleles found. These accessions harboured also seven unique alleles not detected in any of the varieties grown by the farmers' included in this study.

Table 12 Distribution of alleles of 23 SSR loci in Vietnamese cassava and CIAT collection, *one rare allele included (<1% in the sampled collection of cassava plants)

SSR locus	Vietnam	Northern	Central	Local	Improved	CIAT collection	
	no of alleles	no of alleles	no of alleles	no of alleles	no of alleles	no of alleles	no of unique alleles
SSRY 4	2	2	2	2	2	2	0
SSRY 9	5	5	4	5	4	4	0
SSRY 12	4	4	4	4	4	3	0
SSRY 19	8	8	6	8	8	5	0
SSRY 20	5	5	4	5	4	5	1
SSRY 21	7	7	6	7	6	3	0
SSRY 34	4	4	4	4	4	3	0
SSRY 47	4	4	3	4	3	2	0
SSRY 59	3	3	3	3	3	3	0
SSRY 63	3	3	3	3	3	3	0
SSRY 64	7	7	5	7	5	3	0
SSRY 68	4	4	4	4	4	4	0
SSRY 69	4	4	4	3	3	5	1
SSRY 82	5	5	4	5	4	4	0
SSRY 100	7	5	3	5	3	5	1
SSRY 102	2	2	2	2	2	2	0
SSRY 103	4	4	4	4	4	5	1
SSRY 105	4	4	4	3	4	3	0
SSRY 106	4	4	4	4	4	4	1
SSRY 108	4	4	4	4	4	3	0
SSRY 148	6	6	4	6	5	3	0
SSRY 171	5*	5*	4	4	5*	3*	0
SSRY 181	3	3	3	2	3	5	2
Total no of alleles	104	102	88	98	86	79	7
Mean no of alleles	4,52	4,43	3,83	4,26	3,74	3,43	0,30

3.5.2 Genotypic composition of varieties

The whole sampled collection of Vietnamese varieties showed an average heterozygosity (H_o) of 0.93 (SD=0.09). When comparing the different named varieties, all of them showed a high average H_o , ranging from 0.83 in *sắn my / can tua* to 1.0 (heterozygotes at all loci) in *sắn vỏ đỏ*, *sắn T.134 / sắn lõi vàng* and *Thailand new*. This means that many Vietnamese cassava plants in all varieties carried two alleles for most of the studied SSR loci. A high average H_o has also been seen in cassava grown in Africa and Latin America (e.g. Elias *et al.*, 2000; Fregene *et al.*, 2003; Balyejusa Kizito *et al.*, 2007). In contrast to the high heterozygosity in cassava many cereal crops such as cultivars of barley and wheat are made homozygous (only one allele per locus) through breeding. Samples collected from seed plants in a farmer's field in Central Vietnam and potential hybrids in a field in Northern Vietnam were all heterozygotes for all of the 23 SSR loci.

All plants in each of the named varieties, *sắn vỏ đỏ*, *sắn T.134 / sắn lõi vàng* and *Thailand new*, showed the same genotype at all loci (Table 13). This means that all the investigated plants within each of these varieties are clones. Higher genetic diversity was found in the other varieties. Interestingly, both local and improved varieties consisted of more than one genotype at most of the 23 studied SSR loci. Evidently, cassava plants named as the same variety by the interviewed farmers, were genetically different and therefore not clones of the same multilocus genotype. However, a predominating genotype occurring in more than 60% of the plants in a variety was found for all SSR loci in three improved varieties, *sắn cao sản*, *KM94* and *KM98*, suggesting small genetic differences among plants within these varieties. In contrast, the improved varieties *sắn dù trắng* and *sắn Vedan* in Northern Vietnam showed predominating genotypes in rather few loci.

A few loci such as SSRY4 and SSRY102 showed few different genotypes, compared to the other 21 studied loci. This may indicate that these two loci are strongly linked to traits that consciously or unconsciously have been under intensive selection during domestication and/or breeding, reducing their genetic diversity. They may, however, be neutral genes not linked to any selected trait, but still be affected by exceptionally strong genetic bottleneck(s) as a result of domestication and/or breeding, even reducing the diversity in neutral genes (Doebley *et al.*, 2006).

Most varieties showed several different multilocus genotypes in a farmer's field (Table 13). Some of these different multilocus genotypes could differ in a single SSR locus, others differed in many. However, many clonal plants were found within farmer's field (Table 14). The three farmers in the village in commune Khánh Thượng (village 2) in Northern Vietnam named in total eight different varieties. Interestingly, each of these varieties was maintained as a clone. Also, some farmers in Central Vietnam cultivated single clones of different local and improved varieties. In contrast, in the farmers' fields in the other visited village in Northern Vietnam in commune Mường Chiềng (village 1) more than one multilocus genotype were found in each named variety, except for *sắn cao sản*. Evidently, cassava grown by these farmers show larger genetic diversity than expected provided that plants given the same name are genetically identical.

Table 13 Genotypes at each SSR locus and predominating genotypes (underlined) occurring more than 60% of the studied plants within a variety. N= Northern Vietnam, C= Central Vietnam

Variety	Number of samples	SSRY loci																						
		4	9	12	19	20	21	34	47	59	63	64	68	69	82	100	102	103	105	106	108	148	171	181
Local Varieties:																								
<i>Sắn xanh (N)</i>	10	<u>12</u>	13	33	28	24	13	24	33	11	<u>13</u>	33	11	<u>11</u>	<u>55</u>	11	<u>12</u>	<u>13</u>	<u>44</u>	<u>24</u>	11	14	<u>44</u>	<u>11</u>
			14	35	37	25	17	34	44	12		36	<u>34</u>			16		23		34	13	24		
			34		38	35	34			33						17					14	46		
<i>Sắn dù đỏ / sắn nông (N)</i>	12	<u>22</u>	<u>12</u>	<u>44</u>	<u>11</u>	15	12	12	11	<u>33</u>	11	13	<u>24</u>	22	12	11	11	<u>12</u>	34	<u>23</u>	11	44	14	12
			13	45	18	<u>25</u>	16	22	<u>22</u>		13	25	44	23	<u>13</u>	17	12	23	44	33	<u>12</u>	<u>45</u>	24	22
			53	55	67	23	<u>46</u>	24	33			36			14	57				34	24		44	
<i>Sắn vô đỏ (N)</i>	6	<u>12</u>	<u>34</u>	<u>25</u>	<u>46</u>	<u>23</u>	<u>14</u>	<u>12</u>	-	<u>33</u>	<u>13</u>	<u>44</u>	<u>44</u>	<u>12</u>	<u>15</u>	-	<u>22</u>	<u>34</u>	<u>44</u>	<u>45</u>	<u>13</u>	<u>36</u>	<u>34</u>	<u>12</u>
<i>Sắn mỹ / Can tua (C)</i>	14	<u>22</u>	23	45	26	23	17	14	22	<u>11</u>	<u>11</u>	13	11	12	12	11	<u>12</u>	12	<u>44</u>	34	11	34	34	<u>12</u>
			33	<u>55</u>	46	25	37	24	24	22	12	16	12	<u>22</u>	13	17	22	23	45	45	12	36	44	22
			34		67	33	47	34	<u>33</u>		13	<u>47</u>	<u>44</u>		15	33		24		<u>13</u>	45			
					68	<u>34</u>	56					67		55			34				56			
Improved Varieties:																								
<i>Sắn dù trắng (N)</i>	23	11	13	35	14	<u>23</u>	13	14	22	11	11	16	11	<u>22</u>	13	11	11	14	34	25	11	33	34	<u>12</u>
		<u>22</u>	23	45	24	33	14	<u>24</u>	33	12	13	17	23	23	15	33	22	23	44	34	12	34	35	22
			34	55	26	34	47	34	34	13	23	36	34	44	25			34		45	13	44	<u>44</u>	
					28	35	57		44			47			55						14	46		
																					22			
<i>Sắn T.134 / sắn lõi vàng (N)</i>	7	<u>11</u>	<u>14</u>	<u>35</u>	<u>35</u>	<u>25</u>	<u>37</u>	<u>24</u>	<u>34</u>	<u>33</u>	<u>13</u>	<u>36</u>	<u>44</u>	<u>24</u>	<u>55</u>	<u>77</u>	<u>12</u>	<u>11</u>	<u>14</u>	<u>22</u>	<u>11</u>	<u>24</u>	<u>44</u>	<u>23</u>
<i>Sắn Vedan (N)</i>	14	<u>11</u>	14	<u>35</u>	28	25	13	24	34	12	12	<u>36</u>	11	22	<u>55</u>	11	<u>12</u>	11	14	12	11	24	<u>44</u>	22
			34		35	35	37	34	44	33	13		14	24		77		23	15	34	14	46		23
												44								55				
<i>Sắn cao sản (N + C)</i>	29	<u>11</u>	12	24	<u>28</u>	25	11	24	<u>44</u>	<u>12</u>	11	<u>36</u>	<u>11</u>	<u>22</u>	23	<u>11</u>	11	22	<u>15</u>	33	11	44	14	12
		22	<u>34</u>	<u>35</u>	66	<u>35</u>	<u>13</u>	<u>34</u>		33	<u>12</u>	37	14	34	<u>55</u>	77	<u>12</u>	<u>23</u>	34	<u>34</u>	<u>14</u>	<u>46</u>	<u>44</u>	<u>22</u>
						46					13		23						44	35	24			
											23		44											

Table 13 continue Genotypes at each SSR locus and predominating genotypes (underlined) occurring more than 60% of the studied plants within a variety. N= Northern Vietnam, C= Central Vietnam

Variety	Number of samples	SSRY loci																						
		4	9	12	19	20	21	34	47	59	63	64	68	69	82	100	102	103	105	106	108	148	171	181
Improved Varieties continue:																								
<i>Sản lá tre (N + C)</i>	11	<u>22</u>	12	44	11	23	17	12	22	11	<u>13</u>	13	<u>24</u>	<u>23</u>	12	11	<u>11</u>	12	<u>34</u>	23	11	34	<u>14</u>	<u>22</u>
			23	45	26	24	46	14	33	<u>33</u>		16		13	17		23		34	12	45	34		
			33	55	46	25	47	22	24			46		15	33		24		35	13	46	44		
			34		67	34	56	24				47				37		34		45	22	56		
<i>KM94 (C)</i>	45	<u>11</u>	23	<u>35</u>	<u>28</u>	24	11	12	24	11	11	16	<u>11</u>	<u>22</u>	12	<u>11</u>	11	<u>23</u>	14	<u>34</u>	13	34	11	<u>22</u>
		12	33	45	46	34	<u>13</u>	14	33	<u>12</u>	<u>12</u>	<u>36</u>	14	44	15	17	<u>12</u>	24	<u>15</u>	45	<u>14</u>	<u>46</u>	14	23
			<u>34</u>	55	67	<u>35</u>	17	24	<u>44</u>	33		46			<u>55</u>	37		34				56	34	
						56	<u>34</u>					47												<u>44</u>
<i>KM98 (C)</i>	5	11	<u>34</u>	<u>55</u>	<u>26</u>	<u>23</u>	<u>47</u>	<u>24</u>	<u>33</u>	<u>11</u>	<u>13</u>	<u>47</u>	23	24	<u>15</u>	<u>33</u>	<u>12</u>	<u>23</u>	<u>34</u>	<u>34</u>	<u>11</u>	<u>34</u>	<u>24</u>	22
		<u>22</u>											<u>24</u>	<u>34</u>										<u>33</u>
<i>Thailand new (C)</i>	5	<u>12</u>	<u>33</u>	<u>55</u>	<u>46</u>	<u>34</u>	<u>17</u>	<u>12</u>	<u>33</u>	<u>33</u>	<u>12</u>	<u>46</u>	<u>14</u>	<u>22</u>	<u>15</u>	<u>37</u>	<u>12</u>	<u>34</u>	<u>44</u>	<u>45</u>	<u>13</u>	<u>46</u>	<u>34</u>	<u>33</u>
Other collected samples:																								
<i>Suspected Hybrids (N)</i>	2	<u>12</u>	33	35	28	34	13	14	34	11	<u>11</u>	36	<u>34</u>	<u>22</u>	12	<u>11</u>	<u>22</u>	23	<u>44</u>	<u>45</u>	13	34	34	<u>11</u>
			34	55	46	35	17	34	44	12		47			55			34			14	46	44	
<i>Seedlings (C)</i>	5	<u>11</u>	12	35	11	22	<u>33</u>	22	<u>44</u>	11	<u>12</u>	11	11	<u>22</u>	13	11	<u>11</u>	12	11	23	11	44	11	<u>22</u>
		22	34	44	18	25		24		22		14	22	33	33	17	12	22	15	33	12	46	13	
			44	46	28	33		44	33		36	24		55				23	34	34	44	66	<u>44</u>	
				55	88	35						66												
CIAT collection:																								
<i>VNM 1</i>	1	-	53	23	17	75	-	24	-	33	-	-	11	22	-	19	11	66	14	66	14	34	44	12
<i>VNM 2</i>	1	11	13	23	78	34	-	14	-	11	-	56	13	14	35	17	12	24	44	35	13	46	44	12
<i>VNM 3</i>	1	11	53	25	78	34	-	44	-	13	-	45	14	54	23	17	11	64	44	65	34	36	44	12
<i>VNM 4</i>	1	11	53	35	78	33	-	24	-	33	-	45	14	14	23	57	11	23	44	34	13	33	44	12
<i>VNM 5</i>	1	11	33	25	47	73	15	24	34	33	23	56	14	12	23	11	11	24	44	65	13	46	44	11
<i>VNM 7</i>	1	-	34	55	67	23	-	24	-	22	-	56	24	34	33	11	22	12	34	-	44	46	45	43
<i>VNM 8</i>	1	12	33	25	46	34	17	12	33	33	13	46	34	11	15	37	12	34	44	45	13	46	34	11
<i>VNM 9</i>	1	-	34	55	67	33	11	24	-	22	13	56	22	34	33	11	22	12	34	-	44	46	45	53

Table 14 Number of clonal plants *2 different clones

Village	Farmer	variety	number of plants	number of clonal plants
Northern Vietnam				
Village 1	M1	<i>Sắn xanh</i>	5	4
		<i>Sắn dù trắng</i>	6	5
		<i>Sắn lá tre</i>	6	0
	M2	<i>Sắn cao sản</i>	5	2
		<i>Sắn dù trắng</i>	6	2
		<i>Sắn dù đỏ / sắn nong</i>	5	4
M3	<i>Sắn xanh</i>	5	2	
	<i>Sắn cao sản</i>	5	5	
	<i>Sắn dù trắng</i>	6	3	
Village 2	K1	<i>Sắn T.134 / sắn lõi vàng</i>	7	7
		<i>Sắn vỏ đỏ</i>	6	6
		<i>Sắn Vedan</i>	7	7
	K2	<i>Sắn cao sản</i>	7	7
		<i>Sắn dù trắng</i>	7	7
		<i>Sắn dù đỏ / sắn nong</i>	7	7
	K3	<i>Sắn Vedan</i>	7	6
		<i>Sắn cao sản</i>	7	7
	Central Vietnam			
Village 3	AT1	<i>Sắn my / Can tua</i>	5	5
		<i>KM94</i>	5	4
	AT2	<i>Sắn my / Can tua</i>	5	0
		<i>KM94</i>	5	2/2*
		<i>KM98</i>	5	3
Village 4	AD1	<i>KM94</i>	5	3
	Th1	<i>KM94</i>	5	3
		<i>Thailand new</i>	5	5
	Th2	<i>KM94</i>	5	5
		<i>KM94</i>	5	5
	Th3	<i>Sắn cao sản</i>	5	4
		<i>KM94</i>	5	4
		<i>KM94</i>	5	4
	Th4	<i>Sắn lá tre</i>	5	5
<i>Sắn my / Can tua</i>		4	3	
<i>KM94</i>		5	4	
Village 5	TL1	<i>KM94</i>	5	5
	TL2	<i>KM94</i>	5	5

3.5.3 Genetic relationship of Vietnamese cassava

To evaluate the genetic relationship between all sampled cassava plants named by different farmers in Northern and Central Vietnam hierarchical cluster analysis was performed based on the genotype data of each plant. This analysis shows that genetically similar plants are grouped as visualized in Figure 11. Groups or plants separated with long branches have larger genetic differences than those separated with short branches.

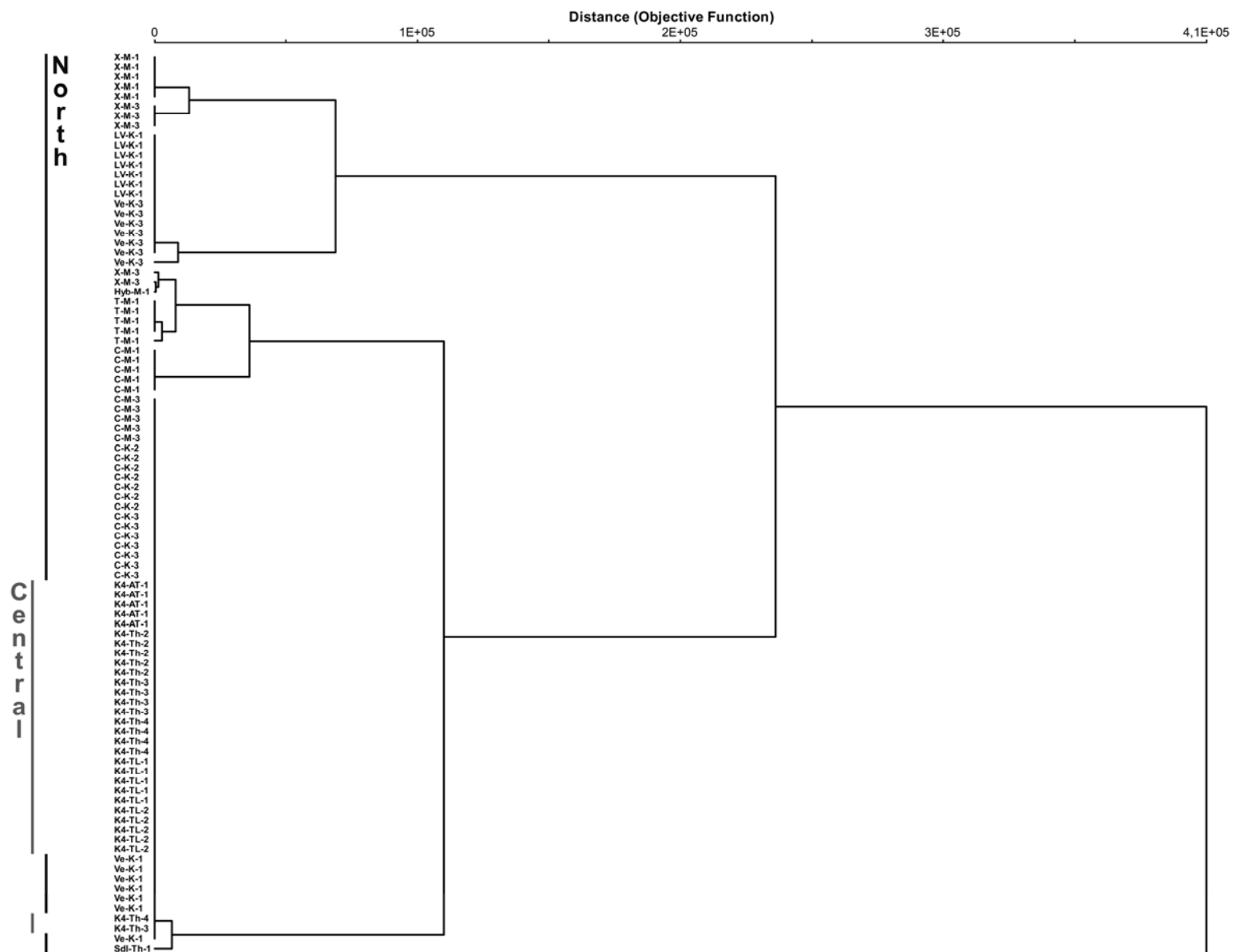
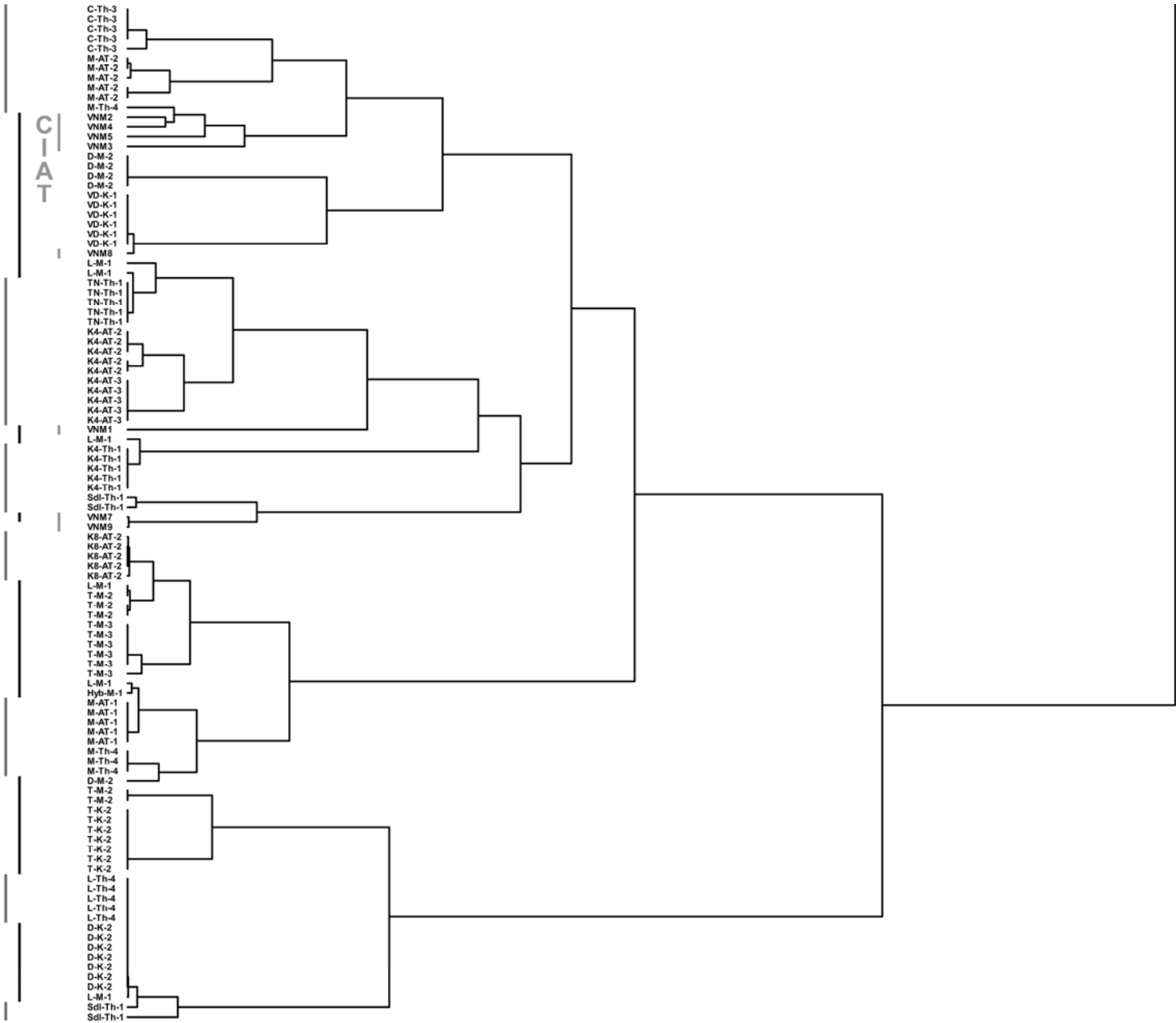


Figure 11 Hierarchical cluster analysis of cassava genotypes in Northern and Central Vietnam. Symbols indicate varieties named by farmers as described in Table 6, seedlings Sdl and possible hybrids Hyb, villages and farmer in each village indicated by number as described in Table 4. X-M1, X-M-3, D-M-1, D-K-2, M-AT-1, M-AT-2, M-Th-4 and VD-K-1 are local varieties, other varieties are improved.



Interestingly, some cassava plants named differently by different farmers such as the improved varieties *sắn cao sản* (C-M-1, C-M-3 and C-K-2) in three farmers' fields and *sắn Vedan* (Ve-K-1) in one field in Northern Vietnam, and *KM94* (K4-AT-1, K4-Th-2, K4-Th-3, K4-TL-1 and K4-TL-1) in five farmers' fields in Central Vietnam were grouped in the hierarchical cluster analysis. Based on the genetic marker data they are genetically identical and therefore considered to be one single clone. Even more surprising is that the improved variety *sắn lá tre* (L-Th-4) grown in a field in Central Vietnam and the variety *sắn dù đỏ / sắn nông* (D-K-2), considered being local by a farmer in Northern Vietnam, clustered. Also, plants of the two improved varieties *sắn T.134 / sắn lõi vàng* (LV-K-1) and *sắn Vedan* (Ve-K-3) grown by different farmers in Northern Vietnam, belonged to the same clone. This means that the plants named *sắn Vedan* by two different farmers in the same village in Northern Vietnam were genetically different. Moreover, clones of the newly improved *KM94* cultivated by farmers in different villages in Central Vietnam appeared in both of the two main clusters, which shows that the clones of *KM94* were genetically very different. Other clones of *sắn cao sản*, one cultivated by a farmer in a village in Northern Vietnam (C-M-3) and another by a farmer in Central Vietnam (C-Th-3) were genetically different from each other and from the single clone with a large number of plants named both *sắn cao sản* and *KM94*, already discussed above. Large genetic variation was also found in the named variety *sắn dù trắng* (T-M-1, T-M-2, T-M-3 and T-K-2), which was described as improved by different farmers in the same village in Northern Vietnam.

It was evident from the cluster analysis that genetic variation could also be found among plants grown in a single farmers' field. For example, the plants named *sắn lá tre* (L-M-1) were scattered in the hierarchical cluster tree, showing large genetic differences among these plants. Cassava fields with the same named variety but cultivated by different farmers in the same village could also show different patterns of genetic variation. Plants of the local variety *sắn xanh* (X-M-1) grown by a farmer in Northern Vietnam were all genetically identical but one which differed in only one locus, while the plants in another field grown by another farmer in the same village (X-M-3) showed genetic variation among plants in several loci. This was consistent with the results described in Table 14, which showed that number of clonal plants of a variety can differ between farmers' fields.

The hierarchical tree showed two main clusters, but no distinct pattern in the distribution of cassava plants at the regional, village or farm level was found in these clusters. Varieties both from Northern and Central Vietnam appeared in each of the two clusters. Also, cassava plants from different villages grouped closely together, for example as for *sắn cao sản* from different villages in Northern Vietnam and *KM94* from different villages in Central Vietnam. Another example is the grouping of *sắn cao sản* (C-Th-3) and *sắn my / can tua* (M-AT-2) grown by farmers in two different villages in Central Vietnam. Neither was there any distinct grouping on the farm level since varieties grown by a single farmer did not cluster separately from other farmers' varieties.

The genetic differentiation on the different geographical levels was also quantified using F -statistics analysis (Weir and Cockerham, 1984). Very small differentiation was found on the regional level between cassava grown in Northern and Central Vietnam ($F_{st}=0.02$). Small genetic differences were also found among cassava grown in different villages ($F_{st}=0.05$), while a larger differentiation was shown among cassava farms ($F_{st}=0.11$). As expected the genetic variation was high among varieties ($F_{st}=0.23$), while the genetic variation between the two groups, local and improved varieties, was smaller ($F_{st}=0.11$).

3.5.4 Genetic relationship among plants within the same field

As described earlier a farmer couple in commune Khánh Thượng (village 2) in Northern Vietnam explained during the interview that they were growing two different varieties in the same field, *sắn dù đỏ / sắn nông* and *sắn dù trắng*. They separated the two varieties based on the colour of the petioles and stems. These two varieties (D-K-2 and T-K-2) are two distinct clones, which group in different clusters in the hierarchical tree (Table 14,

Figure 11), confirming that these farmers are able to differentiate the two varieties based on the morphology. In addition, a farmer in the commune of Mùòng Chiềng (village 1) in Northern Vietnam had during the growing season in 2011 replaced some dead plants of *sắn cao sắn*, with another variety. By genetic marker analysis it was found that those *sắn cao sắn* plants (C-M-1) pointed out by the farmers were genetically identical (Figure 11).

To study the genetic diversity in seedlings found in one farmer's field in Central Vietnam, where the variety *Thailand new* was currently cultivated, leaf material from five seedlings were collected and analyzed for the 23 SSR loci. Last season both this and another variety from Thailand were cultivated. This farmer also currently and previously cultivated *KM94*. A large genetic diversity was found among these seedlings (Table 13). Two of the seedlings clustered near *sắn lá tre* (L-Th-4) and *sắn dù đỏ / sắn nông* (D-K-2), one near the *KM94-sắn cao sắn* clone, and one did not show much genetic similarity with any variety.

In one of the cassava fields in Northern Vietnam the farmer pointed out a few plants which she thought were hybrids between *sắn xanh* and *sắn than do* based on differences in the colour of the cortex of the storage roots, the petiol and stem. Two of these plants were sampled. One of these presumed hybrids were genetically similar to *sắn dù trắng* grown by the same farmer (T-M-1) and to some plants of *sắn xanh* grown by another farmer in the village (X-M-3). The other presumed hybrid clustered near *sắn lá tre*, which was grown by the same farmer (L-M-1).

3.5.5 Genetic relationship among CIAT Vietnamese accessions

The eight Vietnamese accessions (*VNM1-VNM5* and *VNM7-VNM9*) in the Germplasm Bank at CIAT showed different multilocus genotypes (Table 13). However, two of the accessions, *VNM7* and *VNM9*, showed high genetic similarity. Also *VNM2*, *VNM3*, *VNM4* and *VNM6* clustered, while *VNM1* did not cluster with any other VNM accession. All accessions were significantly different from the studied varieties. All accessions appeared only in one of the two main clusters.

4 Discussion

Genetic diversity in all organisms is important for adaptation and evolution - so also in crop plants. As a result of the strong selection during the domestication of crops, starting more than 10 000 years ago, many traits and genes were left behind in their wild relatives. Breeders are therefore left with a smaller gene pool for improvement of crops. This is particularly alarming with respect to the crucial role of high-quality and high-yielding crops needed in view of the growing global human population and ongoing climate change.

Cassava is a drought-tolerant crop and can grow in areas with soils that have low fertility and experience prolonged periods of drought. These areas are considered marginal for most other crops. It also grows well in mountainous areas and sloping hills. In areas with more extreme growing conditions, cassava may therefore potentially replace other starch crops such as maize and rice.

Vietnam is one of the countries predicted to be most affected by changes in the climate. Coastal areas will be flooded as well as the deltas in Southern and Northern Vietnam, which are considered to be among the most fertile soils for crop production. Agriculture will then be restricted to areas in the highlands of Northern and Central Vietnam that will therefore become increasingly important for agricultural production. It can therefore be projected that cassava will be one of the crops that will be increasingly cultivated and to contribute to food security and economic development in Vietnam.

In this thesis, significantly higher genetic diversity of cassava in local farmers' varieties compared to bred varieties was discovered. The local varieties can have an important role for improvement of cassava varieties and in particular for Vietnamese cassava. In the Vietnamese cassava accessions in the CIAT Germplasm Bank, this diversity was not present. This suggests that more local cassava should be conserved to increase the diversity of germplasm banks.

The specific objectives presented in paragraph 1.1 are discussed below.

4.1 Farmers naming and ability to differentiate varieties

In total 12 different varieties were named by the farmers in Northern and Central Vietnam. Some of these were referred to by two different names by a single farmer (e.g. *sắn dù đỏ* / *sắn nông*, Table 6). A few named varieties were only grown by a single farmer (e.g. *sắn vô đỏ*). However, the same variety name was used by different farmers. Some name was used only by farmers within the same village (e.g. *sắn xanh*), others by farmers in different villages within the same region (e.g. *sắn my* / *can tua*) or by farmers in both Northern and Central Vietnam (e.g. *sắn cao sân*). Many of the plants referred to by the same showed the same multilocus genotype based on the genetic marker analysis, and were therefore considered to be a single clone. Some of these clones have been grown for several generations within the family, which suggests that the Vietnamese farmers are able to maintain and preserve cassava varieties and clones over long period of time.

In some farmers' fields more than one multilocus genotype was found among the plants cultivated by the same name. In many cases only one or two plants collected from the same field differed in a single or a few loci. In others, the cassava plants showed variation in a larger number of loci. Larger genetic differences found among plants within some fields showed two different patterns. For example, in one of the farmers' fields in Northern Vietnam two genetically distinct groups of *sắn xanh* (X-M-3, Figure 11) were found, suggesting that two different clones are grown in the same field. *Sắn lá tre* (L-M-1, Figure 11) in a farmers' field in Northern Vietnam showed another pattern, where all plants were genetically very different.

The different pattern of genetic variation found within farmers' fields may be caused by several processes. Spontaneous somatic mutations can occur in meristemic

tissues such as stems and other tissues used for vegetative propagation. In a vegetatively propagated plant such as cassava these mutations will slowly generate over time and result in differentiation among plants within the same clone. If a mutation is linked to a favourable trait (e.g. quality, yield, morphology), the farmers may actively select this phenotype for propagation or just maintain it with the already existing clone. Another explanation for genetic differences found among plants of the same variety may be that the planting material (cuttings), disseminated by the government directly to the farmers or via starch factories, originated from different clones of the same variety. These clones may be derived from selected siblings of the same crossing scheme within a breeding program. A possible reason for the high diversity with no clonal plants found in *sắn lá tre* (L-M-1) is a mix up of cuttings of different genotypes when grown for several generations within the family (Table 6). An additional source of genetic variation can be through sexual reproduction and seed plants have been observed in several farmers' fields. Incorporation of cuttings from these plants into the planting material may also have contributed to the diversity within this particular variety. However, incorporation of seed plants is not common among Vietnamese farmers, based on results from both farmers' interviews and genetic marker analysis. This is in contrast to what has been found in some previous genetic diversity studies on cassava, which documented usage of seed plants (Salick et al., 1997; Chiwona-Karlton et al., 1998; Elias et al., 2000, 2001a; b, Balyejusa Kizito et al. 2006.)

Interestingly, different clones identified by marker analysis were given the same name by the farmers' for some varieties such as the improved *sắn dù trắng*, *sắn cao sản* and the local variety *sắn dù đỏ / sắn nông*. As described in Table 7, the Vietnamese name of these and other varieties describes important characteristics of the plant such as yield, colour of roots, stems and petiols and shape of leaves. Based on the interviews of the farmers and discussions during the field visits, many of the farmers distinguished the different varieties grown by them based on the morphology. The importance of morphological characteristics for the naming may explain why genetically very different clones were referred to by different names. Two different clones of cassava with similar phenotypes may therefore be named the same by different farmers. Other varieties had names given by the breeding programs. However, the Vietnamese farmers' were also using morphological characteristics as well as level of yield for recognition of these improved varieties. The variety *sắn Vedan*, named by the starch factory, was cultivated by two different farmers in one of the villages in Northern Vietnam. Interestingly, the plants grown by different farmers showed distinctly different multilocus genotypes. One of the clones had the same genotype as the improved *KM94* (Figure 11). This indicates either that the starch factory had been distributing different clones under the same name of which one was the improved variety *KM94*, or that the name had been changed or mixed up with *sắn Vedan* by the farmer, since it may have been known that *sắn Vedan* was an improved variety distributed by the factory.

Besides the variety *KM94* and one clone of *sắn Vedan*, plants of the named improved variety *sắn cao sản* belonged to the same clone (Figure 11). Cassava plants of the same clone grown by different farmers under different names may indicate that some farmers' have renamed varieties after dissemination. For example, the farmers' in the two villages in Northern Vietnam used the Vietnamese name *sắn cao sản*, instead of the name *KM94* given by the breeding program. Based on the interviews the Vietnamese name for the variety means high-yielding and describes the main characteristics for improved varieties in general. Thereby it may be more appropriate for some farmers to use the Vietnamese name than a variety code without any meaning for the farmer. The use of distinct characteristics for differentiating between varieties has been discussed above and seemed to be even more used by farmers in Northern Vietnam cultivating a larger number of varieties than the farmers in Central Vietnam. The cassava farmers in Central Vietnam were also more close to the breeding programs and the disseminators of the newly improved varieties, which may influence the level of information received by the farmers.

4.2 No distinct genetic structure on different geographical levels

As described above the same cassava clones of several varieties are grown by different farmers in different villages both in Northern and Central Vietnam. No distinct genetic structure in cassava was therefore seen on different geographical levels. However, the largest differentiation was found among cassava on different farms ($F_{st}=0.11$) compared to the differentiation among villages ($F_{st}=0.05$) and between cassava grown in Northern and Central Vietnam ($F_{st}=0.02$). For increasing the genetic diversity in the cassava germplasm banks and the gene pool for breeding, different cassava fields on a farm should therefore be targeted for collection rather than randomly collecting cassava throughout Vietnam.

4.3 Farmers' preferences and selection criteria

In Central Vietnam, the variety *KM94* was the dominating variety cultivated by farmers, which was understood by the strong connection to the local starch industry. In fact, all newly improved varieties, grown in Central Vietnam, *KM94*, *KM98*, *Thailand*, *new* and *sản Vedan*, were disseminated from the starch factories. As a consequence four out of the nine farmers only grew one variety, *KM94*. The influence of the starch industry in Central Vietnam was highly due to the proximity to the market and factories. The starch industry had therefore a strong influence on the genetic diversity and composition of cassava varieties grown by the farmers in Central Vietnam. In contrast, preferences in Northern Vietnam were more scattered, showing larger number of varieties used for human consumption. Nevertheless, this was not reflected by the size of the area restricted to these varieties, showing that yield overruled other preference.

Interestingly, when farmers were asked to name outstanding characteristics of their varieties, they only once named high yield and never mentioned starch content. But one important trait, resistance against pests and diseases, was mentioned by one third of the farmers and may therefore be considered a strong selection criterion provided that there are resistant varieties available. This is one of the important breeding goals for cassava. Low temperature during the growing season was mentioned as biggest concern for one third of the varieties. Consequently, low temperature can be seen as a natural selection factor for cassava grown in both Central and Northern Vietnam.

Most varieties were described as sweet, but this characteristic was not directly mentioned as a preferred trait (Table 8). Two farmers were not able to clearly distinguish between sweet and bitter varieties. The contrasting statements by farmers might indicate differences in sensitivity and knowledge about this factor. Women are traditionally processing the roots for human consumption, even in Vietnam, which enforces their knowledge and sensitivity towards the distinction between bitter and sweet varieties. Yet gender analysis in this regard did not point into any distinct direction. The contrasting statements might therefore be explained by different personal standards of the categorization and the possibly decreasing relative importance of sweet cassava for human consumption in Northern and Central Vietnam.

4.4 Improved varieties have replaced local varieties

Small-scale farmers in Northern and Central Vietnam had widely adopted newly improved varieties. However, some farmers were still growing old local varieties in more limited areas. All local cultivars were grown for several generations within the families and are both grown in Northern and Central Vietnam.

In Asia, the FAO estimates that improved varieties of cassava are grown on 55% of the area (Howeler *et al.*, 2013). For more than two decades improved varieties were distributed to a large number of households in Vietnam. In 2008, *KM140*, *KM98-7* and *KM21-12* were disseminated to farmers in Northern Vietnam (Kim *et al.*, 2010). However, none of the farmers in this study named any of these improved varieties, but that may be explained by reasons discussed above. In 2009, improved varieties were grown on more than 90% of the total cassava area of Vietnam (Kim *et al.*, 2010). In this study 93% of the area investigated in Northern and Central Vietnam was cultivated with improved varieties, which shows that this dataset does support the above-mentioned number. The trend of

cultivating new varieties in favour of old varieties may reflect a need for better varieties (Balyejusa Kizito, 2006). It is, however, more likely that it is under influence of the decisions made by the government and the market including the starch industry. This has, for example, resulted in the extensive cultivation of *KM94*, grown for the production of starch, covering 51% of the land investigated in this study.

4.5 Local varieties- potential genetic resources in breeding

The reduction of local varieties in farmers' fields after the introduction of high-yielding crops is not unique to Vietnam, but can be seen in many countries, leading to reduced agrobiodiversity. However, the local varieties grown by the Vietnamese farmers in this study showed only slightly higher genetic diversity than the improved ones (Table 12). More important is the genetic differentiation found between the local and improved varieties ($F_{st}=0.11$), which indicates that these two groups of varieties carry partly different genotypes. It is therefore crucial to preserve the existing local variety germplasm before this genetic diversity is lost in favour of continuous adoption of new improved varieties by the farmers. The increased diversity in the breeding gene pool is especially important in view of the growing global human population and ongoing climate change. Further investigations on the importance of local varieties as genetic resources for improvement of traits such as resistance to abiotic (drought, heat, salt) and biotic stresses (pest and diseases) in Vietnamese cassava are needed. Moreover, local varieties are important for their cultural value in e.g. traditional recipes.

4.6 Vietnamese accessions in the CIAT Germplasm Bank

The CIAT germplasm collection aims to build a genetic resource for the breeding of cassava adapted to climate changes and farmers' needs. The genetic composition and diversity of the eight local Vietnamese accessions at the CIAT Germplasm Bank was investigated in this study. Seven of the eight tested accessions originate from Northern Vietnam. Some of the accessions showed genetic similarity. Moreover, all accessions grouped in the same main cluster of the hierarchical tree (Figure 11). This shows that much of the diversity in Vietnamese cassava cultivated today is not represented in the CIAT Germplasm Bank. For further collection of germplasm of Vietnamese local varieties, in order to discover a larger diversity, it may, as discussed above in paragraph 4.2, be more fruitful to put emphasis on collecting samples of cassava from different fields grown by single farmers, rather than to randomly collect samples dispersed over a wider area.

4.7 Cassava cultivation and diversity in a changing climate

Genetic diversity of cassava will be crucial for the adaptation to the predicted climate changes as different genotypes will most likely demonstrate different responses to new pests and diseases and to extreme weather events such as drought and flooding (Jump & Penuelas, 2005; Howeler et al., 2013). In Vietnam this is of special importance, as it will be tremendously affected by climate change. Cassava may therefore increase in its cultivation and use due to its low-input system, low intensive cultivation, good adaptation to dry and warm environments and the predicted increasing availability of land suitable for cassava at higher altitudes. It may even be an alternative starch crop for marginal areas where maize and rice cultivation may not be possible anymore or due to e.g. flooding in coastal areas and deltas. If cassava will be needed to grow on more hillsides with unpredictable rainfalls, problems with soil fertility and erosion are important to address in the future as cassava is considered to be an erosion prone crop. More sustainable cultivation techniques may need to be implemented. In Northern and Central Vietnam farmers have not mentioned problems with soil fertility or erosion and sustainable cultivation techniques like intercropping were observed. However, over the last years cassava cultivation has already shifted towards Central and Southeast Vietnam due to soil fertility and erosion problems (Kim *et al.*, 2010).

4.8 The conservation dilemma

Conservation of genetic resources is a very important issue and it is yet a challenging one as it encompasses the conservation of populations that are subjects to ongoing evolutionary processes constantly changing the diversity in the field.

In order to save threatened genetic diversity from loss and enable its availability in the future, *ex situ* collections are very important. However, germplasm collections need to be screened for genetic information to increase knowledge and ensure a good representation of the diversity in the distribution area of the species. Through this study a need for extending the conservation of Vietnamese germplasm was identified.

Ex situ conservation is certainly important, yet limitations in costs, time and space are omnipresent, and, in addition, a constant adaptation to a changing climate is not given. To encounter some of those limitations CIAT started to produce cassava seeds through self-pollination for conservation purposes (Commission on Genetic Resources for Food and Agriculture, 2010).

Nearly all cassava-growing countries depend primarily on field-grown plants next to an *in vitro* collection (Commission on Genetic Resources for Food and Agriculture, 2010). Creation of *in situ* reserves of wild cassava relatives has not yet been realized but may play a key role for preserving genetic resources for future breeding (Commission on Genetic Resources for Food and Agriculture, 2010). In Vietnam the on-farm conservation of cassava can ensure a constant adaptation to a changing climate. This is however faced with the problem of interests from many stakeholders including farmers, the starch industry, breeders and the Vietnamese government. As we found most genetic differentiation among farmer's fields, small-scale farmers may show high potential as target groups for on-farm conservation programs. In order to conserve and even enhance local knowledge and genetic diversity, while enabling the availability of on-farm diversity, new approaches are needed.

4.9 Access on-farm diversity for breeding

For breeders, access and information about the varieties available for breeding is crucial in their work. As pointed out earlier (see 1.5.2. Cassava breeding in Vietnam), in Vietnam breeding material from other countries dominated in earlier breeding programs (Kim *et al.*, 2010). Yet a number of local Vietnamese varieties with unique genetic diversity, may be of high importance as genetic resources for future breeding. Participatory Plant breeding (PPB) may be one approach to access the diversity on farmers' fields while including the farmers in the breeding process. In PPB, farmers are motivated to experiment, breed and thereby develop their own variety adapted to the farmers' needs, preferences and agro-ecological zones, in collaboration with professional breeders (Almekinders *et al.*, 2008). This exchange between farmers and breeders may also enhance knowledge about farmers' own varieties as well as about breeders' varieties, benefiting both parties.

Agriculture in Vietnam has been increasingly feminized with 62% of women and 52% of men working in agricultural production (World Bank, 2010), which is a consequence of male migration due to difficulties in agricultural development, natural disasters and environmental crisis (Trung, 2013). Woman and children are often left behind in small-scale agriculture which changes women's work tasks, while facing inequalities in access to land and water (World Bank, 2010). It is a predominantly female task to process the cassava for human consumption in households also in Vietnam. In PPB, programs need to consider this gender perspective on communication patterns, time schedules and gender relations. Since cassava in Asia is mostly sold to the starch industry, female entrepreneurship in this regards is a future possible perspective and could be promoted by the government and fostered by starch factories in Vietnam.

5 Conclusion

In Vietnam, cassava is nowadays predominantly grown as a raw material for the starch industry and ethanol production, which showed to boost rural development. However, in the face of a changing climate and growing population size its importance as a staple crop for food security may return due to its drought tolerance, low-input system and ability to grow at high altitudes on sloping soils. This shows again how complex the future needs of small-scale farmers in Vietnam are, enhancing the importance of genetic diversity in order to breed and conserve for a sustainable future. The understanding of interactions between farmers' management and genetic diversity on the farmers' fields is crucial for the development of strategies for conservation and breeding which will serve future needs of small-scale farmers. The interdisciplinary approach used in this study is necessary to detect the actual genetic composition and to explore farmers' cultivation practises. By comparing and contrasting these datasets several conclusions can be drawn.

Vietnamese farmers cultivated clones to a great extent and were able to distinguish their varieties. However, genetic variation was detected in some farmers' fields. Farmers were able to conserve their varieties as clones over long periods of time. In some cases plants of the same clone could be referred to by different names by different farmers. In contrast, plants of the same clone were in some other cases named differently by different farmers in Central and Northern Vietnam.

Farmers were growing local and improved varieties. According to the interviews, farmers in Northern Vietnam appreciated local varieties yet improved varieties were grown over much larger areas. Starch factories heavily influenced the decision over what was grown in the farmers' fields in Central Vietnam, showing that they were key players in this process.

Two distinct selection criteria were mentioned by the farmers, resistance to pests and diseases and resistance to low temperatures during the growing season, which shows that these are two important traits possibly affecting the genetic composition and diversity of cassava.

Genetic differentiation between local and improved cassava suggests that local varieties harbor partly unique diversity, which make them potential genetic resources for breeding and therefore needed to preserve. In addition, this will greatly increase the small diversity found in the Vietnamese accessions in the CIAT Germplasm Bank.

The number of stakeholders in Vietnamese cassava is very high and includes farmers, breeders, consumers, the starch industry and the Vietnamese government. This indicates a challenge for all future conservation and breeding approaches.

Thanks to the approach used in this study with a combination of interviews and genetic marker analysis, it was possible to detect more clones and diversity using the genetic analysis than what was expected solely from the naming by the farmers. The approach used in this study was based on the varieties named and provided in the fields. If this had not been available, the genetic part of the study had been designed differently and a significant part of the information for the interpretation and relevance of the results had been lacking. The approach also made it possible to understand the structure of diversity on different geographic levels and thereby provide information about varieties/clones that could be used for breeding and conservation.

The results of this study enhance the importance of understanding the farmers' influence on the genetic composition of cassava. It also highlights the interaction of farmers' management and genetic diversity to accurately identify the genetic composition and organisation of cassava plants in the field, as more clones and diversity were detected using the genetic analysis than expected solely from the naming by the farmers.

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