PRODUCTION STATUS AND GENETIC CHARACTERIZATION OF ERITREAN PEPPER (*Capsicum spp*.)

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DOCTOR OF PHILOSOPHY

(Biotechnology)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2016

Production Status and Genetic Characterization of Eritrean Pepper (*Capsicum spp*.)

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A Thesis Submitted in Fulfillment for the Degree of Doctor of Philosophy in Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2016

DECLARATION

This thesis is my original work and has not been presented for a degree in any university.

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DEDICATION

I would like to dedicate this work to my beloved mother, Rahma Hassen**,** who devoted her life to me and continuously supported me throught my education. To my beloved late father, Khiar Saleh, who did not get chance to see this achievement.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof. Aggrey Bernard Nyende, Dr. Remmy Kasili, Dr.George Edward Mamati and Prof. Woldeamlak Araia for their supervision, guidance, critical comments, and unlimited supported throughout the work period.

My deep gratitude to Mr. Semere Amlesom, Dean Hamelmalo Agricultural College for his continous support and encouragement and great efforts for securing experimental plot for the second site. Thanks to Prof. Zemenfes Tsige, for manging the fellowship throughout the study program and Semere Simon and Dawit for facilitating the financial issues in Eritrea. Extremely deep gratefulness to Abddela Omer, Abdulrezak Hamde and Kiflom Tesfamichael for their field and lab assistance and endless moral support. My Gratitude extends to Ato Kahsai Gebrehiwet, Governor of the Central Zone and Ato Afwerki for provision of experimental land in Asmara and Ato Abraha G/Meskel for securing space for after harvest data collection. Thanks to Girmay and Amanuel Mahdere for the genebank storage support. Thanks to Dr. Iyassu G. Director General of NARI, Tsegay Berhane, Mussie Fekadu and Andom Kafel for provision of seed and documents, and providing soil analysis services.

I acknowledge the support of the staff in the Department of Horticulture, Department of Plant Protection and Administration and Finance of Hamelmalo Agricultural College for provision of land, irrigation facilities and laboratory service for all the field experiments at Hamelmalo. Special thanks to the Staff of Department of Meteorolgy, Asmara International Airport for provision Meteorological data. My thanks also extends to all staff members of the Ministry of Agriculture at the headquarters, regional and sub-regional offices for provision of documents, data and technical support during the survey and seed collection. Thanks also to Ministry of Finance, Department of Customes, Ministry of Trade and Industry and National Statistics office for provision of secondary data.

I thank all the technical and administrative staff at the Institute of Biotechnology Research and the Department of Horticulture of the Jomo Kenyatta University Agriculture and Technology for their support, encouragement and technical advise. I would also like to thank the African Development Bank (ADB) and the National Commission for Higher Education (NCHE) of Eritrea for the funding my study. The African biosciences challenge fund (ABCF) in the Biosciences eastern and central Africa (BecA) for funding the molecular characterization experiments and the staff of BecA and the Internationl Livestock Reseach Institute (ILRI) for their technical and moral support.

At last but not leaset I would like to express my deep appreciation and thanks to my wife Lemlem Saleh for taking responsibility of our family and tolerating my absence for extended periods and to my children Amel, Nesredin, Majed and Sefae who tolerated my absence. Finally thanks to my brother Saleh Khiar and his family for the continuous support and encouragement.

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LIST OF ABRIVATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
AVRDC	Asian Vegetable Research and Development Centre
FAO	Food and Agriculture Organization of the United Nations, Rome.
FAOSTAT	Statistical division of FAO
GD	Gene diversity
HAC	Hamelmalo Agricultural College
H_o	Observed heterozygosity
IBPGR	International Board for Plant Genetic Resources
IPGRI	International Plant Genetic Resource Institute
KALRO	Kenyan Agricultural and Livestock Research Organization
MAF	Major allele frequency
MoA	Ministry of Agriculture, Eritrea
MoWLE	Ministry of Water, Land and Environment
Na	Number of alleles
NARI	National Agricultural Research Institute, Eritrea
Nm	Number of effective emigrants
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats

ABSTRACT

Pepper is among the most widely grown and consumed spice and one of the most important vegetable crops in Eritrea as well as the world. In Eritrea it is an ingredient in almost all traditional dishes of the country, thus it is characterized by its high demand throughout the year. Locally produced pepper is of low quality and low productivity which necessitates intervention for improving it. Thus this research aimed: To study the current status and opportunities of pepper production in Eritrea, the morphological and molecular characterization of locally available germplasm and its relatedness to selected reference germplasm from other countries and to evaluate the breeding potential of a local variety crossed with exotic varieties.

Current status of pepper production in Eritrea was assessed using a participatory rural appraisal method, collection of secondary data, key informants interviews, focus group discussions and formal household survey The major constraints identified were unavailability of improved and quality seed, inputs and services, insect pests and diseases, small acreage and discouraging land tenure system, improper marketing chain, poor extension service and shortage of water. Opportunities were identified as availability of vast lands, favourable climate, domestic and export markets and high willingness of farmers to grow pepper.

During the survey a total of 129 seed samples were collected from farmers and institutions for diversity studies. The collected germplasm was evaluated at two sites; Hamelmalo and Asmara located in two different agro-climatic regions of Eritrea. A randomized complete block design was used in each evaluation. Data was collected on 39 quantitative and qualitative characters. Data were subjected to Analysis of variance, Principal Component Analysis, Principal Coordinate Analysis and Hierarchical clustering with Euclidean distance. Phonological attributes and fruit characteristics (number of fruits per plant, fruit weight, fruit wall thickness, calyx annular constriction and Fruit shape at both pedicel attachment and blossom end), were found to contribute most of the variation. Genotype and location had significant effect on majority of the characteristics evaluated; but the interaction between them was not. The highest Coefficient of Variation was related to fruit characteristics.

Based on the combined data from the two sites the collections were grouped into four clusters. Cluster one was characterized by intermediate to erect growth habit, presence of calyx annular constriction, elongate fruit shape, mainly light red or dark red but sometimes orange red, light brown and brown mature fruit colour and neck at base of fruit was present or absent. Fruits of this group were relatively short, medium in fruit width and pericarp thickness and an average fruit weight of 15.06 g, number of fruits/plant of 45.28 and yield/plant of 419.68 g. Cluster two was characterized by mainly erect with intermediate growth habit, calyx annular constriction was rarely present, fruit shape was elongate, mature fruit colour was usually light red but also dark red, brown and rarely light brown and neck at base of fruit was present or absent. Fruits were intermediate in length, slim and relatively thin pericarp with an average fruit weight of 10.56g, number of fruits/plant of 62.63 and yield/plant 392.48. Cluster four was characterized by Erect or intermediate growth habit, calyx annular constriction was present but some times absent, triangular or elongate fruit shape, light red mature fruit colour, sometimes dark red and rarely brown and neck at base of fruit was usually absent but sometimes mixed. Fruits in this group were intermediate in length, wide and relatively thick pericarp with an average fruit weight of 25.46g, number of fruits/plant of 39.24 and yield/plant of 637.44g.

A total of 150 seed collection were evaluated using 28 SSR markers. The 28 polymorphic markers revealed existence of high genetic variation among Eritrea genotypes and that germplasm maintained in situ by farmers are heterogeneous. A total of 352 alleles were obtained with an average of 13 alleles per marker, Mean Polymorphic Information Content was 0.62, mean Genetic Diversity was 0.65 and mean Observed Heterozygosity was 0.4. A large number of rare alleles were also observed. A PCoA analysis, neighbour joining clustering and the model based clustering (Structure) classified the collections into 3 groups. However, in the model based clustering; increasing the number of populations to 4 (K=4) moved all the non-Eritrean genotypes in a separate cluster. This suggests that the Eritrean populations are specific since the collections studied had a large number of private alleles.

CHAPTER ONE

INTRODUCTION

1.1. Background

Pepper (Capsicum spp.) is one of the oldest domesticated and utilized crops. Its use dates back to more than 7000 years in Mexico and is believed to have originated in tropical America (Andrews 1999 and Bosland 1996) Pepper is a perennial small shrub in suitable climatic conditions, living for a decade or more in tropical South and Central America (Bosland et al., 1996). Pepper types usually are classified by fruit characteristics such as pungency, colour, shape, flavour, size, and their use (Bosland, 1996). Most commercially cultivated pepper lines in the world belong to the species, C. annuum which is characterised by its wide range of variability. However, cultivars belonging to C. frutescens and C. chinense are also currently widely cultivated (Eshbaugh, 1993). Based on pungency peppers are divided into hot and bell or sweet. The hot peppers are referred to as chilis. The major difference of the two types is capsaicin content which is responsible for the pungency, however within each type variety of sizes, shapes and colours exist (Berke et al., 1999).

1.2. Production

Pepper is one of the important vegetable and spice crops. According to Bosland et al. (2012), pepper is produced in most countries of the world and has showed substantial increase over the years as the production of the top 20 producers of the world increased from 16.74 million tons in 1998 to 25.59 million tons in 2008. Recent data show that the increase in production and productivity is still continuing (Tables 1.1 and 1.2). The world production of green and dry pepper was approximately 31.2 and 3.4 million, tons harvested from 1,91 and 1.99 million hectares respectively, with an average yield per ha of 16.3 tons green and 1.7 tons dry pepper (Tables 1.1 and 1.2).

For green pepper, China with 16 million tons was the most important producer and had the largest cultivated area (709,150 ha). Among the top 10 producers, the highest productivity (56.6 t/ha) was recorded in Spain, while the lowest (2.7 t/ha) was

recorded by Ethiopia (Table 1.1). India was the most important dry pepper producer (1.3 million tons) and has the largest cultivated area (793,590 ha). Peru (10.9 t/ha) recorded the highest productivity among the top 10 producers, while the lowest (0.33 t/ha) was recorded in Ethiopia (Table 1.2).

Table 1.1: Green pepper production, cultivated area and yield/ha of the top 10 producers in the world.

Country	Production (ton)		Area (ha)		Yield (t/ha)	
	2011	2012	2011	2012	2011	2012
China	15,541,611	16,023,500	707,086	709,150	22.0	22.6
Mexico	2,131,740	2,379,736	144,391	136,132	14.8	17.5
Turkey	1,975,269	2,072,132	93,826	96,000	21.1	21.6
Indonesia	1,903,229	1,656,615	239,770	242,196	7.9	6.8
U SA	991,370	1,064,800	301,10	30,880	32.9	34.5
Spain	921,089	1,023,700	17,739	18,100	51.9	56.6
Egypt	670,434	650,054	39,666	39,819	16.9	16.3
Nigeria	449,594	500,000	57,382	60,000	7.8	8.3
Algeria	384,267	426,566	21,272	22,605	18.1	18.9
Ethiopia	305,221	402,109	115777	147,092	2.6	2.7
World	30,063,389	31,171,567	1,865,626	1,914,685	16.1	16.3

Source: *FAOSTAT, 2015* *USA= United States of America*

Table 1.2: Dry pepper production, cultivated area and yield/ha of the top 10 producers of the world.

Country	Production (ton)		Area (ha)		Yield (t/ha)	
	2011	2012	2011	2012	2011	2012
India	1,276,301	1,299,940	804,792	793,590	1.6	1.6
China	282,342	290,000	42,773	43,000	6.6	6.7
Peru	171,929	175,000	15,683	16,000	11.0	10.9
Bangladesh	176,134	172,000	104,967	99,000	1.7	1.7
Pakistan	140,414	150,000	64,776	65,000	2.2	2.3
Thailand	152,000	145,000	68,000	65,000	2.2	2.2
Myanmar	124,321	128,000	131,783	132,000	0.9	1.0
Ghana	88,000	100,000	13500	14000	6.5	7.1
Ethiopia	95,000	100,000	330,000	350,000	0.3	0.3
Benin	38,542	67,760	19,722	24,351	2.0	2.8
World	3,244,251	3,352,163	1,976,351	1,989,664	1.6	1.7

Source: ***FAOSTAT, 2015***

1.3. Problem Statement

Only small amount of the pepper consumed in Eritrea is produced locally and is mainly consumed as fresh pods. The bulk of the dry pepper is imported from Ethiopia, Yemen and recently China, demanding foreign currency and resulting in scarcity and price escalation in many occasions. Attempts are ongoing to substitute the imported pepper with locally produced ones. In recent years both the cultivated land and production notably increased, however yield per hectare and quality is still low (Table 1.3). Data in Table 1.4 show a dramatic decrease in productivity from 10.6 tons/ha in 2008 and 2009 to 3.7 tons/ha in 2011. This is 45% of the yield/ha recorded in 1968 (8.2 t/ha). Considering differences in production technologies between 1968 and 2011 the observed decline in productivity is huge. The current yield per hectare is not only far below the world averages, but also below the average of Africa and lower than most of the eastern African countries (Table 1.4). Similarly average yield/ha for Africa and all eastern African countries (except Tanzania) is considered very low compared to the world average (Table 1.4).

In Eritrea poor agronomic practices by farmers and unavailability of improved varieties among others are of the main reasons for the low productivity (MoA, 2002 & 2006). However, in the absence of high yielding, disease and insect resistant or tolerant cultivar(s) that meet consumer preferences, the role of improved agronomic practices in improving yield and quality is limited. This partially helps explain why efforts of the Ministry of Agriculture to improve agronomic practices could not improve the yield and fruit quality. Instead yield/ha decreased to 3.7 t/ha in 2011, thus intervention is an at most necessity for making improved varieties available (Ministry of Agriculture, 2011).

Farmers in many parts of Eritrea have been growing pepper for a long time; many of them saved their own seeds. A survey conducted in pepper markets in Asmara and Keren showed that the local pepper offered in the market is of mixed pods containing wide range of fruit size, colour, pungency etc. Although this reduces the market value of the commodity which affects the income of growers; it reflects the rich

genetic variation apparent observed and not confirmed existing in the local germplasm, hence high breeding potential by selection and hybridization to combine desirable characteristics. However, the magnitude of this diversity has not yet been studied. In addition to that no documentation is available that estimate and describe the existing diversity of pepper genotypes saved by farmers and research institutions in different parts of Eritrea.

Table 1.3: Green pepper cultivated area production and productivity in Eritrea 1957-2011

Year	Area (ha)	Production (ton)	Yield (ton/ha)
1957†	136.4	99	7.25
1958†	117.4	70	5.9
1959†	117.4	84	7.2
1960†	161.5	84	5.2
1968†	1045	8482	8.12
2003‡	1093	11021	10.1
2004‡	1168	11681	10.0
2005‡	2734.2	20303.4	7.4
2006‡	2188.2	19453.2	8.9
2007‡	2740.3	14575.7	5.3
2008‡	2854.2	30309.4	10.6
2009‡	1744.1	18571	10.6
2010‡	2,874	21,010	7.3
2011‡	4,132	15,118	3.7

†*Source: Ministry of Agriculture, Ethiopia (Annual agricultural reports, 1958-1969)*
‡*Source: Ministry of Agriculture, Eritrea (2012)*

Table 1.4:Pepper cultivated area, production and yield for some east African countries compared to the world and African averages in 2010 and 2011

Country	Commodity	Area (ha)		Production (ton)		Yield (ton/ha)	
		2010	2011	2010	2011	2010	2011
Eritrea†	Green	2,873.55	4,132	21,009.9	15,118	7.3	3.70
Ethiopia‡	Dry	431,000	330,000	141,200	95,000	0.33	0.29
	Green	97,712	115,777	237,700	305,221	2.43	2.64
Kenya‡	Dry	4,400	2,755	4,800	2,832	1.1	1.03
	Green	1,300	757	5,900	4,230	4.54	5.59
Sudan‡	Dry	7,247	9,440	6,726	10,393	0.93	1.10
	Green	1,560	1,716	12,700	13,335	8.2	7.77
Tanzania‡	Dry	4,409	3,324	6,300	7,000	1.43	2.11
	Green	500	456	11,500	13,880	23	30.44
Uganda‡	Dry	3,700	2,269	3,700	2,152	1	0.95
Africa‡	Dry	618,478	518,042	536,657	558,688	0.87	1.1
	Green	358,320	321,053	2,684,451	2,525,649	7.49	7.9
World‡	Dry	1,918,203	1,976,351	3,054,861	3,244,251	1.6	1.6
	Green	1,859,597	1,865,626	27,552,507	30,063,389	14.82	16.1

† Source: Ministry of Agriculture, Eritrea (2012) ‡ Source: FAOSTAT (2015)

1.4. Justification

Pepper is an important ingredient in the daily use of Eritreans foods. A survey conducted in 2002 shows that the average weekly household consumption of dry pepper was about at 140 grams (National Office of Statistics, 2002). This implies that positive or negative changes in the supply of this commodity to markets and its quality will affect the prices and in turn will affect the consumer. On the other hand, the majority of pepper growers in Eritrea are small scale farmers. Thus large numbers of farmers are affected by the low productivity and quality of the pepper they grow.

Although exact figures are not available; Eritrea imports large quantities of dry pepper which requires a considerable amount of foreign currency. In 2011, the value of the imported pepper was more than US$ 10 million (Ministry of Finance, 2012). This can be saved if enough quantity and quality pepper can be produced locally. Potential export markets were also identified in the neighboring Middle East countries and some distant European countries if a good quantity and quality pepper produced. This can help in foreign exchange.

In Eritrea pepper breeding and improvement have been limited. As a result farmers are still dependent on non-improved local genotypes that are poor yield. This is one of the factors that contribute to the low production and productivity as well as fruit quality of pepper. Eritrea has six agro-ecological zones. Pepper is grown almost in all the zones. Therefore, it is expected that genotypes adapted in a different location or zones are available. Due to exchange of germplasm among the different regions; some genetic relationship may exist among the genotypes growing in different agro-ecological zones; however, the magnitude of these relationships is unknown. Existence of wide genetic diversity gives Eritrea an advantage for developing new improved varieties from locally available genotypes. Therefore, there is need for information regarding the genetic diversity of the local genotypes and the current status of pepper production in the country, so that an effective breeding program is planned. Studies were conducted in different parts of the world for studying diversity of genotypes. Examples are Baral and Bosland (2002b) in Nepal, Votava et al.

(2005) in New Mexico, Adetula and Olakojo (2006) in Nigeria, Fonseca et al, (2008) in Brazil and Balkaya and Karaagc (2009) in Turkey.

Thus there is an urgent need for identifying the existing local genotypes, characterize them morphologically and molecularly and evaluate the breeding value of selected genotypes for using them in variety development and improvement programs. In addition to the updating information on the current status, constraints, and potentials of pepper production is required for better planning of breeding and improvement programs.

1.5. Objectives

General objective

To evaluate the potential for improving Pepper using available germplasm in Eritrea.

Specific objectives

1. To document the current production opportunities and constraints of pepper in Eritrea.
2. To evaluate local hot pepper germplasm for diversity using morphological means
3. To evaluate local pepper germplasm for diversity using molecular techniques.

1.6. Null Hypothesis

- No opportunities or constraints for pepper production exist in Eritrea
- No genetic difference exists among local pepper germplas

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and distribution

Pepper (*Capsicum spp*.) is one of the oldest crop plants used by humans. It was domesticated in the Americas almost 7000 years ago (Bosland, 2010). It is difficult to precisely define the original geographic distribution of peppers (Walsh and Hoot, 2001), however, it is believed that the pre-Columbian distribution of Capsicum extends from southern United States to the southern part of South America (Moscone et al., 2007). *Capsicum* probably evolved from an ancestral form in the Bolivia/Peru area (Eshbaugh, 1993). Before the arrival of Columbus, the five domesticated *Capsicum* species were widely cultivated in tropical America (Pickersgill, 1997) Later they had worldwide distribution and the hot pepper spread into tropical Asia and tropical Africa, while the sweet pepper spread into the temperate zone of Europe and Northern America (Pickersgill, 1997 and Eshbaugh, 1993). Of the five domesticated species three (*C. annuum, C. chinense* and *C. frutescens*) are worldwide cultivated, while *C. baccatum* var. *pendulum* and *C. pubescens* are confined to Southern America (Pickersgill, 1997). Eshbaugh (1983) found that pepper was introduced to Africa through three routes viz., the new world to Europe then secondary introduction to Africa, through the slave routes and through botanical gardens. Among the cultivated varieties *C. annuum* is the most variable (IBPGR, 1983). Although wide variety of fruit size, shape, colour, pungency are observed, most of the peppers cultivated in the world belong to *C. annuum*, however, *C. chinense* and *C. frutescens*, which are closely related to *C. annuum* are also widely cultivated (Mongkolporn and Taylor, 2011).

2.2. Taxonomy

Pepper belongs to the genus *Capsicum* which is a member of the Solanaceae family that includes tomato, potato, tobacco, eggplant, and petunia. There is a lot of argument in the taxonomy of the genus *Capsicum*; the number of species was reported to be 20 (Pickersgill, 1971), 25 species (Eshbaugh, 1993) or approximately

27 (Bosland, 1996). Baral and Bosland (2002a) updated the synthesis of the genus *Capsicum* and found a lot of iteration in its nomenclature, however, they reported existence at least of 27 species, while Moscone et al. (2007) found these to be at least 31 species. Generally it is accepted the genus *Capsicum* consists five domesticated species; *C. annuum, C. baccatum, C. chinense, C. frutescens*, and *C. pubescens (*Baral and Bosland, 2002a). However, Eshbaugh, (1993 and 1980) argued that the *C. annuum, C. chinense and C. frutescens* are not distinct species but accepted only four species considering *C. frutescens* can fit with either *C. annuum* or *C. chinense.* The domesticated species of *Capsicum* belongs to three distinct and separate genetic lineages (Moscone et al., 2007 and). Capsicum species are organized into three complexes viz, the annuum complex, the baccatum complex and the eximium complex (Moscone et al., 2007). The annuum complex is composed of *C. annuum, C. chinense, C. frutescens* and the wild species C. galapagoense and *C. chacoense.* Members of the baccatum complex are *C. baccatum* and the wild species *C. tovarii* and *C. praetermissum.* The eximium complex members are *C. pubescens* and the wild species *C. eximium and C. cardenasii* (Bosland et al., 2012, Mongkolporn and Taylor, 2011 and Moscone et al., 2007). Hybridization among species within the same complex is easy while crossing among species from different complexes ranges from difficult to not possible unless a bridge species is used (Mongkolporn and Taylor, 2011).

2.3. Plant description

Capsicum is a perennial small shrub in suitable climatic conditions, living for a decade or more in tropical South and Central America (Bosland, 1996), however, the annum complex species may exhibit biennial herbaceous forms and other species may grow to a tree (Moscone et al., 2007). Plant forms include prostrate, compact or erect with stem and leaf pubescence ranging from glabrous to abundant (IBPGR, 1983). Corolla colour in *Capsicum* is variable including white, yellow, purple, violet or mixture, however corolla of the cultivated species is white or cream white in the *annuum* complex species, white with yellow spot at the base in *C.baccatum* and violet/purple or white diffused with violet/purple in case of *C.pubescens* (Moscone et

al., 2007, Eshabough, 1993 and IBPGR, 1983). Pepper fruits are considered vegetables, but are berries botanically (Bosland, 1996). Fruit flesh is firm in all cultivated *Capsicum* species except in *C. frutescens* and some varieties of *C.annuum* it is soft (IBPGR, 1983). Bosland (1996) mentioned the existence of hundreds of pepper pods of the cultivated species with a wide range of fruit shape, size and colour with some of them cultivated for fresh consumption and others used in dry forms. Fruit pungency; the most important flavour traits of peppers is characteristic of the genus *Capsicum* and it is due to a mixture of compounds known as capsaicinoid (Rodriguez-Burruezo et al., 2010). With respect to pungency *C.annuum* is the most variable and both *C. chinense* and *C. frutescens* are the highest and *C.baccatum* the lowest while *C.pubescens* is mild (Rodriguez-Burruezo et al., 2010).

2.4. Flower biology and pollination

Flowers in pepper are protogynous but plants are self pollinated (Pickersgill, 1971), however, out-cross in pepper ranges 0.5% to 91% (Bosland et al., 2012, do Rego, et al., 2012 and Bosland 1996). Due to this high out-cross pepper is considered not purely self-pollinated but facultative cross-pollinating plant. Knowledge of the amount of cross pollination is necessary for determining the precaution required in seed production and breeding (Bosland, 1996). Cross pollination is usually by bees, less often by other insects and rarely by wind, thus proper isolation technique is required for producing pure lines (Berke, 2000).

Crossability among species within the complex is easy compared to crossing among species from different complexes (Mongkolporn and Taylor, 2011).The degree of crossability among species from different complexes varies according to the crossing combinations (Moscone et al., 2007 and Pickersgill, 1971). In some cases even when viable seed is produced additional barriers may prevent gene exchange among the species (Pickersgill, 1971).However, crossing among species that are too difficult to cross may require the use of bridge species. The use of *C. chinense* has been used as a bridge between *C. annuum and C. baccatum* is an example for solving that problem (Mongkolporn and Taylor, 2011). Success of intercross between *C. annuum* and *C.bccatum* using double pollination and treatment of female gametophyte with

nitrous oxide (N2O) gas four to six hours before pollination has been described by Greenleaf (1986).

Flower opening (anthesis) in pepper takes place during the first three hours after sunrise, however, a smaller anthesis may happen in the afternoon time and the flowers remains open until evening then close to open next morning (OCED, 2006 and Raw, 2000). Anthesis is basically influenced by daylight (OCED, 2006) and temperature (Raw, 2000). Dehiscence may be delayed to late morning or even sometimes anthers may fail to dehisce (OCED, 2006 and Raw, 2000). Usually anther opens one hour after anthesis and continues for about ten hours. Receptivity of stigma extends 5-8 days starting a few days before flower opening to few days after opening with a peak in the day of opening (OCED, 2006). Environmental factors, especially air temperature affect pollen formation and viability. Optimum temperature is 20-25 oC, temperatures above 30 oC may cause sterility, while temperatures lower than 12 oC reduce number and germinability of pollen (OCED, 2006). Pëkozdi et al. (2002) evaluated nine lines with cytoplasm male sterility, four restorers and their F1 hybrids over three seasons (May to September) for the parents and two seasons (August to October) for the hybrids and reported pollen viability was higher in September and October. Similarly, Dhall et al. (2011) evaluated pollen viability in four different planting dates and found pollen viability percentage to increase from June to September.

Hand pollination is required for selfing or hybridization for producing seed in breeding programs. Pickersgill (1971) applied no isolation technique after crossing considering that emasculated flower will not be attractive to insects, but others mentioned that covering pollinated flowers is a key requirement for producing genuine hybrids (Greenleaf, 1986) Some of the materials are double layer cheesecloth (Greenleaf, 1986), aluminum foil or white glue (do Rego, et.al., 2012). Greenleaf (1986) indicated that crosses can be done any time of the day but early morning or late afternoon is the best. Pickersgill (1971) crossed pepper flowers immediately after emasculation and in recent study Dhall et al. (2011) reported

percentage fruit set was higher when flowers crossed at anthesis compared to crossing 12 hrs after antethis or 24 hrs after anthesis.

Due to the high out-cross levels in pepper, production of pure lines requires isolation techniques to be applied. A minimum of 200 m distance among different varieties to be maintained (Berke 2000), however, the distance between any two varieties depends on the foraging area that can be covered by the pollinating insects, closer distances can be maintained when dominant pollinators in the area can travel only small distance (Raw 2000). Distance also is determined by the purity level of the intended seed to be produced. It ranges from 1.6 Km for foundation seed to 0.4 Km. for certified seed (Bosland 1993). Covering plants with plastic, growing in greenhouse or screenhouse, covering individual flowers are among other techniques applied for isolation (Berke 2000).

2.5. Importance and uses

Pepper is the most widely used spice and condiment in the world and is greatly priced for its pungency and adding special flavor to many cuisines throughout the world (Andrews, 1999). Historically it was used mainly for seasoning and as medicinal plant, but today its use extended to fresh and processed vegetable, spice, dried forms, used as food dye, bred as ornamental plant and production of extracts for various pharmaceutical and cosmetics industry (Paran and Kaanab, 2007 and Djian-Caporalino et al., 2006). It is an ingredient in preparation for almost all Eritrean dishes. Average weekly household consumption of dry pepper in Eritrea is estimated at 140 grams (National office of statistics, 2012). It is consumed as powder prepared from dry pods called '*berbere*' which is added to dishes as a food dye and spice. The dry red pods are also the main component for preparing '*shiro*' powder, which is a popular sauce in Eritrea. The green pods are eaten raw as a salad or appetizer.

It is an attractive potential export crop due to the demand for it is steadily increasing. It has high price elasticity in addition; it is classified as high-value agricultural products that tend to require two to four times more labour than cereal crops (TIPS

and AUSAID, 2015). According to Grubben and El Tahir (2004), in Africa capsicum production is usually practised on small-scale farms on plots of 0.1–0.5 ha and if properly managed, it is labour intensive, especially planting, weed control and the repeated harvests. The greatest part of the hot pepper area in tropical Africa, however, is cultivated in an extensive way as a low input system (Grubben and El Tahir 2004).

2.6. Genetic diversity in pepper

Significant amount of diversity exists within species and among species of Capsicum and breeders have only recently started to exploit this diversity (Pickersgill, 1997). The diversity includes a wide range of fruit forms and colour (Daskalov, 1986). Among the cultivated species C.annuum enjoys the highest morphometric diversity and is cultivated almost all over the world (Murillo-Amador et al., 2015). Diversity studies are an essential step and pre-requisite in plant breeding and could produce valuable knowledge for crop improvement programmes (Mohammadi & Prasanna, 2003 ; Roch et al., 2010). Genetic diversity studies are also useful for conservation, evaluation and utilization of genetic resources and for determining the uniqueness and distinctness of genotypes (Franco et al., 2001).Understanding the genetic relationships between pepper accessions may provide an effective management tool for their conservation, as well as help inform plant breeding efforts (Votava et al., 2005). Several methods have been used in diversity studies of the genus Capsicum. Eshabugh (1976) described the contribution of floral morphology, genetic and biochemical systematic studies to better understand the relationship among the cultivated species. Assessment of genetic diversity of crop plants is a common practice. Methods used for assessment are morphological characterization, biochemical characterization and molecular marker analysis (Govindaraj et al. 2015).

2.6.1. Morphological characterization

Description of the cultivated and wild species of Capsicums dates back to the 18th century when Linnaues described two cultivated species *C. annuum* and *C. frutescens* in Species Plantarum (1753) and in Mantesa plantarum (1767) two wild

species were described (Baral & Bosland, 2002a ; Smith & Heiser, 1951). Smith and Heiser, (1951) presented a detailed morphological description of *C. annuum* and *C. frutescens* as a basis for differentiation between the two species. Smith and Heiser (1957) presented a detailed morphological description C.sinense .Eshabough (1987) described in detail C. annuum.var.aviculare (Synonym, *C. annuum var. glabriusculum*) and compared it with the three cultivated species of the C.annuum complex. Morphological description for differentiating the five cultivated species is also available (IBPGR, 1983). Descriptors for capsicum (*Capsicum spp*.). Is the commonly used reference for diversity studies in capsicum (IPGRI et al., 1995).

The wide range of distribution of peppers has created an opportunity for local germplasm leading to varieties and landraces to exist. Landraces are important genetic resources because they have unique gene pools and serve as important reservoirs of genetic diversity for breeding and conserving biodiversity (Bosland, 2010). The use of morphological characterization for studying genetic diversity of local pepper germplasm including landraces, accessions and cultivated varieties has long been utilized for identifying the potential for breeding to meet desirable traits. Many scientists around the world have studied variability germplasm and clustering them into genetically related groups for selecting superior genotypes and utilization in future breeding and crop improvement programmes (Nsabiyera et al.,2013; Nkansah et al., 2011; Ortiz et al., 2010; Madosa et al., 2009; Adetula & Olakojo, 2006). Morphological characterization has also been used for identifying species and duplications in germplasm collection in different parts of the world (Stavělíková et al., 2010; Jarret, 2007).

2.6.2. Molecular characterization

Molecular markers are pieces of DNA with known or unknown position in the chromosome and are useful tools for diversity studies and they are more precise compared to morphological tools. Several molecular markers with different applications are available. Considering several factors, including equipment availability, simplicity, cost, anticipated polymorphism level, quantity and quality of DNA, marker inheritance and availability of adequate skills and equipment, Semagn

et al. (2006) found that, RFLP, SSR, RAPD, AFLP, and ISSR are markers that could be used for a wide range of applications in plants.

In recent years, molecular markers have been used intensively in molecular characterization of different plant species. However, only a few studies attempting to characterize a broad selection of cultivated C. annuum genetic diversity using molecular markers have been reported (Hill et. al., 2013). RAPD markers were one of the most popular molecular markers used for diversity studies to compare populations in pepper by number of scientists in different places and were reported to be useful. Baral and Bosland (2002b), Sanatombi (2010), Bhadragoudar and Patil (2011), Akbar et.al., (2010) used RAPDs for diversity studies of genotypes from Nepal, India and Pakistan. RAPDs were also used for varietal identification and to investigate genetic purity of genotypes from Turkey (Ilbi, 2003) and for characterizing and comparing the genetic structure of landraces and wild populations. (Votava et al., 2005; Oyama et al., 2006). Similarly AFLP markers were used by Geleta et al. (2005) for investigating pepper genotypes from Ethiopia and other countries and Aktas et al. (2009) for studying genotypes from Turkey and established that AFLP markers were useful for revealing genetic diversity among pepper genotypes.

SSR markers are simple sequence repeats composed of 1-6 nucleotides. SSR markers are widely used in genetic studies of pepper and other plants. The information value of SSRs is higher compared to RFLPs, AFLPs and RAPDs (Lee et al., 2004). Semagn et al (2006) in reviewing the use of molecular markers found that several names and definitions were given to SSR markers by different authors. They were defined as 1-5, 1-6, 2-6 and 2-8 bp repeats. They are known as Microsatellites, simple sequence repeats, short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs). SSR markers are genome specific, abundant, highly polymorphic, co-dominant, easily detected with high potential for automation (Ijaz, 2011). Due to these advantages they have been widely used in plant species including cereals, vegetables, fruits and others; not only for genetic diversity studies, population genetics and evolutionary studies, but also in fundamental researches like

genome analysis, gene mapping and marker-assisted selection. (Kalia et al., 2011). Compared to SNP markers which can only be transferred to different mapping populations within the same species, due to multiple alleles, cost-effectiveness, and transferability, SSR markers will continue to play an important role in different genetic studies in many minor plant species (Wang et.al., 2009). Use of SSR markers in genetic studies of Capsicum varied from constructing several pepper linkage maps (Lee et al., 2004, Yi et al., 2006, Barchi et al., 2007, Mimura et al., 2012 and Sugita et al., 2013), tests of distinctiveness (Kwon et al., 2005), genetic diversity and structure (Melendez et al., 2009, Rodrigues and Tam 2010, Tilahun et al., 2013, Rai et al., 2013; Dhaliwal et al., 2014), genetic relationship in Capsicum cultivars (Patel et al., 2011), in studying the origin of 11 species and their genetic relationship (Nicholi et al., 2013). SSR markers were also used in genetic analysis of number of other crops: QI-Lun et al., (2008) in Maiz, Backes et al., (2009) in Barley, Mu˜noz-Falcón et al. (2011) in Eggplant, Azmat and Khan (2010) in Cotton, Sajib et al., (2012) in Aromatic rice, Emanuelli et al. 2013 in (Grape), Sow et al. (2014) in Rice.

2.7. Pepper improvement

Based on number of chromosomes the genus Capsicum is separated into two groups, the first with 2n=2x=24 and the second is 2n=2x=26, *C. annuum var. glabriusculum* is the only one that is tetraploid with 2n=4x=48 (Moscone et.al., 2007). All the cultivated capsicum belongs to the first group (Pozzobon, 2006 and IBPGR, 1983).

There are weedy or wild forms of the cultivated species (Pickersgill, 1971), and crossability among species was reported (Greenleaf, 1986; Smith and Heiser, 1957). This diverse gene pool has been utilized in the improvement of peppers, however, most of the improvement efforts have been directed towards *C. annuum* (Mongkolporn & Taylor, 2011). Bosland (1996) stated that the strategy of the chilli pepper breeder is to assemble into a cultivar the superior genetic potential for yield, protection against production hazards, and improved quality. Pepper breeding methods focussed on using natural source of germplasm, cross breeding and exploitation of heterosis of F1 hybrids (Daskalov, 1986). Greenleaf (1986) reviewed breeding methods in pepper and found that pedigree breeding with selection,

pedigree breeding following hybridization, transfer of single genes and intercrosses of different backcross families with different recurrent parents and with different target genes methods were used for developing pepper cultivars. Growing interest on induced mutation for pepper improvement was also mentioned by Dascalove (1986) and he described mutagen treatment procedures. Hand-emasculation, genic male-sterility, and cytoplasmic male-sterility are used for hybridization (Bosland, 1996).

Many pepper traits such as fruit size, yield, and adaptation to environmental conditions are inherited quantitatively or depend upon the accumulation of many genes; each contributing a small content to the total expression (Greenleaf, 1986).

Pepper breeding programs for improving yield, quality and resistance to biotic and abiotic stress have been running for a long period in different parts of the world. Many national and International institutions are running breeding and improvement programs. The New Mexico State University (NMSU) has the longest continuous program of hot pepper improvement in the world that began in 1888 (Bosland, 2012). The Caribbean Agricultural Research and Development Institute (CARDI) has been running several pepper breeding and evaluation works that resulted in developing 3 varieties, 25 new lines potential for new cultivars, stabilization and purification of 10 landraces (Roberts, 2004). Similarly the breeding unit at the Asian Vegetable Research and Development Centre (AVRDC) is running a program for breeding improved varieties suitable for the tropics and sub-tropics with potential disease and pest resistance, abiotic stress tolerance, and quality (Berke & Engle, 2012). Zewdie (1994) reported about The International Hot Pepper Trial Net Work (INTHOPE), which is coordinated by the AVRDC, and was initiated with the objective of facilitating the exchange and evaluation of popular hot pepper landraces and elite germplasm across international test environments.

CHAPTER THREE

ASSESMENT OF THE CURRENT STATUS AND FUTURE OPPORTUNITIES OF PEPPER PRODUCTION IN ERITREA

Abstract

Pepper has been grown in Eritrea for a long period. The crop was well utilized during the 1950s up to 1970s when the export demand was high. Yield per hectare of pepper has continued to decline from 10.6 t/ha in 2008 to 3.7t/ha in 2011. The reason for decline in productivity hae not beed documented. A study was conducted in 10 major pepper growing sub-regions to determine the major constraints and opportunities of pepper production in Eritrea. A participatory rural appraisal method that included the collection of secondary data, key informants interviews, focus group discussions and formal household survey was used. The major constraints identified were the unavailability of improved varieties, poor quality seed, inputs and services, insect pests and diseases, small acreage and discouraging land tenure system, improper marketing system, poor extension service and persistent drought that affect water availability. Opportunities identified were availability of land, favourable climate, domestic and export markets and experienced farmers with high willingness to grow pepper. Average number of years in growing pepper was 16.4 years. The respondents were 40.7% green pepper growers, 25.8% dry pepper and 33.5% produce both types. Average land size was 3.66 ha and area allotted to pepper 1ha. Farmers who saved their own seed and produced their own seedlings were 69.2% and 82.4% respectively. Days from sowing to transplant ranged from 20-90days with an average of 44 days. Majority of farmers (86.3%) grow pepper once a year, 73.6% of them plough the land 2 or 3 times, 51.6% use animal driven equipments for plough and 83.5% transplant into narrow ridge. Average spacing was 51.4cm between rows and 29.6cm between plants in row resulting in 51,154 plants/ha. Majority of the farmers applied fertilizers; however, the amount is far below the recommended. Severity of insect pests, diseases and weed problems were found to be 58.8, 56.6 and 42.3%

respectively. These indicate that Eritrea has great potential for pepper production however, constraints need to be overcome and opportunities maximized.

3.1 Introduction

Pepper is one of the important vegetable crops of Eritrea (MoA, 2006). The exact time when pepper was introduced in Eritrea is unknown, however, it has been grown and used in Eritrea for a very long time. During the 1950s to mid 1970s Eritrea was an exporter of green pepper to Europe and the Middle East. Report of the Bank of Ethiopia (1962) mentioned that there was a great increase in green pepper production in the previous years due to high demand for export. Annual agricultural reports of Ethiopia (1957-1960 & 1969) show that the production of green pepper increased greatly from 99 tons in 1957 to 8,482 tons in 1968.

Yield and quality of the locally produced pepper are quite low. Several factors contributed to the low yield and quality of pepper produced in Eritrea affecting both the producers and consumers. However, information regarding constraints and opportunities is not available. Asgedom et al., (2011) stated that in Eritrea, the few surveys conducted in the past, are general and covered all horticultural crops. Such surveys did not identify the current status, constraints and opportunities at the crop level. Thus the current study was undertaken in order to identify constraints and opportunities of pepper production in order to make informed decisions that may help find solutions for the major constraints and maximize the existing opportunities for improving pepper production and quality in Eritrea. The objective of this study was to document the current status and opportunities of pepper production in Eritrea.

3.2 Materials and Methods

3.2.1 Location

The study was conducted in 10 sub-zobas (sub-regions) in four zobas (administrative regions) of Eritrea. The regions surveyed were Debub (Southern), Anseba, Semenawi Keih Bahri (Northern Red Sea) and Gash-Barka (Table 3.1). Survey locations and number of respondents in each region and sub-region were determined based on

number of farmers and analysis of information about history and current pepper growing areas in Eritrea. A total of 193 households were planned to be interviewed in the surveyed areas, however, for logistical reasons only 182 were reached (Table 3.1). Agro-climatic regions coverage was also considered.

Table 3.1: Surveyed sub-regions number of respondents and participants in group discussions

Survey area characteristics			No. of Households		
Region	Sub-region	Agro-climatic regions	Plan-ned	Intervi-ewed	NPFGD
Anseba	Elabered	Western Escarpment	20	18	8
	Geleb	Northern Central Highlands	15	16	15
	Mendefera	Southern Central Highlands	20	19	13
Southern (Debub)	Dbarwa	Southern Central Highlands	20	20	14
	Adi-quala	Western Escarpment	15	11	-
	Dekemhare	Southern Central Highlands	20	17	13
Northern Red Sea	Foro	Coastal Plains	15	14	12
	Gindae	Green Belt & Coastal Plains	33	32	12
	Afabet	Coastal Plains	20	20	10
Gash-Barka	Akurdat	Western Lowlands	15	15	10
Total			193	182	107

NPFGD= Number of participants in focus group discussions

3.2.2 Secondary data

Published and non-published documents on pepper production and related topics that would help in analysing the current situation and future prospects of pepper production in Eritrea were collected. Information was collected from the Ministry of Agriculture Headquarters and the regional offices, National Agricultural Research Institute, Ministry of Trade and Industry, Department of Customs and National Office of Statistics. The collected data included policies and regulations in

agriculture and import export guidelines, production areas, introduced varieties, seed and inputs distribution, pepper quantity produced locally, value of imported pepper, pepper consumption and annual reports, projects and consultancy reports (Appendix 1).

3.2.3 Key informant discussions

A total of 25 interviews were conducted with key informants familiar with pepper production. The interviewed experts were staff of the Ministry of Agriculture headquarters, staff of the Ministry of Agriculture regional and sub-regional offices, Staff of the National Agricultural Research Institute and experts in other organizations (Appendix 2). A check list was used to initiate and guide the discussion but a free flow was allowed for extracting as much information as possible (Appendix 3). The discussion focused on the major issues of pepper production in the country or specific places such as history and development of pepper production, current trend, and major factors affecting production

3.2.4 Focus group discussions

Nine focus group discussions were held in nine of the ten sub-regions surveyed. The discussions included farmers of different age groups and some extension workers. The number of participants in each discussion was in the range of 8-15 participants (Table 3.1). Number of participants in each group was decided based on consultation with staff of Ministry of Agriculture in each region and sub region, and resources available. A check list (Appendix 4) was used to guide the discussion but was conducted in informal way to encourage free flow of discussion. The discussions mainly focused on pepper production history and development and current constraints and opportunities of pepper production at farmer level.

3.2.5 Formal household surveys

Surveys were conducted in selected major hot pepper growing areas. Based on previously collected information and discussions with staff of the Ministry of Agriculture in the region, the most important pepper growing sub-region and areas

within sub-region were selected. Thereafter, farmers in each area were randomly selected. A formal semi-structured questionnaire (Appendix 5) was used for collecting data at individual farmer level. Number of farmers to be interviewed in each area was determined based on number of producers in the area. The head of the household was the person interviewed.

Data collected included general information about farmers, cultivated areas, varieties in use, source of seed, application of different cultural practices, yields, cost and availability of inputs, marketing of products, prices, and distances to markets, major constraints and opportunities.

3.2.6 Tools used for data collection

Nokia E5 mobile and HP Pavilion 6 laptop were used to record the interviews of the key informants and the focus group discussion respectively to avoid information leakage.

3.2.7 Data analysis

Excel 2010 was used for entering and arranging the quantitative data collected in form of frequencies, percentages and averages. The qualitative data from the interviews and discussions as well as the secondary sources was subject to logical analysis for supporting the quantitative data analysis. SPSS statistical software, version 20 (IBM, 2011) was used for formal statistical analysis.

3.3 Results and discussion

3.3.1 Gender and household characteristics

Average family size in the surveyed areas was 8.2 persons per family with significant ($P<0.05$) difference among the surveyed sub-rgions. The highest average was 10.3 recorded in Afabet followed by 9.9 in Geleb, while the lowest was 6.6 recorded in Gindae (Table 3.2). This average number of persons in the surveyed areas is high compared to the average of 5 persons per family reported by Mapeba and Pitoro, (2009) for both Malawi and Zimbabwe. The reason could be the social and

economical factors that appreciate large families and members as source for farm labour. However, in Afabet and Geleb , the two sub-regions that showed the higher average number of persons per family compared to the remaining surveyed sub-regions could be due to majority of the respondents in Afabet and many in Geleb had more than one wife. The results in Table 3.2 also show higher average number of male members compared to female members in the family (1.1:1 ratio). This is slightly different comprated to previous report (United Nations, 2004) that showed a 1:1.01 male to female ratio. The highest ratio was in Geleb (1.48:1) and the lowest in (0.96:1) in Dbarwa followed by 0.98:1 in Afabet (Table 3.2).

Average number of working persons in the family was 3. The highest was 4.5 in Adi-quala and the lowest was 2 in Afabet and Akurdat (Table 3.2). Women make essential contributions to agriculture in developing countries, but their roles differ significantly by region (FAO, 2011). The results of the current study show participation of women in farming activities, however FAO, (2011) reported that in sub-Saharan Africa women contribute 50% of labour in agriculture, while the average number of working female members of the family in the surveyed areas was 0.85 persons compared 2.1 male persons with the highest 1.5 persons in Adiquala

Table 3.2: Family characteristics

*Significant at 0.05 % ^{NS} Not significant

Sub-region	Family size			No of working persons		Childeren in School	
	Male	Female	M to F ratio	Male	Female	Male	Female
Elabered	3.8	3.0	1.27:1	1.9	1.2	1.6	1.1
Geleb	5.9	4.0	1.48:1	2.2	0.6	2.5	1.4
Mendefera	4.1	3.3	1.24:1	2.4	1.4	1.4	0.8
Dbarwa	4.6	4.8	0.96:1	2.4	1.3	1.6	2.5
Adi-quala	5.0	5.0	1:1	3.0	1.5	2.1	1.3
Dekemhare	3.9	3.6	1.08:1	2.1	1.1	1.6	1.6
Foro	4.1	3.0	1.37:1	2.2	0.14	1.4	0.4
Gindae	3.3	3.3	1:1	1.5	0.7	1.3	1.2
Afabet	5.1	5.2	0.98:1	1.7	0.3	1.8	1.3
Akurdat	4.5	3.5	1.29:1	1.9	0.14	2.1	1.8
Mean	4.3 *	3.9*	1.1:1	2.1^{NS}	0.85.3*	1.7 ^{NS}	1.3*
s.e.	0.147	0.154		0.097	0.97	0.115	0.111

and lowest was 0.14 person in Akurdat and Foro. The difference among the sub-regions for average number of working females in a family was significant (P>0.05) (Table 3.2). It was also observed that women participation in farm work was lower in sub-regions Foro, Afabet, Akurdat and Geleb where women usually do house work and girls may participate in herding goats and sheep. This is in agreement with Green and Baden (1994) who reported that gender division of labour in Eritrea is affected by agro-ecological, socio-cultural and socio-economic factors. They explained that women participation in farm work is less in the semi-nomads Muslim communities of the lowlands compared to the Christian communities of the southern highlands who practice settled agriculture.

Average number of male children going to school was 1.7 compared to 1.3 of female children. However, the difference among the sub-region for female children was significant. The highest number of male and female children going to school was 2.5 recorded in Geleb and Dbarwa respectively (Table 3.2).

The results show that 98% of the households were headed by men. The lowest percentage (93.8%) was in Gindae and the highest (100%) in seven of the surveyed sub-regions. This was confirmed by chi-squared analysis that showed no association between the different sub-regions and gender of household head (Table 3.3). This shows that households in rural areas are man dominated. The 2% women headed households are mainly due to being widowed. This is much lower than the results reported by Njuguna (2011) in Kenya who found women headed household to be 14% who are widows, divorced or being single mother. However, both studies showed that households in rural areas are man dominated.

The age of the respondents ranged from 24 to 86 with average of 53.2 years. The results also show that 41.2% of the respondents were in the age range of 50- 64 years; 22%, 65 years or greater including elders greater than 75 years old and only 1.6 % less than 30 years. The highest percentage (65%) of the age group 50-64 was in Afabet and the lowest (31.6%) in Mendefera, while age group (65 years or greater) was higher (42.1%) in Mendefera compared to the lowest (7.1%) in Foro, however, the difference among the sub-region for distribution was not significant (Table 3.3). This indicates that most of the respondents are in the last active age stage or even beyond the active age. This is in contrast to the results of Njuguna (2011) in

Kenya and Tuteja, (2013) in India who found 75% and 56.99 % of respondents respectively to be in the active age. This indicates that young people in Eritrea are somewhat away from farm work. The reason partially could be due to young people have more tendencies to move out of agriculture, but it is mainly due to engaging them in military service. This could be one of the main reasons for the unavailability and high labour cost considered in some of the surveyed areas as one of the production constraints.

Table 3.2: Gender and age groups of the respondents

Sub-region	Gender		Age groups (year)					N
	Male (%)	Female (%)	< 30 (%)	30-39 (%)	40-49 (%)	50-64 (%)	=>65 (%)	
Elabered	94.44	5.56	0.00	22.22	5.56	44.44	27.78	18
Geleb	100.00	0.00	0.00	6.25	25.00	50.00	18.75	16
Mendefera	100.00	0.00	0.00	15.79	10.53	31.58	42.11	19
Dbarwa	100.00	0.00	0.00	0.00	18.18	54.55	27.27	11
Adi-quala	100.00	0.00	0.00	0.00	40.00	35.00	25.00	20
Dekemhare	94.12	5.88	0.00	29.41	17.65	35.29	17.65	17
Foro	100.00	0.00	7.14	7.14	42.86	35.71	7.14	14
Gindae	93.75	6.25	6.25	21.88	21.88	31.25	18.75	32
Afabet	100.00	0.00	0.00	0.00	15.00	65.00	20.00	20
Akurdat	100.00	0.00	0.00	6.67	40.00	40.00	13.33	15
Total	97.80	2.20	1.65	12.09	23.08	41.21	21.98	182
Chi square	15.148		47.559					
Significance	0.738		0.87					

Education is an important tool for development. Particularly in agriculture, it is important for farmers to understand and adopt improved technologies that ultimately lead to higher yield and better product quality. The result of the current study show that 15.9% of the respondents are illiterate, 11 % can read and write and 73 % had formal education. The highest percentage of respondents who had no formal education was in Afabet (85%) and the lowest (5.9%) was in Dekemhare. The

differences among the sub-regions was significant (Table 3.4). The high percentage in Afabet is due to the pepper production area in this sub-region had no access to school until very recent years. The percentage of farmers who had formal education was higher than that of tomato growers reported by Asgedom *et al*., (2011). National literacy rate in Eritrea is 67% and male and female literacy rates are 73.6 and 56.3% respectively (UNESCO, 2012). Since 98% of the respondents in the current study are male, the results in agreement with that of UNESCO (2012) who also indicated wide disparity among the different regions and between male and female. Disparity among regions can be observed in Table 3.4, while differences between male and female could be observed in the number of children going to school (Table 3.2). The results also showed that only 6% of respondents have education greater than high school (Table 3.4) which indicates that people beyond the high secondary school usually do not go for farm work; instead they prefer to be employed by the government or other employers. This is similar to that found by Mariyono, *et al*. (2009) who reported that in Central Java, highly educated people prefer to go to more comfortable business than be engaged in labour intensive chilli production.

Table 3.3: Educational level of the respondents

	Education level							
Sub-region	Ill (%)	RW (%)	P (%)	J (%)	S (%)	C (%)	G (%)	N
Elabered	27.78	5.56	33.33	11.11	16.67	5.56	0.00	18
Geleb	6.25	6.25	56.25	6.25	12.50	6.25	6.25	16
Mendefera	15.79	0.00	47.37	0.00	31.58	0.00	5.26	19
Dbarwa	9.09	9.09	81.82	36.36	18.18	0.00	0.00	11
Adi-quala	20.00	5.00	10.00	20.00	15.00	0.00	0.00	20
Dekemhare	5.88	0.00	52.94	17.65	17.65	5.88	0.00	17
Foro	28.57	0.00	50.00	14.29	7.14	0.00	0.00	14
Gindae	15.63	6.25	28.13	12.50	25.00	3.13	9.38	32
Afabet	20.00	65.00	5.00	10.00	0.00	0.00	0.00	20
Akurdat	6.67	6.67	40.00	6.67	26.67	6.67	6.67	15
	15.93	10.99	36.81	12.64	17.58	2.75	3.30	182
Chi square	111.638							
Significance	0.000							

Ill= Illiterate C= Certificate RW= Read & write P=Primary J= Junior S= Secondary G= Graduate (degree or diploma)

3.3.1 Farming activity and experience

Different farming activities are practiced in the surveyed areas for income generation. The results in Table 3.5 shows that 49.5% of the respondents were active in vegetable and cereals production, while 31.3% were vegetable growers and only 1.1% were engaged in outside farm activities. The highest percentage (100%) of cereals and vegetables producers was in Mendefera and Dbarwa and the lowest (0%) was in Akurdat. The highest for vegetable producers (65%) was in Afabet and the lowest (0%) in Mendefera and Dbarwa. The Chi-squared test confirmed the difference among the sub-regions as significant (Table 3.5). Akurdat recorded the highest percentage of respondents engaged in fruit and vegetable production (66.7%) and vegetable, fruit, vegetables and cereals (20%), while the lowest (0%) for the former was in Mendefera, Adi-quala, Dbarwa and Foro and for the later in seven sub-regions. The reason is Akurdat is a centre for banana production while in most of the southern region areas fruit production is not common.

Table 3.5: Farming activities of the households

	Income generating activity				
Sub-region	Veg (%)	C & veg (%)	Fr.& veg (%)	Veg, Fr. & C (%)	Other (%)
Elabered	44.4	22.2	33.3	0.0	0.0
Geleb	25.0	18.8	50	6.3	0.0
Mendefera	0.0	100	0.0	0.0	0.0
Adi-quala	30.0	65	0.0	5.0	0.0
Dbarwa	0.0	100	0.0	0.0	0.0
Dekemhare	35.3	58.8	5.9	0.0	0.0
Foro	50.0	50	0.0	0.0	0.0
Gindae	34.4	56.3	6.3	0.0	3.1
Afabet	65	25	5.0	0.0	5.0
Akurdat	13.3	0.0	66.7	20.0	0.0
Grand *mean*	31.3	49.5	15.4	2.7	1.1
Chi square			135.983		
Significance			0.000		

veg = *Vegetables* ***C = Cereals*** ***Fr= Fruits***

Farmers in Eritrea have long history of pepper production. Number of years in pepper production of the respondents ranged from 1 to 66 years and the average was 16.4 years. The results show that 51.1% of the respondents had less than 10 years experience with the highest in Foro (100%) followed by Afabet (85%) which are new pepper growing areas, while the lowest was in Mendefera (5.3%) which one of the oldest pepper producing areas in the country. The difference among the sub-regions was significant (Table 3.6). The results also show that 7.9% and 3.4% are in the 36-50 and >50 years category indicating long history of growing pepper in many of the surveyed areas and more engagement of old people in production.

Table 3.4: Experience in pepper production

Number of years in pepper production							
Sub-region	10 <= (%)	11-15 %	16-25 %	26-35 %	36-50 %	> 50 %	N
Elabered	29.4	17.6	23.5	17.6	5.9	5.9	17
Geleb	68.8	18.8	12.5	0.0	0.0	0.0	16
Mendefera	5.3	10.5	26.3	21.1	21.1	15.8	19
Adi-quala	15.8	10.5	31.6	42.1	0.0	0.0	19
Dbarwa	63.6	36.4	0.0	0.0	0.0	0.0	11
Dekemhare	41.2	0.0	17.6	11.8	29.4	0.0	17
Foro	100	0.0	0.0	0.0	0.0	0.0	14
Gindae	63.4	3.3	20.0	3.3	6.7	3.3	30
Afabet	85	15.0	0.0	0.0	0.0	0.0	20
Akurdat	46.6	6.7	26.7	0.0	13.3	6.7	15
Grand *mean*	51.1	10.7	16.9	10.1	7.9	3.4	178
Chi square	159.869						
Significance	0.000						

3.3.2 Acreage and area allotted for pepper production

Land size is an important component in expansion and increased production of any crop. Generally in Eritrea acreage per household is very small, however, in lowlands it is larger compared to the highlands and midlands. The result of the current study show that average land size of the surveyed area is 3.66 ha with minimum land size of 0.025 ha in Afabet and maximum of 85 ha in Akurdat. The highest mean land size was in Akurdat (14.28 ha) and the lowest (1.1ha) in Afabet (Table 3.7). The small land size in Afabet is specifically for villages along the Mogae River which is the pepper growing area of Afabet, while in the upper lands of the sub-zoba larger land sizes could exist. The difference in land holding size among the sub-regions was statistically significant (Table 3.7) due usually in lowland areas vast potential agricultural lands available,

Average area allotted for pepper is 1 ha with minimum of 0.015 ha in Afabet and maximum of 25 ha in Gindae. The highest average area allotted for pepper (2.31) was in Gindae and the lowest (0.2) was in Geleb. The difference was statistically significant (Table 3.7).This is almost similar to that mentioned by Grubben and El Tahir, (2004) who stated that in Africa capsicum production is usually practised on small-scale farms on plots of 0.1–0.5 ha, but much smaller than the average reported by Asgedom et at. (2011) for tomato growers in Eritrea (3.26 ha). Cereals, tomato, potato, onion, fruits and some other minor vegetables are competitors of pepper in farm land. The degree of competition between pepper and the other crops varies from place to place. Total acreage depends on availability of cultivable land and population density, while allocation of land to different crops depend on farmers decisions; depending on the importance of each crop as household food or cash crop and its adaptability to the conditions of the area.

Table 3.5: Land resources allocation for pepper compared to other crops

	Total Farm Area (ha)			Area Allotted for Pepper (ha)			Mean Area Allotted for Competing Crops (ha)					
Sub-region	Mean	Max	Min	Mean	Max	Min	Tomato	Potato	Onion	Other veg.	Cereals	Fruits
Elabered	1.72	5.80	1.00	0.23	0.50	0.10	0.58	0.00	0.05	0.00	0.36	0.36
Geleb	1.77	4.00	0.50	0.20	0.50	0.10	0.40	0.34	0.40	0.06	0.26	0.38
Mendefera	2.92	6.50	0.50	0.56	2.00	0.10	0.46	0.86	0.12	0.56	1.36	0.00
Dbarwa	3.60	8.00	1.00	0.74	3.50	0.13	0.86	0.88	0.32	0.74	1.07	0.00
Adi-quala	1.55	3.00	0.50	0.24	0.75	0.13	0.26	0.26	0.10	0.13	1.32	0.00
Dekemhare	2.40	5.00	1.00	0.53	1.00	0.25	0.65	0.49	0.00	0.35	0.61	0.05
Foro	2.93	12.00	0.50	1.91	7.00	0.50	0.16	0.00	0.12	0.21	0.47	0.00
Gindae	4.80	50.00	0.25	2.31	25.0	0.10	0.48	0.00	0.00	0.10	0.63	0.20
Afabet	1.10	7.50	0.03	0.70	2.00	0.02	0.05	0.01	0.05	0.03	0.32	0.01
Akurdat	14.28	85.00	3.00	1.45	3.50	0.25	1.73	0.00	1.33	1.30	1.55	3.10
Grand mean	3.66	85.00	0.03	1.00	25.0	0.02	0.58	0.28	0.22	0.33	0.76	1.22
s.e.	1.064			0.286								
Significance	0.017			0.003								

Mariyono et *al*. (2009) found farmers who had large land size allotted larger area for pepper compared to those who had smaller land. This is somewhat true for the current study where farmers with large acreage tend to grow larger area of pepper, except in Akurdat where greater attention is given to banana, onion and may be other vegetables such as pumpkin (Table 3.7).

3.3.3 Pepper varieties and seed source

Generally pepper grown in Eritrea is for both green (fresh) and dry consumption. The results show that farmers know cultivars or types suitable for dry or green peppers, but most of them (77.5%) could not mention the name of the variety under cultivation. Even the mentioned names were not real variety names but the place where they came from (eg. Sawa or Adis), the shape of fruit (eg. Kerni-irab) or the organization that introduced it (eg. Amrach). The highest percentage of farmers who did not know name of varieties they grew ranged from 100% in Geleb to 40% in

Dbarwa (Table 3.8). The reason for the high percentage for not knowing the variety name could be attributed to the last 20 years where no introduction of new varieties or seed distributions for pepper resulting in most of the farmers (69.2%) using their own seed or purchase seed usually of unknown quality from other farmers or consumption market (26.9%) and the rest 3.8% depend on seed exchange (Table 3.8).

Although more dry pepper is consumed in Eritrea, until recent years its production has been limited compared to green pepper. Of the respondents 40.7% produce green pepper, 25.8% dry pepper and 33.5% both green and dry peppers either in separate plots (10.4%) or successively from the same crop (23.1%). This is a mechanism for earning early income. The highest green pepper producers (68.8%) were in Geleb and the lowest (0%) in Afabet, while for dry pepper the highest was in Afabet (95%) and the lowest (0%) in Mendefera and Dbarwa. The differences among the different sub-regions regarding knowing varieties, source of seed and type of pepper grown was significant (Table 3.8).

3.3.5 Seedling quality

Seedling quality is one of the most important factors that affect productivity. In agreement with Grubben and El Tahir , (2004) who stated that direct seeding in pepper is rarely practiced, all the respondents use seedling method for growing pepper. Table 3.9 show that majority of the respondents (82.4%) produce their own seedlings compared to 8.8% who purchase seedlings and 8.8% who produce their own seedlings or sometimes purchase. The highest percentage of farmers who produce their own seedlings (100%) was in Dekemhare, Gindae and Akurdat, while

Table 3.6: Knowledge of varieties grown, seed source and type of pepper production by farmers

	Knowledge of variety grown		Seed source			Production type				
Sub-region	Yes (%)	No (%)	OS (%)	Pur (%)	SEx (%)	G (%)	D (%)	GDSC (%)	GDDP %	N
Elabered	11.1	88.9	66.7	22.2	11.1	44.4	22.2	33.3	0.0	18
Geleb	0.0	100	25	56.3	18.8	68.8	25.0	6.3	0.0	16
Mendefera	31.6	68.4	94.7	5.3	0.0	52.6	0.0	42.1	5.3	19
Dbarwa	60.0	40	75	25.0	0.0	63.6	0.0	36.4	0.0	11
Adi-quala	45.5	54.5	27.3	72.7	0.0	40.0	10.0	10.0	40.0	20
Dekemhare	23.5	76.5	94.1	0.0	5.9	41.2	5.9	41.2	11.8	17
Foro	28.6	71.4	50	50	0.0	7.1	50.0	14.3	28.6	14
Gindae	12.5	87.5	90.6	9.4	0.0	65.6	9.4	15.6	9.4	32
Afabet	15.0	85	85	10.0	5.0	0.0	95.0	5.0	0.0	20
Akurdat	6.7	93.3	33.3	66.7	0.0	6.7	46.7	40.0	6.7	15
Grand mean	22.5	77.5	69.2	26.9	3.8	40.7	25.8	23.1	10.4	182
Chi square	31.251		72.448			142.492				
Significance	0.000		0.000			0.000				

OS= Own seedlings Pur= Purchased SEx = Seed exchange G= Green pepper D= Dry pepper GDSC= G & D pepper from the same crop GDDP= G&D pepper from deferent plots

the lowest was in Adi-quala (36.4%). Currently the source of seedlings available for sale is the surplus from farmers. Information from the key informants and focus group discussions show that no specialised nurseries that produce quality seedlings for sale are available. Information collected from key informants indicated, during the 1990s the Ministry of Agriculture used to produce seedlings and sale them to farmers at reasonable prices hows. Farmers in the surveyed areas usually use beds on a plot in the middle or one side of the field to be used as nursery which may affect the health of the seedlings produced due to infection from the surrounding fields.

Age of the seedling is an important quality factor. The number of days from sowing to transplanting practiced by the respondents ranged from 20 days in Gindae to 90

days in Mendefera and Adi-quala with average of 44±13 days. The differences among the sub-regions was significant (Table 3.9). The Asian Vegetable Research and Development Canter's (AVRDC) suggested cultural practices for chilli pepper (Berke et al. 1999) consider 30 days after sowing as optimum for transplanting under favourable conditions. At this age seedlings form 4 to 5 leaves. Grubben and El Tahir (2004) consider transplanting 30-40 days old seedlings with 8 to 10 leaves as usual in tropical regions. Only 29.1% of the respondents in the current study transplant seedlings at 30 days after sowing, while 14.8% transplant 35-40 days after transplanting. A total of 54.3% of the respondents found to transplant above 45 days and out of them 12.6% are even transplant at the age greater than 60 days (Table 3.9). This implies that aged seedlings used are difficult to establish and be productive. This could be the reason for several replanting mentioned by farmers in these areas.

Table 3.7: Source of seedlings and number of days to transplant

Sub-region	Source of seedlings			Number of days to transplant				
	OSd (%)	Pur (%)	S.Pur (%)	30 (%)	35-40 (%)	45-55 (%)	60 (%)	Other (%)
Elabered	88.90	11.10	0.00	33.30	44.40	22.20	0.00	0.00
Geleb	75.00	25.00	0.00	50.00	18.80	31.30	0.00	0.00
Mendefera	63.20	5.30	31.60	0.00	0.00	5.30	78.90	15.80
Adi-quala	36.40	54.50	9.10	0.00	15.00	20.00	30.00	35.00
Dbarwa	90.00	5.00	5.00	9.10	9.10	0.00	72.70	9.10
Dekemhare	100.00	0.00	0.00	5.90	0.00	41.20	41.20	11.80
Foro	50.00	14.30	35.70	64.30	14.30	7.10	7.10	7.10
Gindae	100.00	0.00	0.00	56.30	12.50	12.50	0.00	18.80
Afabet	85.00	0.00	15.00	25.00	10.00	50.00	5.00	10.00
Akurdat	100.00	0.00	0.00	33.30	26.70	33.30	0.00	6.70
Mean	82.4	8.80	8.80	29.10	14.80	22.50	20.90	12.60
Chi square	80.360			154.053				
Significance	0.000			0.000				

OSd= Own seedlings Pur= Purchased S.Pur= Sometimes purchase

3.3.4 Growing seasons and land preparation

Selection of growing season depends on climatic conditions, availability of water, market, pests and other factors. In Eritrean highlands and midlands pepper can be grown at least two times per year. However, the results of the current study show that 86.3% of the farmers grow pepper once a year. The highest was 100% in Gindae, Afabet and Akurdat and the lowest 60% in Dbarwa (Table 3.10). Discussions with farmers and key informants revealed that shortage of irrigation water was the major reason for not growing pepper in the second season.

Land preparation for pepper should include enough tillage that enables seedlings to establish well and provide proper soil texture for root growth and development (Kelley & Boyhan, 2006). Most of the respondents consider land preparation an important practice for getting good yield and protecting plants against soil borne diseases. The results of the current study show that 35.7% and 37.9% of the respondents plough the land 2 or 3 times respectively and only 4.9% plough their land once. The highest for ploughing 3 times was 90.9% in Adi-quala and the lowest was 5.9% in Dekemhare, while the highest for one plough was 30% in Afabet (Table 3.10). This shows how much the farmers consider repeated plough important for their crop.

Animal driven plough equipment was the most widely used method in the surveyed areas. It was used by 51.6% of the respondents. Tractor users constitute 23.7% while 24.7% of the respondents use both tractor and animal driven equipment. The highest users of animal driven equipments were in Afabet (100%) and the lowest (3.1%) in Gindae (Table 3.10). The reason for dependency on animals for plough in the highlands and midlands is mainly due to small land size unsuitable for large tractors which are common in Eritrea, while in the lowlands shortages of tractor services is the problem. The chi-squared test confirmed the association of number of pepper growing seasons per year, number of ploughs and plough method with sub-regions (Table 3.10).

Prior to transplanting farmers divide the land into smaller plots and start preparing the ridges where pepper plants are to be planted. The results show that majority of the respondents (83.5%) plant pepper on narrow ridges which is common in Eritrea

Table 3. 8: Type and frequency of ploughing

	Number of seasons per year		Number of Ploughs					Ploughing method			
Sub-region	One (%)	Two (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	AD (%)	TR (%)	AD & TR (%)	N
Elabered	94.40	5.60	0.00	44.40	50.00	5.60	0.00	77.80	16.70	5.6	18
Geleb	81.3 0	18.80	0.00	43.80	56.30	0.00	0.00	93.80	6.30	0.0	16
Mendefera	73.70	26.30	0.00	20.00	25.00	50.00	5.00	25.00	25.00	45	20
Dbarwa	60.00	40.00	0.00	0.00	52.60	31.60	15.80	68.40	10.50	26.3	19
Adi-quala	72.70	27.30	0.00	0.00	90.90	9.10	0.00	72.70	27.30	0.0	11
Dekemhare	76.50	23.50	0.00	0.00	5.90	41.20	52.90	35.30	0.00	64.7	17
Foro	100.00	0.00	0.00	57.10	42.90	0.00	0.00	78.60	0.00	21.4	14
Gindae	96.90	3.10	9.40	62.50	28.10	0.00	0.00	3.10	53.10	43.8	32
Afabet	100.00	0.00	30.00	60.00	10.00	0.00	0.00	100.0	0.00	0.0	20
Akurdat	100.00	0.00	0.00	40.00	53.30	0.00	6.70	6.70	80.00	13.3	15
Mean	86.30	13.70	4.90	35.70	37.90	13.70	7.70	51.60	23.60	24.70	182
Chi square	29.456		187.795					127.108			
Significance	0.001		0.000					0.000			

AD= Animal Driven *TR= Tructor*

for solanacious crops. The highest was 100% found in Elabered, Adi-quala, Dekemhare and Foro, while the lowest (20%) was in Afabet. Other variations in bed preparation are basin (4.9%), flat ridge (6.6%) in addition to zigzag narrow ridge locally known as *Sebaa-Themanya* method (4.9%) which is common in Afabet only. The difference among sub-regions was statistically significant (Table 3.11).

Table 3.9: Types of ridge used for growing pepper in the surveyed areas

	Type of ridge				
Sub-region	Basin (%)	NR (%)	RFB (%)	ZNR (%)	N
Elabered	0.00	100.00	0.00	0.00	18
Geleb	25.00	75.00	0.00	0.00	16
Mendefera	0.00	80.00	15.00	0.00	20
Dbarwa	0.00	84.20	21.10	0.00	19
Adi-quala	0.00	100.00	0.00	0.00	11
Dekemhare	0.00	100.00	0.00	0.00	17
Foro	0.00	100.00	0.00	0.00	14
Gindae	0.00	93.80	6.30	0.00	32
Afabet	25.00	20.00	10.00	45.00	20
Akurdat	0.00	93.00	6.70	0.00	15
Mean	4.90	83.50	6.60	4.90	182
Chi square	135.015				
Significance	0.000				

NR= Narrow ridge RFB= Raised Flat Bed ZNR= Zigzag narrow ridge

Inter and intra-row spacing determines the number of plants per unit area. Most of the respondents (65.9%) apply specific intra and inter-row spacing. The highest (90.9%) was in Adi-quala and the lowest (20%) in Afabet. Average spacing between rows was 51.4±13.2 cm and between plants in row 29.6±12.2 cm. The highest average spacing between rows (77.5±26.3cm) was in Afabet and the lowest (46.5±8.8cm) in Adi-quala, while the highest spacing between plants in row (60 cm) was in Afabet and the lowest (21.3cm) in Elabered. The result of the above spacing was an average plant population of 51,154 plants/ha with the highest in Elabered (84,507 plants/ha) and lowest (19,355 plants/ha) in Afabet. Application of specific

spacing and spacing between raws was significantly differen among sub-regions (Table 3.12).

Recommended spacing varies based on cropping system, soil type and variety. In AVRDC a total population of 26670 plants/ha is adapted (Berke et al.1999) and in Southern Australia 30,000 plants/ha is considered good (Burt, 1999). In Africa

Table 3.10: Spacing and plant densities in the surveyed areas

	ASS		Average spacing (Cm)				Average number of plants/ha	N
Sub-region	Yes (%)	No (%)	Intra-row	StDv	Inter-row	StDv		
Elabered	77.80	22.20	50.00	7.79	21.30	4.16	84507	18
Geleb	75.00	25.00	48.33	5.37	34.20	14.43	54450	16
Mendefera	85.00	10.00	49.70	20.42	29.90	6.87	60564	20
Dbarwa	73.70	31.60	52.90	6.11	31.20	6.82	54741	19
Adi-quala	90.90	9.10	46.50	8.83	30.80	8.35	62841	11
Dekemhare	82.40	17.60	48.12	9.63	23.80	6.47	78585	17
Foro	57.10	42.90	50.60	4.17	29.10	13.49	61123	14
Gindae	59.40	40.60	50.30	9.03	26.70	10.63	67014	32
Afabet	20.00	80.00	77.50	26.29	60.00	27.08	19355	20
Akurdat	53.30	46.70	64.30	19.02	33.13	14.13	42251	15
Grand mean	65.90	34.10	51.40	13.20	29.60	12.24	59154	182
Chi square	32.582		s.e: 1.987		1.063			
Significance	0.000		0.021		0.314			

ASS= Application of specific spacing

50,000-80,000 plants/ha is normal. In Mauritius 55,000 pl/ha gave the highest yield (6.2 t/ha), in Ethiopia 10 plants/m^2 (90,000 plants/ha) is optimum and in Zimbabwe 30,000-55,000 plants/ha is adapted for chilli (Grubben & El Tahir, 2004).

The results of the current study show that spacing and plant population although variable but are similar to those common in other African countries. Moreover closer spacing gives high yield in short period while wider spacing allow picking over longer period (Burt, 1999). In many of the surveyed areas the growing season is short and farmers look for faster returns which could be the reason for adoption high plant population.

3.3.5 Fertilization

Availability of adequate nutrients in the soil is crucial for obtaining good yield. The results of this study show that 76.9% and 70.9 of the respondents apply organic and mineral fertilizers respectively (Table 3.13), while 9.9% do not apply fertilizer but they annually divert floods into their fields for adding silt that is rich in organic matter (Plate 4). The highest percentage for application of organic fertilizer (100%) was in Geleb, Mendefera, Dbarwa and Dekemhare. Similarly the highest for mineral fertilizer was 100% recorded in Mendefera, Dbarwa and Adi-quala. Respondents in Foro followed by Afabet were the lowest in fertilizer application with 7.1% and 10% of organic fertilizer respectively and 0% of mineral fertilizer. The reason for these low percentages is that in Foro 92.9% of the respondents use siltation and in Afabet farmers used to be dependent on diverting floods which is not currently possible due to unavailability of diversion structures that fit the current depth of the Mogae river. Sub-regions were significantly differen for application both organic and mineral fertilizers (Table 3.13 and Plate 1).

Table 3.11: Manure and mineral fertilizer application

	Manure			Mineral fertilizer		
Sub-region	Yes (%)	No (%)	Siltation (%)	Yes (%)	No (%)	N
Elabered	94.40	0.00	5.60	66.70	33.30	18
Geleb	100.00	0.00	0.00	75.00	25.00	16
Mendefera	100.00	0.00	0.00	100.00	0.00	19
Dbarwa	100.00	0.00	0.00	100.00	0.00	20
Adi-quala	90.90	9.10	0.00	100.00	0.00	11
Dekemhare	100.00	0.00	0.00	88.20	11.80	17
Foro	7.10	0.00	92.90	0.00	100.00	14
Gindae	84.40	6.30	9.40	78.10	21.90	32
Afabet	10.00	85.00	5.00	0.00	100.00	20
Akurdat	73.30	26.70	0.00	100.00	0.00	15
Grand mean	76.90	13.20	9.90	70.90	29.10	182
Chi square		230.018		113.042		
Significance		0.000		0.000		

The results in Table 3.14 show that 56.4 of the respondents use purchased organic fertilizer with the highest in Dekemhare (88.2%) and lowest in Geleb (18.8%). The results also show that 52.1% of the respondent reported that organic fertilizer is not available, with the highest 82.4% in Dekemhare and the lowest 18.8% in Geleb (excluding Foro and Afabet where only 1 &2 respondents respectively apply fertilizer). However, this varies from place to place depending on availability of animal resources in the area, number of users and whether the farmer uses his own

A **B**

Plate 1: Diversion of flood water to the fields in Dogali (A) and a farmer showing amount of silt accumulated in one season in the same farm

animal manure or purchases it. It is evident that there is positive relationship between using their own manure and its availability (Table 3.14).

The amount of organic fertilizer applied by the respondents ranged from 0.6 t/ha to 19.2 t/ha (Table 3.14). Collectively 79.1 % of the respondents apply an amount of less than 10 t/ha. Out of these 31.2% of the respondents apply even less than 2.5 t/ha. Respondents applying less than 2.5 t/ha ranged from 100% in Foro to 0% in Elabered. The percent of respondents who do not know the exact amount they apply was 17.1% of which the 7.1% add organic fertilizer indirectly by allowing 200-300 goats to graze and stay on the land for about 2 to 3 months. This method is specifically applied in Demas area of the Gindae sub-region. Both the grower and goat owner are benefiting by exchange of grazing permission with manure left in the ground (Table 3.14).

Most of the respondents (58.57%) apply mixed animal manure, with the highest in Foro (100%) and the lowest 20% in Adi-quala (Table 3.15). Differences in type of manure are generally due to farmer's preference and type of animals common in the area. The results in Table 3.15 also show that 50.4% of the farmers in the surveyed areas apply manure during land preparation with the highest in Dekemhare (94.12%) and lowest in Foro and Afabet (0%). The rest (49.64) apply it in different stages from transplanting to cultivation and even with irrigation water. This explains why farmers

Table 3.12: Source, availability and amount of manure applied

	Source		Availability		Amount (ton)					
Sub-region	Own (%)	Pur (%)	Av (%)	Nav (%)	<2.5 (%)	3.5-5 (%)	7-10 (%)	14-20 (%)	Unkn (%)	N
Elabered	64.70	35.30	58.80	41.20	0.00	64.70	11.80	0.00	23.50	17
Geleb	81.30	18.80	81.30	18.80	62.60	12.60	0.00	0.00	25.00	16
Mendefera	47.40	52.60	42.10	57.90	21.10	15.80	57.90	5.30	0.00	19
Dbarwa	35.00	65.00	35.00	65.00	40.00	20.00	20.00	15.00	5.00	20
Adi-quala	60.00	40.00	70.00	30.00	21.40	28.60	50.00	0.00	0.00	10
Dekemhare	11.80	88.20	17.60	82.40	11.80	35.30	35.30	11.80	5.90	17
Foro	100.00	0.00	0.00	100.00	100.00	0.00	0.00	0.00	0.00	1
Gindae	29.60	70.40	44.40	55.60	33.30	25.90	3.70	0.00	37.00	27
Afabet	100.00	0.00	100.00	0.00	50.00	0.00	0.00	0.00	50.00	2
Akurdat	18.20	81.80	45.50	54.50	63.60	9.10	0.00	0.00	27.30	11
Mean	43.60	56.40	47.90	52.10	31.2	26.4	21.50	4.20	17.10	140

Pur= Purchased Av = Available Nav= Not available Unkn= Unknown

in many of the surveyed areas believe that manure applied in the current season is only beneficial in the following season or year.

Table 3.13: Type of manure and time of application

	Type of manure			Time of application					
Sub-region	CD (%)	SRD (%)	Mix (%)	DLP (%)	BP&AT (%)	On ridge (%)	DC (%)	WIW (%)	N
Elabered	11.76	5.88	82.35	29.41	29.41	5.88	35.29	0.00	17
Geleb	37.50	6.25	56.25	56.25	0.00	37.50	6.25	0.00	16
Mendefera	26.32	5.26	68.42	31.58	0.00	47.37	21.05	0.00	19
Dbarwa	20.00	10.00	70.00	25.00	0.00	55.00	20.00	0.00	20
Adi-quala	70.00	10.00	20.00	40.00	0.00	40.00	10.00	10.00	10
Dekemhare	17.65	5.88	76.47	94.12	0.00	5.88	0.00	0.00	17
Foro	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	1
Gindae	7.41	55.56	37.04	85.19	7.41	3.70	3.70	0.00	27
Afabet	50.00	0.00	50.00	0.00	0.00	100.00	0.00	0.00	2
Akurdat	9.00	45.45	45.45	18.18	0.00	27.27	36.36	18.18	11
Mean	22.14	19.29	58.57	50.36	5.04	27.34	15.11	2.16	140

CD= Cattle dung SRD=Small ruminants dung Mix= Mixed animal manure DLP= During land preparation BP&AT= Before plough & after transplant On ridge= Before & during transplant on ridge DC= During cultivation WIW= With irrigation water

The results in Table 3.16 show that 59.1% of the respondents use urea and DAP together with the highest in Dekemhare (80%) and lowest in Foro and Afabet (0%). The results also shows that 31.8% apply only urea with the highest in Adi-quala (54.5%) and lowest in Foro and Afabet (0%) (Table 3.16). The reason for using only these two types is that they are the only known mineral fertilizers to farmers in Eritrea except in Akurdat where a complete foliar fertilizer is used. Average amount applied is 57±35.5 and 43.6±29.2 Kg/ha of nitrogen and phosphorus respectively, 70% of the applied nitrogen is in the form of urea; while the rest come from DAP. The highest average amount of applied nitrogen and phosphorus was in Dbarwa (71.1±46.7 and 56.3±43.3 kg/ha), while the lowest (0%) was in Foro and Afabet. Application method of mineral fertilizers is equally shared between broadcasting and side dressing where each method is applied by 44.2% of the respondents. The highest

Table 3.14: Type of mineral fertilizer, application method and mean amount

	Type of fertilizer				Application method				Amount				
Sub-region	U (%)	D (%)	U&D (%)	U&F (%)	BD (%)	SD (%)	BD&SD (%)	F (%)	TN (Kg)	NU (%)	ND (%)	P (Kg)	N
Elabered	25.00	8.30	66.70	0.00	25.00	66.70	8.30	0.00	45.2±26.65	75.00	25.00	28.5±16.35	12
Geleb	41.70	0.00	58.30	0.00	50.00	33.30	16.70	0.00	29.4±16.65	69.00	31.00	23.0±13.54	12
Mendefera	31.60	0.00	68.40	0.00	57.90	31.60	10.50	0.00	56.5±22.52	69.00	31.00	44.3±29.16	19
Dbarwa	25.00	0.00	75.00	0.00	45.00	35.00	20.00	0.00	71.1±46.69	69.00	31.00	56.3±43.3	20
Adi-quala	54.50	0.00	45.50	0.00	36.40	63.60	0.00	0.00	61.1±32.37	72.00	28.00	43.2±30.1	11
Dekemhare	13.30	6.70	80.00	0.00	53.30	40.00	6.70	0.00	51.2±28.63	65.00	35.00	46.0±25.87	15
Foro	0.00	0.00	0.00	0.00	0.00	0 .00	0.00	0.00	0.00	0.00	0.00	0.00	0
Gindae	24.00	24.00	52.00	0.00	56.00	40.00	4.00	0.00	59.7±41.68	73.00	27.00	41.0±32.21	25
Afabet	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Akurdat	53.30	0.00	26.70	20.00	13.30	60.00	6.70	20.00	62.0±24.25	70.00	30.00	48.3±27.11	15
Grand mean	31.80	6.20	59.70	2.30	44.20	44.20	9.30	2.3	57±35.48	70.00	30.00	43.6±29.22	129

U= Urea D= DAP UD= Urea & DAP F=Foliar UF=Urea & Foliar BD=Broadcasting SD=Side dressing BD&SD=Broadcasting & Side dressing TN= Total nitrogen P=Phosphorus Nu=Percent nitrogen in urea form ND=Percent nitrogen in DAP form

for broadcasting (57.9%) as in Mendefera and for side dressing (66.7%) in Elabered, while the lowest for both mehtods was 0% in Foro and Afabet (Table 3.16).

The amount of fertilizer to be applied depends on soil fertility and climatic conditions, thus the recommended amounts vary from place to place. However, in Eritrea there is no recommended amount of fertilized to be applied for pepper. Grubben and El Tahir, (2004), recommendation for pepper in tropical Africa is a supply of 10–20 t/ha of organic fertilizer and 130 kg/ha of N, 80 kg/ha of P and 110 kg/ha of K; in addition to Boron at the rate of 10 kg/ha. Pepper growers in Eritrea are aware of the importance of both organic and mineral fertilizers for improving yield and quality of their crop. However, the results of this study show that the amount applied is far below that recommended by Grubben and El Tahir, (2004). The main reasons for this could be unavailability of fertilizers (Table 3.14 and 3.20) and the high cost described during discussions with farmers and key informants; as not affordable by most small scale farmers.

3.3.6 Insect pests, diseases and weeds

The draft policy of agriculture (MoA, 2002), predicted that the build-up of insect pests and disease will be a problem in future that necessitates setting strategies for managing it. The results of the current study show that 58.8% and 56.6% of the respondents have severe insect pest and disease problems respectively. The highest severity for insect pests and diseases was recorded in Akurdat (100% and 93.3% respectively), while the lowest was in Adi-quala with 27.3% and 0% respectively (Fig. 3.1). The results also show that 74.7% of the respondents use chemical control for combating insect pests and diseases. The highest was in Adi-quala (100%) and the lowest in Afabet (40%). A negative relationship was observed between intensity of chemical control and severity of insect pests and diseases excluding Akurdat where 93.3 of the respondents depend on chemical control yet recorded the highest insect pests and diseases problems (Fig. 3.1). The high dependency on chemical control could have negative environmental and economical consequences where globally the trend is towards organic production which will negatively affect opportunities for accessing export markets.

The most common insect pests mentioned by farmers and experts were white fly, African ball worm, aphids, rust mites and termites in addition to birds. Purple blotch, fusarium wilt, powdery mildew, downy mildew, leaf spot, cereosspora disease and anthracnose were the most serious diseases.

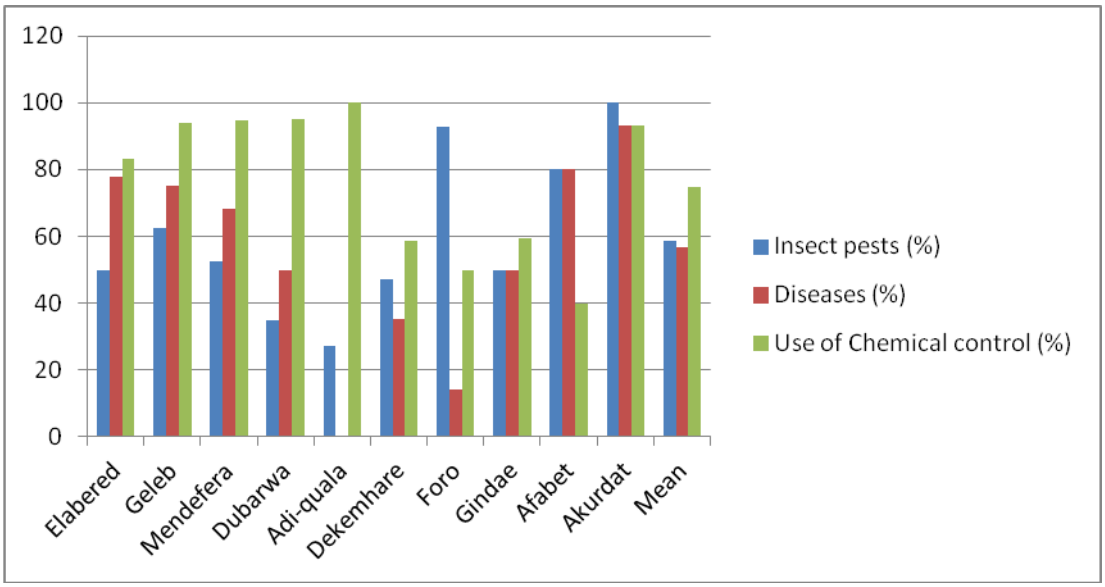

Figure 3.1: Effect of chemical control on severity of Insects pests and diseases in different sub-regions

Similar to insect pests and diseases 42.3% and 39.6% of the respondents suffer severe and medium weed problems respectively. The highest for severe weed problem was in Akurdat (100%) and the lowest in Adi-quala (0%), while for medium weed problem the highest (73.7%) was in Mendefera and the lowest (0%) in Akurdat (Table 3.17). Generally severity of weeds was higher in areas where flooding of fields is used for irrigation like Foro, Gindae and Afabet (71.4, 59.4-& 60% respectively) or areas where most of the fields are on river banks like Akurdat (100%). Majority of the farmers (65.5%) use both cultivation and hand weeding, with highest (96.9%) in Gindae and lowest (35%) in Afabet were hand weeding using *Nejama* (a small local hand tool) is common (Table 3.17).

3.3.7 Productivity

The results of the current study show that average yield for green pepper was 3.4±2.7 t/ha. The highest mean yield was in Dbarwa (6.1±3.4t/ha) followed by Mendefera (4.8±4t/ha) and the lowest mean yield was in Afabet (1t/ha). The highest yield was

Table 3.15: Severity of weed problem and control methods

	Severity				Control method			
Sub-region	NoPro (%)	Low (%)	Med (%)	Sev (%)	Cul (%)	HW (%)	Both M (%)	N
Elabered	0.00	27.80	44.40	27.80	50.00	5.60	44.40	18
Geleb	6.30	6.30	50.00	37.50	62.50	0.00	37.50	16
Mendefera	0.00	15.80	73.70	10.50	10.50	5.30	84.20	19
Dbarwa	10.00	40.00	45.00	5.00	30.00	0.00	70.00	20
Adi-quala	0.00	81.80	18.20	0.00	63.60	0.00	36.40	11
Dekemhare	0.00	5.90	52.90	41.20	23.50	0.00	76.50	17
Foro	0.00	0.00	28.60	71.40	28.60	7.10	64.30	14
Gindae	0.00	9.40	31.30	59.40	0.00	3.10	96.90	32
Afabet	0.00	0.00	40.00	60.00	0.00	65.00	35.00	20
Akurdat	0.00	0.00	0.00	100.00	13.30	0.00	86.70	15
Grand mean	1.60	16.50	39.60	42.30	24.20	9.30	66.50	182

NoPro= No problem *Med= Medium* *Sev= Severe* *Cul=Cultivation*
HW=Hand Weeding *Both M=Both methods (cultivation and hand weeding)*

Table 3.16: Yield for green and dry pepper

	Green pepper (ton/ha)			Dry pepper (ton/ha)		
Sub-region	Mean	Max	Min	Mean	Max	Min
Elabered	2.9±2.20	7.50	0.50	0.51±0.38	1.00	0.10
Geleb	2.5±1.21	4.80	1.00	0.55±0.67	1.60	0.10
Mendefera	4.8±4	180	1.00	0.35±0.36	1.00	0.03
Dbarwa	6.1±3.42	12.00	3.00	1.60±1.04	3.00	0.15
Adi-quala	2.9±1.56	6.00	1.00	0.54±0.36	1.00	0.15
Dekemhare	2.7±1.32	6.00	1.00	0.61±0.36	1.00	0.10
Foro	2.5±0.97	4.00	1.20	0.97±0.84	3.00	0.30
Gindae	2.4±1.65	8.00	0.40	1.17±1.15	3.00	0.02
Afabet	1.0±0	1.00	1.00	0.60±0.32	1.30	0.20
Akurdat	2.4±1.58	5.00	0.30	1.38±0.92	4.00	0.70
Grand mean	3.4±2.72	18.00	0.30	0.87±0.81	4.00	0.02
s.e.	0.2386			0.0808		
Significance	0.000			0.001		

recorded in Mendefera (18t/ha) and the lowest in Afabet (1t/ha) while the highest minimum yield was 3t/ha recorded in Dbarwa and the lowest 0.3t/ha recorded in Akurdat. For dry pepper average yield was 0.87±0.8t/ha with the highest mean yield of 1.6±1.04t/ha in Dbarwa and lowest mean yield of 0.35±0.36t/ha in Mendefra. The highest maximum yield was 4t/ha recorded in Akurdat and the lowest 1t/ha recorded in Elabered, Mendefera, Adi-quala and Dekemhare, while the highest minimum was 0.7t/ha recorded in Akurdat and the lowest 0.02t/ha obtained in Gindae (Table 3.18). This very low yield of dry pepper in Gindae was recorded in an area of rainfed pepper production that has been experiencing drought conditions for the last few years in addition to that farmers tend to harvest the early fruits as green crop before allowing it to fully mature and harvested dry. Average yields found in this study are very low compared to the 2011 world and African averages (FAOSTAT, 2012). However, it reflects the reality that productivity of pepper in Eritrea is greatly

declining. For green pepper; Eritrea that used to have an average yield of 8.12t/ha in 1968 (Table 1.3) which is greater than the average of Africa in 2011 (FAOSAT 2012), is today among the lowest in yield per hectare. In the last few years yield of green pepper declined from 10.6 t/ha in 2008 to 7.3 t/ha in 2010 to 3.7 t/ha in 2011 (Table 1.4).

3.3.8 Constraints and opportunities for pepper production in Eritrea

Constraints

Up to 1974 Eritrean average annual export of horticultural crops was US$1.02 million (Bank of Ethiopia,1965-1974). During this period pepper production and export in Eritrea was increasing and mean yield per ha as per 1968 (8.2 ton/ha) was much greater than the 2011 average of 3.7 ton/ha (Table 1.3). It was also greater than average yield/ha of Africa for 2011 which is 7.47 (FAOSTAT, 2015). This indicates to several constraints contributing to the current low production and productivity of pepper in Eritrea as well as its quality. Constraints identified by the Eritrean national agricultural development strategy and policy (MoA, 2006) are; small land size and discouraging tenure system, declining soil fertility, seed, weeds, insect pests and diseases, labour, lack of skill based extension support, insufficient access to water, in adequate supply of inputs and services and high post-harvest loss. Similar results were identified in the current study. Results extracted from contacting 25 key imformants, conducting 9 farmer group discussions and formal questionnaire of 182 housholds are summarized in Table 3.19.

Table 3.17: Summary of the majore constraints based on the number of times each constraint mentioned in group discussions, key informants interviews and response of the individual farmers on the questionnaire.

	Constraints	discussion	N	informants	N	farmers	N
1	Inputs and services	8	9	19	25	148	182
2	Improved and quality seed	7	9	18	25	94	182
3	Pests and diseases	9	9	12	25	56	182
4	Water scarcity	5	9	9	25	34	182
5	Land tenure & holding size	5	9	9	25	33	182
6	Extension service	5	9	8	25	25	182
7	Labour	3	9	11	25	0	182
8	Marketing chain	3	9	2	25	13	182

Inputs: The results show for more than 88% of the respondents; inputs were considered unavailable (Table 3.20) resulting in escalating prices and reduced quality of some commodities. Similarly discussions with farmers and key informants revealed that other inputs such as small tools and fuel and services like maintenance service, tractor and machinery services are not available. Previously the Ministry of Agriculture was the major supplier of inputs and service; during the last few years unknown sources are dominating.

Table 3.18: Availability of mineral fertilizer, fungicide and insecticide

	Mineral fertilizer (%)		Fungicide (%)		Insecticide (%)	
Sub-region	Available	Not available	Available	Not available	Available	Not available
Elabered	0.0%	100.0%	7.7%	92.3%	15.4%	84.6%
Geleb	27.3%	72.7%	15.4%	84.6%	15.4%	84.6%
Mendefera	5.3%	94.7%	5.3%	94.7%	5.3%	94.7%
Dbarwa	15.0%	85.0%	15.8%	84.2%	25.0%	75.0%
Adi-quala	9.1%	90.9%	33.3%	66.7%	0.0%	100.0%
Dekemhare	0.0%	100.0%	0.0%	100.0%	9.1%	90.9%
Foro	NA	NA	33.3%	66.7%	42.9%	57.1%
Gindae	20.0%	80.0%	15.8%	84.2%	10.5%	89.5%
Afabet	NA	NA	0.0%	100.0%	0.0%	100.0%
Akurdat	13.3%	86.7%	0.0%	100.0%	0.0%	100.0%
Grand mean	11.7%	88.3%	11.2%	88.8%	11.9%	88.1%

NA= Not applicable because farmers in the two regions do not apply mineral fertilizer

Seed: Improved varieties and quality seed is the basis for any improvement in production and quality. The results showed that pepper is major vegetable that had no attention from the ministry to provide farmers with improved and good quality seed. The National Agricultural Research Institute released 5 lines (NARI, 2005-2011), however, due to absence of seed production system only an average of 17 kg of seed is annually distributed by the institute. Consequently farmers use their own seed or purchase unknown quality seed from the dry consumption market instead of the improved seed.

Pests and diseases: Insect pests and diseases problem were a major concern of farmers as well as experts (Table 3.19). Total crop failure due to termites in Foro and unknown disease in Dekemhare have been reported. Available chemicals are not

reliable due to unknown source and expiry date and being used for long time that may result in pests developing resistance.

Land: The results showed, the main issues of land as a constraint; are the tenure system and size of land (Table 3.19). The three land tenure systems of Eritrea are Diesa, Risti and Dominale. The latter two systems have no problem in maintaining the quality of soil, however, the Deisa system in which all lands are communal property of the village and redistributed equally to all members of the village every 5-7 years may have some disadvantages regarding the land-improvement point of view. The farmers have no incentive to long-term investment on land. Land is exposed to wind erosion due to communal grazing out of season and cannot be used as security to obtain credit (Negassi et al., 2000). The results also revealed that small land size in the Diesa and Resti systems push producers to use rented land in the absence of regulating law between the land owner and renter which interfere with proper investment on land.

Water: Water was discussed as one of the major problems that cause partial or sometimes total crop loss in some areas. Generally in Eritrea rainfall is low and significantly varies from year to year (Hurni & Koller, 2002). Average annual rainfall is about 380 mm, varying from less than 200 mm in the semi-desert areas to over 1000 mm in the sub-humid area (Frenken, 2005). However, Frenken, (2005) reported that only 1 percent of the total area to receive more than 650 mm annual rainfall, while over 90 percent receives less than 450 mm. The results in Table 3.21 show that 81.9% of respondents grow irrigated pepper. It shows also that 90.8% of the respondents use underground water from boreholes. Although the quantities and qualities are not as desired, ground water is the basis for domestic water supply in all parts of the country (Frenken, 2005). Streams are few and all the other water sources available for the growers depend on the amount of rainfall and length of the rainy season for recharging them (Table 3.21). Therefore, farmers reported increased depth of wells and reduced water in the rivers due to reduced rainfall water. Based on water availability the irrigation potential can be estimated at 187 500 ha (Frenken, 2005). The current irrigated land is only 28,000 ha (MoA, 2012). The results of the current

study show that all the respondents use surface irrigation that use large amount of water, especially under the climatic condition of Eritrea where annual evapotranspiration rates range from 1900 mm to 8000 mm (Frenken, 2005). Thus, at country level water shortage may not be currently the problem but efficiency of irrigation methods.

Table 3.19: Type of crop and source of water

	Type of crop			Source of Water			
Sub-region	Irrigated (%)	Rain-fed (%)	entary Irrigation (%)	Borehole (%)	River/stream (%)	Dam (%)	N
Elabered	55.60	11.10	33.30	87.50	12.50	0.00	18
Geleb	100.00	0.00	0.00	100.00	0.00	0.00	16
Mendefera	73.70	0.00	26.30	100.00	0.00	0.00	19
Dbarwa	65.00	0.00	35.00	90.00	0.00	100	20
Adi-quala	90.90	0.00	9.10	100.00	0.00	0.00	11
Dekemhare	100.00	0.00	0.00	100.00	0.00	0.00	17
Foro	100.00	0.00	0.00	77.80	22.20	0.00	14
Gindae	62.50	37.50	0.00	95.20	0.00	4.80	32
Afabet	100.00	0.00	0.00	65.00	35	0.00	20
Akurdat	100.00	0.00	0.00	100.00	0.00	0.00	15
Grand mean	81.90	7.70	10.40	90.80	7.50	1.70	182

Extension service: Farmers need technical support for improving their production technologies and skills. The results of the current study show there are no adequate extension services. The ratio of extension agents to farmers in 2001 was 1:2,800, which is below the average for all developing countries (MoA, 2002). Currently the number may not be a problem, but the function and skill. Too many function of extension agents and low level of education and training of extension agents combined with low level of farmer education are some of the major problems identified during field visits of a strategy team in Eritrea (Steele, 2002). The current

number of agents is not very low, however, they are production oriented and service providers and not farmer support agents (MoA, 2006).

Labour: Cheap family labour is one of the opportunities for small scale farming (Wiggins et al. 2011; Dixie, 2005). In Eritrea the primary source of labour for small scale production is family labour; while commercial producers depend on hired labour. However the results show that only 18% of the respondents use family labour, 46% depend on both hired and family labour and 17% on hired labour; while the rest 20% exchange labour for half of the yield. This indicates to lower input of family labour, loosing one of the important opportunities of small scale farming against large scale systems and leading to small scale farmers competing for hired labour with commercial farmers, as a result labour is unavailable and expensive. Seasonal labour no longer arrives from Northern Ethiopia, and a very large proportion of Eritrea's economically active men are in military service (MoA, 2006).

Marketing: Eritrea has a rich experience in the marketing of horticultural products locally and for export, however, it has been drastically eroded over the years. There is a need for building up an efficient marketing system (MoA, 2006). Discussions with farmers and extension workers show that middlemen are the most beneficiaries of the products under the current marketing system where farmers carry their harvest, pay for transportation up to the whole sale shop and accept the offered price. In rare cases under scarcity conditions middlemen may collect the produce at the farm. According to Dixie (2005) rural businesses are said to be exploiting farmers and making unfair profits, however, these businesses are required for linking farmers to markets, thus improving marketing chain is required. Wiggins, et al. (2011) suggested contracts of farmer association with agri-buisness for giving opportunities to small farmers for a link in rewarding supply chains. Currently post-harvest losses are not a problem for Eritrean pepper farmers, they usually market their product the same day or the next day after harvest; thus less causes of spoilage. Dry pepper may be stored for some time with no spoilage if well dried.

Opportunities

Favourable climate, availability of land, market, experience and willingness to grow pepper are the major opportunities identified through the discussions with farmers and experts and the collected secondary data as discussed below.

Favourable climate: Although a small country; Eritrea has six agro-climatic zones that allow growing variety of species and crop varieties. Most of the regions are suitable for growing pepper at least in one growing season. Exception to that is the southern part of the coastal plains zone where limited agricultural land exist and high temperatures that may not be suitable for commercial pepper production.

The results of the formal household survey showed dominance of small acreage, however, the secondary data show availability of land in all the surveyed areas (Table 3.22). Total potential irrigable land of Eritrea is 600,000 ha (MoA, 2012). Based on water availability the irrigation potential can be estimated at 187,500 ha. (Frenken, 2005). The current area under irrigated cultivation is only 28,000 ha. Thus there is an opportunity for pepper to compete in these lands. This is similar to the underdeveloped large areas in Africa such as the cropping of 10% of the 400 million hectares potential lands in Guinea Savannah (Wiggins et al. 2011).

Table 3.20: Irrigated land under cultivation and potential irrigable land in the four surveyedregion

	Potential	Developed Area(ha)	
Region	Area(ha)	Area (ha)	%
Gashbarka	93465	11880	12.7
Anseba	66352	1604.15	2.4
S/K/Bahri	4220	1663	74.95
Debub	17043	3732.5	21.9
Total	181,080	18,879.7	10

Market: Availability of huge domestic markets and close distance to export market as an opportunity for vegetable sector (Saavedra et al., 2014). Wiggins, et al. (2011)

found the fast growing domestic markets and new export markets provide an opportunity for commercialization of small farms in Africa. According to the Customs Department the value, of the imported dry pepper in 2010 was around US$ 8 million, this is apart from the illegal importations (Ministry of Finance, 2012). So the local market in Eritrea can still absorb huge quantities if good quality pepper produced. On the other hand pepper was identified as one of the crops that potentially has comparative advantage in export markets (MoA, 2006), therefore the closeness of the country to export markets can be an additional potential if export requirements satisfied.

Experience and Willingness: Pepper has been grown in Eritrea for a very long time resulting accumulated experience in growing pepper (Table 3.4). These experiences can be exploited in improving production technologies.

Profit is a major motive for farmers to continue growing any crop. The results of this study show that 76.6% and 82.4% of the respondents think that both green and dry pepper respectively are profitable and 91% are willing to continue growing it even if it is not profitable (Table 3.23). The reason for that is Eritrean farmers grow pepper not only for market but also for home consumption and many consider it way of life. Thus, pepper will continue as one of the important crops of Eritrea which necessitates mechanisms for improving it.

Table 3.21: Profitability of both green and dry pepper and willingness of farmers to continue growing pepper in the future

	Profitability of green pepper		Profitability of dry pepper		Willingness to grow pepper in future	
Sub-zoba	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Elabered	29.4	70.6	57.1	42.9	61.1	38.9
Geleb	92.3	7.7	100.0	0.0	87.5	12.5
Mendefera	94.7	5.3	62.5	37.5	100.0	0.0
Dubarwa	88.9	11.1	83.3	16.7	95.0	5.0
Adi-quala	72.7	27.3	25.0	75.0	81.8	18.2
Dekemhare	81.3	18.8	60.0	40.0	94.1	5.9
Foro	57.1	42.9	100.0	0.0	100.0	0.0
Gindae	86.2	13.8	100.0	0.0	93.8	6.3
Afabet	0.0	100.0	100.0	0.0	100.0	0.0
Akurdat	66.7	33.3	92.9	7.1	93.3	6.7
Total	76.6	23.4	82.4	17.6	91.2	8.8

3.4 Conclusions and recomendations

Eritrea has vast lands, favourable climate, domestic and export markets and farmers growing pepper for long period with high willingness to continue growing pepper are the major opportunities for pepper production. Constraints are unavailability of improved and quality seed, inputs and support services, insect pests and diseases, small acreage, unfavourable land tenure system, improper marketing chain, poor extension service and persistent drought that affect availability of water.

A wide range of pepper genotypes are grown in Eritrea which are traditionally passed from one farmer to another and transversely generations.

Pepper in Eritrea has great potential, however, constraints need to be overcome and opportunities maximized. Improving the existing pepper genotypes could have significant contribution in addressing some of the major challenges in pepper production, such as the productivity, quality, and resistance to major pests or diseases, drought tolerance. Therefore the following recommendations are suggested:

- Breeding program that focuses on the existing local pepper genotypes that has been in cultivation for long time should be strengthened.
- Imroving provision of agricultural inputs and services should be considered.
- Agricultural extension service should provide farmers with technical support to improve their practices regarding maintaining or obtaining good quality seed, nursery practices, irrigation methods, insect pest and disease control.
- The land tenure system problem although complicated, actions can be taken to solve some problems. For example policies and regulation should be put in place to organize the relationship between owners and renters.
- Farmers association initiatives should be strengthened in order to help solve marketing problems and production related issues.

CHAPTER FOUR

ASSESMENT OF THE MORPHOLOGICAL DIVERSITY OF ERITREAN PEPPER

Abstract

Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics. The aim of this study was to assess the diversity of Eritrean pepper germplasm in order to obtain information for improving it. A total of 129 pepper (*Capsicum* spp.) seed sample collections were obtained from farmers and institutions in Eritrea. The collections were evaluated at two sites located in two different agro-climatic regions. Of the 129 collection, 95 were tested at Hamelmalo site and 60 in Asmara. The collections were assessed using 16 quantitative and 23 qualitative descriptors. A Randomized Complete Block Design was used for the evaluation. Quantitative and qualitative data of the two sites were subjected to Principal Component Analysis, Principal Coordinate Analysis, Hierarchal clustering, Analysis of variance and Correlation. The distributions of characters of the different quantitative and qualitative traits and the performance of the collections showed existence of variable characters distributed among the collections indicating considerable diversity. For quantitative variables, the first three components were able to explain 61%, 58% and 67% of the total variation in Hamelmalo, Asmara and combined data of the two sites respectively. While for the qualitative variables the first three components were able to explain a variation of 58% in Hamelmalo, 49%, in Asmara and 55% combined data of the two sites. Phenological attributes and fruit characteristics were found to contribute more to the variation. Majority of the traits evaluated were significant and the highest Coefficient of Variation was related to fruit characteristics. The results of this study showed that there is sufficient variability within the Eritrean genotypes that can be used in future pepper breeding programs.

4.1. Introduction

Plant genetic diversity is the heritable variation within a plant species (Rao & Hodgkin, 2002). Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer- and breeders preferred traits (Govindaraj et al., 2015). Understanding the genetic relationships between chilli accessions may provide an effective management tool for their conservation, as well as to help in a plant breeding program (Votava et al., 2005). Variation in local germplasm has long been utilized for identifying the potential for breeding to meet desirable traits. The findings of Adetula and Olakojo (2006) in Nigeria, Balkaya and Karaagc (2009) and Bozakalfa et al. (2009) in Turkey and Naujeer (2009) in Mauritius working in eggplant, are proper examples of identifying variability within the locally available germplasm that can be utilized for future breeding program. In Eritrea, the National Agricultural Research Institute have been running a selection program from local germplasm for several years that resulted in five breeding lines, however, the program did not study the magnitude of diversity within local pepper germplasm (NARI, 2013). Also there is no documentation for any previous studies regarding genetic diversity of Eritrean pepper. Genetic diversity studies are the first basic step in meaningful breeding programme and therefore require accurate and reliable means for estimation (Aremu, 2012). Presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids (Govindaraj et al., 2015). In Eritrea usually farmers save their own seed and transfer it from generation to the next. However, proper seed production methods including isolation techniques are not in practice within and among farms, giving chance to out-cross and introgression forces to take place. In addition seed exchange across the border with Ethiopia was active for long period of time and since the Italian colonial period numerous exotic varieties were introduced. This is the reason as to why local pepper sold in the market is of mixed pods containing wide range of fruit size, colour, pungency etc. reflecting the rich genetic variation existing in the local genotypes. Thus the aim of the current study was to evaluate local pepper genotypes for diversity using morphological characteristics,

and make the necessary information available for future pepper breeding programs in Eritrean.

4.2. Methodology

4.2.1. Study locations

The experimental locations were Hamelmalo Agricultural College located at 15^{o} 52' 35" N and 38^{o} 27' 45" E at an elevation of 1264 m above sea level and Asmara (Halibet) located at 15°18' 42" N and 38^{o} 56' 15' E and an elevation of 2335 m above sea level. The meteorological data for Asmara show the average annual rainfall and temperature for the period 2008-2013 was 408mm and 8.8 ^{o}C respectively, while for Keren; the nearest station to Hamelmalo the average for the period 2010-2013 was 415mm and 21.7 ^{o}C. Average monthly rainfall and temperature for the experimental period (April-October) in the two sites is shown in Table 4.1. The soil in Hamelmalo was loamy, while in Asmara it was sandy loam. Detailed soil properties of the two sites are in Table 4.2.

Table 4.1: Average monthly rainfall and temperature in the study regions

Month	Hamelmalo[a]				Asmara			
	Average rainfall	Ave Temp	Min Temp	Max Temp	Average rainfall	Ave Temp	Min Temp	Max Temp
April	13	30	21	37	67	18	9	27
May	12	32	25	39	46	18.4	11	26
June	76	34	27	41	60.7	18.7	12	25
July	94	34	30	39	40.4	18	13	24
August	154	33	27	39	44.9	17.5	12	23
September	35	34	28	40	10[b]	16.9[b]	9[b]	23[b]
October	0	34	28	40	1.6[b]	15.7[b]	8[b]	23[b]

[a] = Temperature at Hamelmalo is averages of daily temperatures recorded at 7:00 am and 2:00 pm

[b]= Rainfall and temperature for September and October in Asmara are averages for the previous 5 years

Table 4.2: Soil properties of the two experimental sites

Site	Soil type	N (%)	P(ppm)	OM (%)	EC dS/cm	pH	K	Na	Ca	Mg	CEC
ASM	S.loam	0.03	16.2	0.52	0.77	8	0.93	1.21	49	12	64.2
		Low	Low	Low	NS	SA	Suff	high	Suff	Suff	Suff
HAC	Loam	0.13	70.9	4.14	0.3	7.8	0.06	0.40	23	7	31.5
		Sufft	High	Suff	SS	SA	Low	low	Suff	Suff	Suff

ASM=Asmara HAC=Hamelmalo Agricultural College S.Loam=Sandy loam Suff= Sufficient OM=Organic matter NS=Non saline SS=Slightly saline SA=Slightly alkaline

4.2.2. Plant material

A total of 129 seed samples collected from farmers and institutions were used in this study. The 129 seed samples were 91 from farmers, 32 breeding lines from Hamelmalo Agricultural College and six breeding lines from the Nationa Agriculural Research Institute Seed collection were conducted simultaneous with household survey (Chapter 3). Both geographic and agro-climatic distributions were considered. The plant material from individual farmers was collected randomely from 24 villages in 7 sub-regions of 4 administrative regions of Eritrea (Fig. 4.1 Table 4.3). Villages from where the seed was collected in each sub-region are in close distance ranging from 4 to 10km except in Gindae where the three villages are in three distinct areas (Midland, lowland and coastal). Most of the farmers are small scale farmers who grow pepper in close proximity to each other in lands usually less than one hectare and as small as 0.015 ha. (Fig 4.1).

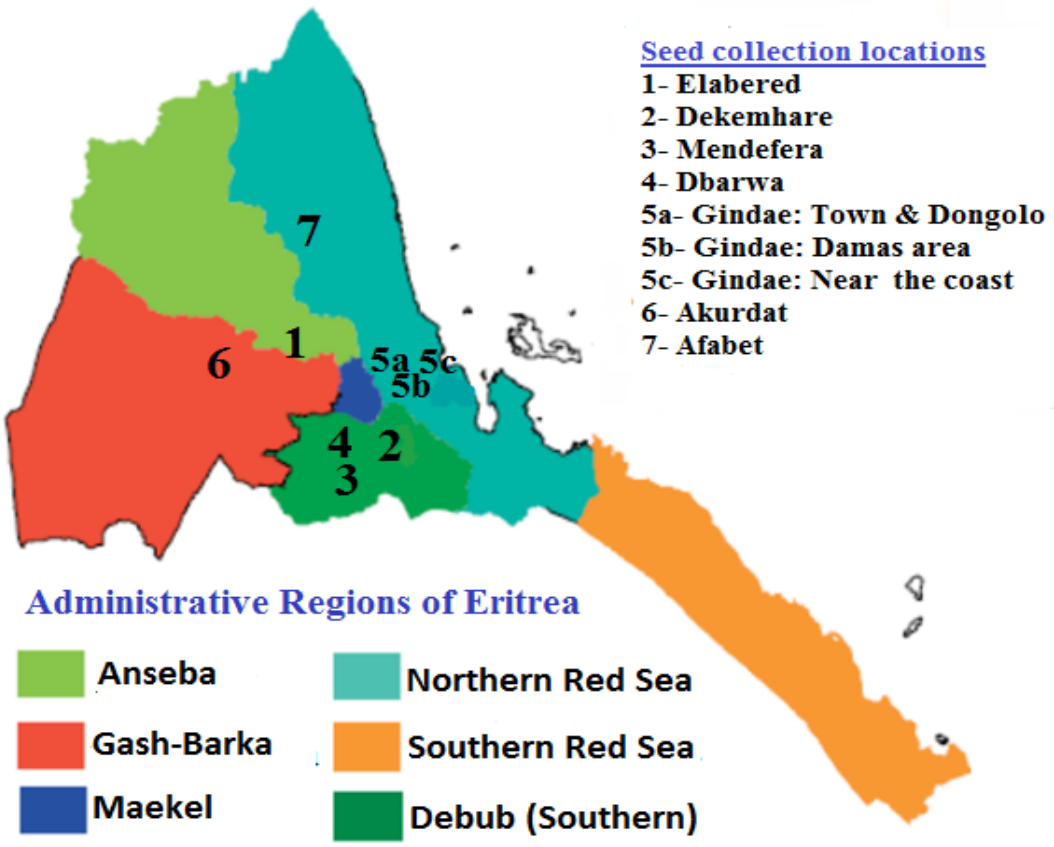

Figure 4. 1: Seed collection locations sites in different administrative regions and agro-climatic zones of Eritrea

Table 4.3: Features of seed collection used in the study

Seed source	Type	Region	NV	NSC	Collections (Code number)
Elabered	Sub-region	Anseba	4	11	ANE01, ANE02, ANE03, ANE04, ANE05, ANE06, ANE07, ANE08, ANE09, ANE10, ANE011
Dekemhare	Sub-region	Debub	1	6	DDK01, DDK02, DDK03, DDK04, DDK05, DDK06
Mendefera	Sub-region	Debub	4	13	DME01, DME02, DME03, DME04, DME05, DME06, DME07, DME08, DME09, DME10, DME11. DME12, DME13
Dbarwa	Sub-region	Debub	4	6	DDB01, DDB02, DDB03, DDB04, DDB05, DDB06
Gindae	Sub-region	NRS	8	29	NRSG01, NRSG02, NRSG03, NRSG04, NRSG05, NRSG06, NRSG07, NRSG08, NRSG09, NRSG11, NRSG12, NRSG13, NRSG14, NRSG15, NRSG16, NRSG17, NRSG18, NRSG19, NRSG20, NRSG21, NRSG22, NRSG23, NRSG24, NRSG25, NRSG26, NRSG27, NRSG28, NRSF01, NRSF02, NRSF03, NRSF04
Akurdat	Sub-region	Gash-Barka	1	3	GBA01, GBA02, GBA03
Afabet	Sub-region	NRS	2	20	NRSAF01, NRSAF02, NRSAF03, NRSAF04, NRSAF05, NRSAF06, NRSAF07, NRSAF08, NRSAF09, NRSAF10, NRSAF11, NRSAF12, NRSAF13, NRSAF14, NRSAF15, NRSAF16, NRSAF17, NRSAF18, NRSAF19, NRSAF20
NARI	Institute	Debun	-	6	Red Long, Chocolate, Red short, Group IA, Group IB, Gahtielay
HAC	Institute	Anseba	-	32	HD0005, HD0009, HD0013, HD0015, HD0023, HD0031, HD0033, HD0036, HD0062, HD0070, HD0074, HD0075, HD0076, HD0077, HD0079, HD0083, HD0090, HD0095, HD0108, HD0112, HD0116, HD0117, HD0118, HD0123, HD0128, HD0134, HD0143, HD0144, HG0018, HG0028, HG0033, HG0077
Total			**24**	**129**	

NV= Number of villages from where seed was collected NSC= Number of seed collections NRS= Northern Red Sea
ANE= Anseba, Elabered DDK= Debub, Dekemhare DME= Debub, Mendefera DDB= Debub, Dbarwa NRSG= Northern Red Sea, Gindae
GBA= Gash-Barka Akurdat NRSAF= Northern Red Sea, Afabet HD= Hamelmalo dry HG= Hamelmalo green

Data regarding specific location and other important information were recorded (Appendix 6) and an average of 50g of seed was collected for farmer variety of which half was sent to the genebank in NARI and the rest used in the experiment.

The collected seed materials are not named varieties or named landraces but sort of heirlooms maintained by farmers through selecting the best pods or plants and transferred from generation to the next. These are exchanged among farmers within the village, sub-region and beyond. Under such conditions, genotypes have an opportunity to interbreed and create a common gene pool leading to the formation of populations.. Breeding lines of each NARI and HAC (Table 4.3) are a result of mass selection from local seed in two separate programs. This means genotypes of each of the two institutions have its own common ancestor, thus each of them forms a population. The term collection will be used for describing each seed sample collected and used in this study.

4.2.3. Nursery and transplanting

Nursery beds were prepared by digging the soil, adding organic manure and well mixing with soil and the land was levelled and sunken beds of 50 x 50cm prepared. In each bed, 10 grams seed of each of the 129 farmer varieties and breeding lines were sown in rows 15cm apart and covered with a shade net. Water was applied daily to the beds using hose and rose and when seed started germination the shed net was raised and fixed at 1.5 metre above the beds for protecting the emerging seedling from direct sunlight. Thining was applied after germination leaving 30 seedlings per row for allowing sufficient spacing for the growing seedlings and 10 days before transplanting the net was totally removed as part of the hardening process.

Out of 129 collections, 27 were extremely weak in germination and failed to produce sufficient number of seedlings for the experiment. Therefore, they were excluded from the experiment, while the rest 102 were prepared for transplanting.

At Hamelmalo seedlings were transplanted in to the field beds at age of 6 weeks (4-6 true leaves), while in Asmara they were transplanted at the age of 7 weeks.

4.2.4. Experimental design and field layout

A Randomized Complete Block Design (RCBD) with three replications in each sit(Hamelmalo and Asmara) was used for evaluating the germplasm.

Hamelmalo: At Hamelmalo all the 102 entries that performed well in nursery were included. Thus each block was formed by 102 plots. Each block was further divided into three sub-blocks each to accommodate 34 plots. Plots were formed by 1 m wide and 2.9 m long raised beds. Each bed included 12 pepper plants arranged into two rows with inter and intra row spacing of 50cm (Fig.4.2). Details of entries distribution in the three blocks are in Appendix 7. However, of the 102 collections seven could not survive extra ordinary rain and hail damage. Thus data was collected only on the survived 95 collections. The seven collections are; DDB05, NRSG11, NRSG13, NRSG28, HD0074 and HD0090.

Asmara: In Asmara due unavailability of adequate land size only 60 entries were included. Thus each block was formed of 60 plots. Each block was further divided into three sub-blocks each to accommodate 20 plots. Plots were formed by 1 m wide and 2.9 m long raised beds. Each bed included 12 pepper plants arranged into two rows with inter and intra row spacing of 50cm (Fig.4.2). Details of entries distribution in the three blocks are in Appendix 8.

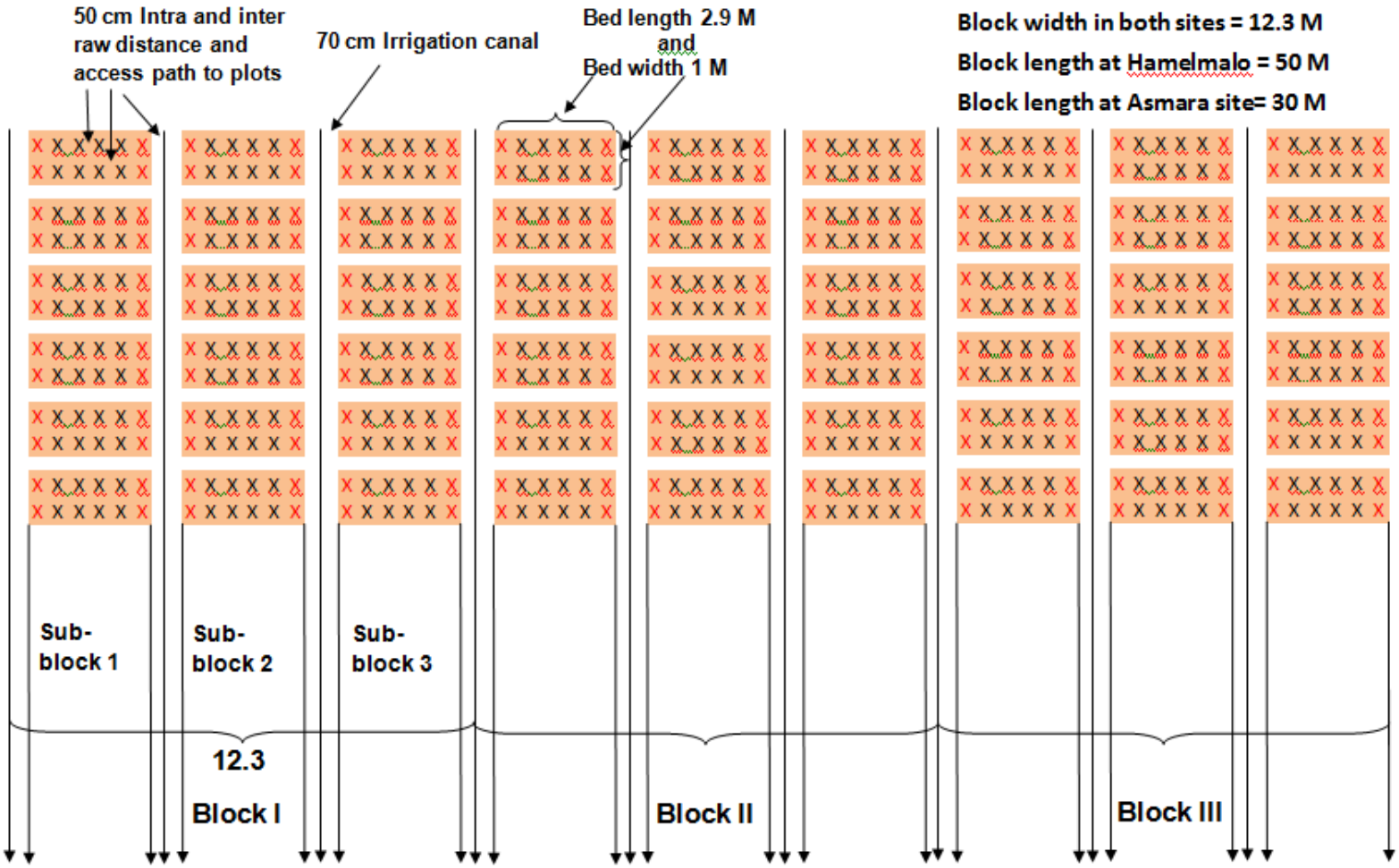

Figure 4. 2: Experimental design used for evaluating the germplasm in both Hamelmalo and Asmara experimental sites

4.2.5. Data collection

A total of 39 quantitative and qualitative morphological data were recorded using the descriptiors for capsicum (IPGRI et al., 1995). These were seedling , phenology, vegetative, flower and fruit characteristics (Table 4.4).

- Cotyledone data were recorded on 10 randomely selected seedlings from central two rows in the nursery.
- Field data were recorded from four randomly selected plants from the centre of the plot excluding the two border plants in each side of the plot.
- Phynological data were recorded as 50% of the seeds germination, 50% of the plants in a plot flowered and 50% of the plants beared fruit.
- Flower characteristics data were recorded on fully open flowers from the first flowring
- Fruit characteristics data were recorded on randomely selected 10 fruits from the second harvest

4.2.6. Data analysis

GENSTAT Discovery edition 4 was used for analysis of the data. The data was subjected to analysis of variance (ANOVA), principal component analysis (PCA), principal coordinate analysis (PCoA) and hierarchal clustering with Euclidean distance. The PCA was run using the 16 quantitative and 23 qualitative data sets and the resulted scree diagram was used to identify the number of components to be used for analysis. Following that principal component analysis was run using six components for the quantitative data and five for the qualitative. A varimax rotation was used for getting clear structure of the components. Then variants scored lower than 0.4 were excluded for improving the percentage explained by the six components. Consequently all the 16 quantitative traits were found to explain enough variation in the data. While for qualitative characters only 10 characteristics scored 0.4 or higher were selected. Based on the result of PCA, the 16 quantitative and 10 qualitative characteristics were used for hierarchical cluster nalysis of the collections conducted based on correlation similarity matrix for handling the different scales of measurements of the variables (Harding and Payne, 2012).

Table 4.4: Descriptors used for evaluating the germplasm (IPGRI et al., 1995)

Descriptor	Code	Value
Seedling stage		
Days to 50% germination	DG	Number of days from sowing to germination
Hypocotyl colour	HC	White= 1 1Green=2 Purple=3
Hypocotyle pubsecence	HP	Sparse=3 Intermediate=5 Dense=7
Cotyledonous leaf shape	CLSh	Deltoid=1 Ovate=2 Lanceolate=3 Elong-deltoid=4
Vegetative growth		
Plant growth habit:	PGH	Prostrate=3 Intermediate=5 Erect=7 Other(Specify)
Stem pubescence	SP	Sparse= 3 Intermediate=5 Dense=7
Leaf colour	LC	Yellow= 1 Light green=2 Green= Dark green=3 Light purple=4 Purple=5 Variegated=6 Other (specify)
Leaf shape	LSh	Deltoid=1 Ovate=2 Lanceolate=3
Lamina margin	LM	Entire=1 Undulate=2 Ciliate=3
Leaf pubescence	LP	Sparse=3 Intermediate=5 Dense=7
Flower and fruit		
Number of flowers per axil:	NFA	1=One 2=Two 3=Three or more 4=Many flowers in bunches but each in individual axil 5= Other (specify
Flower position	FP	Pendant=3 Intermediate=5 Erect=7
Corolla colour	CC	White=1 Light yellow=2 Yellow=3 Yellow-green=4 Purple with white base=5 White with purple base=6 White with purple margin=7 Purple=8 Other (specify)
Calix margin	CM	Entire=1 Intermediate=2 Dentate=3 Other (specify
Calix annular constriction	CAC	Absent=0 Present=1
Fruit colour at intermediate stage:	FCIS	White=1 Yellow=2 Green=3 Orange= 4 Purple=5 Deep purple=6 Other (specify)
Fruit colour at mature stage:	FCMS	White=1 Lemon-yellow=2 Pale orange-yellow=3 Orange yellow=4 Pale orange=5 Orange=6 Light red=7 Red=8 Dark red=9 Purple= 10 Brown= 11 Black= 12 Other (specify)
Fruit shape :	FSh	Elongate=1 Almost round=2 Triangular=3 Campanulate=4 Blocky (Oblong)=5 Other (specify)
Fruit shape at pedicel attachment	FShPA	Acute=1 Obtuse=2 Truncate=3 Cordate=4 Lobate=5
Neck at base of fruit	NBF	Absent=0 Present=1
Fruit shape at blossom end	FShBE	Pointed= 1 Blunt=2 Sunken=3 Sunken & Pointed= 4 Other(specify)
Fruit blossom end appendage	FBEA	Absent=0 Present=1
Fruit surface:	FS	Smooth=1 Semi-wrinkled=2 Wrinkled=3
Fruit cross sectional corrugation	FCSC	Slightly corrugated =3 Intermediate = 5 Corrugated=7

4.3. Results

4.3.1. Morphological description of the germplasm

The distribution of the characteristics within the traits for the 23 qualitative traits showed wide range of variation for most of the traits (Table 4.5). Only five traits showed no variation. These are leaf margin, leaf pubescence, calyx margin, corolla colour and blossom end appendage These predominant characters (100% occurrence) are entire leaf margin, sparse leaf pubescence, Dentate calyx margin, white corolla colour and absence of fruit blossom end appendage. Other traits showed low variation such as cotyledon leaf shape of which 90.5% of the collections were lanceolate. Some traits showed a mixture of values thus described as mixed. In most traits the distribution of the characters was even.

Table 4.5: Percentage distribution of characters for 14 qualitative traits that showed variation

Trait	Percentage occurrence of traits				CV%
HC	Purple= 64.2	Green=32.6	White=3.2		23.1
HP	Sparse= 43.2	Intermediate= 50.5	Dense=7.4		29.7
PGH	Intermediate= 36.8	Erect=63.2			14.2
SP	Sparse= 75.8	Intermediate= 24.2			22.2
LSh	Ovate= 50.5	Lanceolate= 29.5	Mixed= 17.9		23.5
CAC	Present= 45.3	Absent=43.2	Mixed=11.6		87.8
FCMS	Light red= 57.9	Dark red= 23.2	Light brown= 7.4	Brown=9.5	41.9
FSh	Elongate= 73.7	Triangle= 15.8	Mixed=10.5		53.6
FP	Erect=34.7	Intermediate= 53.7	Mixed= 11.6		16.4
FShBE	Blunt= 61.1	Pointed=37.9	Sunken= 1.1		42.7
FShPA	Truncate= 47.4	Obtuse= 34.7	Cordate= 6.3	Mixed=11.6	28.7
FS	Smooth= 42.1	Semi-Wrinkled= 50.5	Mixed= 7.4		29.6
NBF	Absent = 54.7	Mixed=45.3			180
FCSC	Semi-Corrugated= 50.5	Intermediate= 34.7	Mixed=14.7		26.3

HC= Cotyledon color HP= Cotyledon pubescence CLSh= Cotyledon leaf shape CAC=Calyx annular constriction FCMS=Fruit color at mature stage FCSC=Fruit cross sectional corrigation FP=Flower position FShBE=Fruit shape at blossom end FShPA=Fruit shape at pedicel attachment FS= Fruit surface LSh=Leaf shape SP= Stem pubescence PGH=Plant growth habit

However, for some of the traits the values were skewed towards certain characters, for instance Elongate fruit shape (73.7%) and sparse stem pubescence (75.8%), while characters showing low values were white hypoctyle colour (3.2%) and sunken fruit shape at blossom end (1.1%) (Table 4.5).

Hypocotyl colour (HC)for 61 (64.2%) of the collections was purple and only three (3.2%) were white, while the rest 31 (32.6%) had green HC. Majority of the collections with purple HC (30) had intermediate pubescence (HP), while 25 collections had sparse HP and only six with dense HP (Table 4.5 and 4.6). Plant growth habit (PGH) was either erect or intermediate. Majority of the collections (60) were erect. The 30 purple HC and intermediate HP collections were divided into 18 collections with erect and 12 intermediate PGH. Similarly 15 of the collections with purple sparse hypocotyle were erect and the rest intermediate. The 31 collections with green HC were 12 with sparse and 17 intermediate HP and only two dense. All except three of the green intermediate hypocotyle were with erect PGH, while all green sparse except four were with erect PGH (Table 4.6). Sparse stem pubescence (SP) was dominant (75.8% of the collections) to the intermediate SP (24.2%) (Table 4.5). All except two of the 15 collections with purple sparse hypocotyle and erect PGH were with sparse SP, while the 11 purple sparse hypocotyle with intermediate PGH were divided into five sparse and six intermediate SP. All except three of the 12 collections with intermediate PGH had sparse SP, while of the 18 collection with erect PGH and purple interermidiate hypoctyle had sparse SP (Table 4.6). The 14 collections with green intermediate hypocotyle and erect PGH were separated into eight collections with sparse and six intermediate SP. All the eight collections with green sparse hypcotyle and erect PGH had sparse SP, while four collections with green sparse hypocotyle and intermediate PGH were equally separated into sparse and intermediate SP. The two with green dense hypocotyle were sparse SP. Finally the three collections with white sparse which were separated into two erect and one intermediate PGH were all with sparse SP (Table 4.6)

Table 4. 6: Plant characteristics of pepper collections based on pooled data of the two sites

Collection	Farmer	HC	HP	PGH	SP	LSh	STh	LML	LMW	DG	DFl	DFr
ANE01	Izgharia Barua Temnewo	Purple	Sparse	Intermediate	Sparse	Lanceolate	14.57	8.69	11.63	12	53.7	61.5
DME04	Abraham Fkadu Kifleyohanes	Purple	Sparse	Intermediate	Sparse	Lanceolate	13.97	8.76	11.36	12	50.5	60.7
DME05	Asmelash Gebremariam	Purple	Sparse	Intermediate	Sparse	Lanceolate	14.03	9.07	11.55	12	55.5	67.7
ANE07	Bayray Zere Shengebay	Purple	Sparse	Intermediate	Sparse	Ovate	13.01	9.05	11.03	12	51.2	61.5
ANE08	Ysmer Chinie Yohanes	Purple	Sparse	Erect	Sparse	Lanceolate	10.71	7.35	9.03	10	55.7	66
NRSAF12	Hamid Saleh Asenay	Purple	Sparse	Erect	Sparse	Lanceolate	14.72	11.68	13.2	12	56.8	66
NRSAF18	Suleiman Omer Mussa	Purple	Sparse	Erect	Sparse	Lanceolate	15.17	10.14	12.65	13	53.8	68.2
ANE03	Demsas Michael Demsas	Purple	Sparse	Erect	Sparse	Ovate	12.05	8.4	10.23	9	51.7	62.2
DDB04	Samuel Tesfagergish 2	Purple	Sparse	Erect	Sparse	Ovate	12.28	9.2	10.74	11	49.8	60.5
DDB06	Kibrom Gebrihiwet	Purple	Sparse	Erect	Sparse	Ovate	12.24	9.75	11	13	50.2	59.8
NRSG01	Dawit G/Michael Tesema	Purple	Sparse	Erect	Sparse	Ovate	14.32	8.83	11.57	11	51.3	60.7
NRSG12	Habtom Gebar	Purple	Sparse	Erect	Sparse	Ovate	14.08	8.18	11.13	11	52	63.7
NRSG03	Gebregergish Tesfamariam	Purple	Sparse	Erect	Sparse	Mixed	12.81	8.18	10.49	11	54.7	66.2
NRSG06	Habtu G/ezghier Brhane	Purple	Sparse	Erect	Sparse	Mixed	13.75	8.26	11	12	54.8	66
NRSG02	Teklezghi Gebru Tsegai	Purple	Sparse	Erect	Intermediate	Mixed	14.57	9.23	11.9	12	52.2	60.5
DME12	Habte Girmai	Purple	Intermediate	Intermediate	Sparse	Lanceolate	14.21	10.32	12.26	11	51.2	60.8
DDB03	Kidane Tesfay	Purple	Intermediate	Intermediate	Sparse	Lanceolate	13.02	8.76	10.89	12	49.2	60.5
NRSF01	Suleiman Adem	Purple	Intermediate	Intermediate	Sparse	Ovate	14.09	8.77	11.43	11	53.7	66
NRSF04	Ali Abdrahman Saleh	Purple	Intermediate	Intermediate	Sparse	Ovate	15.31	9.42	12.37	12	51.5	61.7
DME09	Tesfasilassie Weldeab Gezai	Purple	Intermediate	Intermediate	Sparse	Mixed	13.59	7.51	10.55	9	46.3	55.7
NRSG15	Nigisti Tekeste Hineshim	Purple	Intermediate	Intermediate	Sparse	Mixed	13.27	9.6	11.43	13	56.7	68.3
NRSF02	Mohammed Khelifa Ahmed	Purple	Intermediate	Intermediate	Sparse	Mixed	15.32	9.33	12.32	12	54.5	66

HC=hypoctyle color HP=hypoctyle pubescence PGH=Plant growth habit SP=Stem pubescence STh=Stem thickness LSh=Leaf shape
LML=Mature leaf length LMW= Mature leaf length DG=Days to Germination DFl=Days to flowering DFr=Days to fruiting

Table 4.6 Cont….

Collection	Farmer	HC	HP	PGH	SP	LSh	STh	LML	LMW	DG	DFl	DFr
NRSAF04	Afa Mussa Asenay	Purple	Intermediate	Intermediate	Sparse	Mixed	15.1	10.77	12.93	12	54.2	68
NRSAF09	Mohammed Abdurehim Afa	Purple	Intermediate	Intermediate	Sparse	Mixed	15.44	10.16	12.8	12	54.7	65.5
DDB02	Samuel Tesfagergish 1	Purple	Intermediate	Intermediate	Intermediate	Lanceolate	9.93	8.7	9.31	12	53	63.3
DME06	Rusom Goitom	Purple	Intermediate	Erect	Sparse	Ovate	12.21	8.62	10.42	12	48.3	62.7
NRSG09	Tesfu Michael	Purple	Intermediate	Erect	Sparse	Mixed	14.05	8.94	11.5	9	52.2	61
NRSG14	Brhane Habte Tewelde	Purple	Intermediate	Erect	Sparse	Lanceolate	15.83	8.83	12.33	13	54	65.7
NRSG17	Tesfai Alemu	Purple	Intermediate	Erect	Sparse	Ovate	13.03	7.64	10.34	11	46.8	56
NRSG18	Gerezghier Hagos	Purple	Intermediate	Erect	Sparse	Lanceolate	11.83	7.8	9.81	11	52.5	64.3
NRSG19	Ali Abdella Ali	Purple	Intermediate	Erect	Sparse	Ovate	14.15	8.36	11.26	10	52.7	61.8
NRSG21	Russom Haile	Purple	Intermediate	Erect	Sparse	Lanceolate	15.39	10.06	12.72	12	53.8	63
NRSG24	Saleh Gulay	Purple	Intermediate	Erect	Sparse	Ovate	14.53	9.45	11.99	13	54.8	67.7
NRSAF01	Ahmed Omer Ali	Purple	Intermediate	Erect	Sparse	Ovate	15.43	10.85	13.14	12	54.3	65.3
NRSAF02	Mohammed Ali Ibrahim	Purple	Intermediate	Erect	Sparse	Mixed	15.98	11.06	13.52	12	55.8	69.3
NRSAF11	Hamid Ahmed Mohammed	Purple	Intermediate	Erect	Sparse	Mixed	15.17	11.07	13.12	12	56.8	71.2
HD0083	HAC	Purple	Intermediate	Erect	Sparse	Ovate	13.32	10.55	11.93	12	54.3	66.5
HD0123	HAC	Purple	Intermediate	Erect	Sparse	Mixed	15.85	11.7	13.78	13	50.3	61.2
NRSAF07	Ali Ismael Ali Khairay	Purple	Dense	Erect	Sparse	Lanceolate	15.12	11.57	13.34	11	55.5	68.2
NRSAF08	Mohammed Mussa Asenay	Purple	Dense	Intermediate	Sparse	Lanceolate	15.5	10.63	13.06	12	52.8	62.5
DME01	Yemane Goitom	Green	Sparse	Intermediate	Sparse	Ovate	13.25	8.27	10.76	9	55.2	64.8
DDK02	Bereket Beyene Gebrekristos	Green	Sparse	Erect	Sparse	Ovate	13.99	10.04	12.02	11	53.2	62.7
DDK03	Negash Misgna	Green	Sparse	Erect	Sparse	Ovate	13.85	8.63	11.24	11	49.2	58.8
DDK05	Isac Mengsteab Zogo	Green	Sparse	Erect	Sparse	Mixed	14.73	14.07	14.4	10	56.3	66.5

HC=hypoctyle color HP=hypoctyle pubescence PGH=Plant growth habit SP=Stem pubescence STh=Stem thickness LSh=Leaf shape
LML=Mature leaf length LMW= Mature leaf length DG=Days to Germination DFl=Days to flowering DFr=Days to fruiting

Table 4.6 Cont….

Collection	Farmer	HC	HP	PGH	SP	LSh	STh	LML	LMW	DG	DFl	DFr
NRSAF06	Mahmud Mesmer Asenay	Green	Sparse	Erect	Sparse	Mixed	17.91	11.24	14.57	10	56.5	68.8
NRSAF10	Osman Afa Mohammed	Green	Sparse	Erect	Sparse	Mixed	13.4	9.66	11.53	12	57.8	73.7
NRSAF19	Ali Faraj Mohammed	Green	Sparse	Erect	Sparse	Lanceolate	14.45	10.03	12.24	13	54	66.5
NRSAF20	Ibrahim Mohammed Ali	Green	Sparse	Erect	Sparse	Mixed	14.99	10.16	12.57	8	54.5	69.2
Red Long	NARI	Green	Sparse	Erect	Sparse	Lanceolate	13.91	10.35	12.13	12	56.8	66.8
NRSG22	Lemlem Tesfankiel	Green	Intermediate	Erect	Sparse	Ovate	13.46	8.57	11.01	13	53.5	65.2
NRSG27	Melake2	Green	Intermediate	Erect	Sparse	Lanceolate	14.93	10.11	12.52	12	54.8	65.2
Red short	NARI	Green	Intermediate	Erect	Sparse	Ovate	14.17	10.82	12.49	12	54.3	64.5
HD0128	HAC	Green	Intermediate	Erect	Sparse	Lanceolate	12.93	10.28	11.6	12	47.7	58.8
HD0031	HAC	Green	Intermediate	Intermediate	Sparse	Ovate	12.72	10.27	11.5	13	51.8	63
HD0108	HAC	Green	Intermediate	Intermediate	Sparse	Mixed	15.76	12.24	14	12	52.5	64.7
HD0134	HAC	Green	Dense	Intermediate	Sparse	Ovate	14.94	11.17	13.06	14	53.7	68.7
NRSAF14	Mahmoud Hamid Seid	Green	Dense	Erect	Sparse	Ovate	15.95	10.5	13.22	11	48.5	60.3
ANE04	Habtesilassie Okubazghi Ghabir	White	Sparse	Erect	Sparse	Lanceolate	14.25	9	11.63	11	49.8	60.3
NRSG04	Tsegai Teklehaimanot	White	Sparse	Erect	Sparse	Ovate	13.25	8.28	10.76	11	51.7	60.5
NRSG05	Hagos Ghirmai Mahanzel	White	Sparse	Intermediate	Sparse	Ovate	12.57	7.81	10.19	11	53.8	67.8

HC=hypoctyle color HP=hypoctyle pubescence PGH=Plant growth habit SP=Stem pubescence STh=Stem thickness LSh=Leaf shape
LML=Mature leaf length LMW= Mature leaf length DG=Days to Germination DFl=Days to flowering DFr=Days to fruiting

Qualitatitive flower and fruit traits showed considerable variability among the collections, however, many of the fruit traits showed mixture of two or more characters (Table 4.5). The results in Table 4.7 show the flower position (FP) for 33 (34.7%) was erect, 51 (53.7%) was intermediate and the rest 11.6% was mixed of the two traits. All except the collections with erect FP were elongate fruit shape except two were triangular and one was with mixed fruit. The distribution in the collections with the mixed flower position was the same. While the collections with intermediate FP included 11 triangular and 8 mixed fuit shaped collections (Table 4.7).

The 30 collections with erect flower position and elongated fruit were separated into six dark red coloured, 16 light red colered, three brown and five light brown colered fruits. The dark red fruits were equally divided into smooth and semi-wrinkled fruit surface (FS), while the the light red fruits except three smooth all had semi-wrinkled fruit surface. Majority (24) of the intermediate elongated fruited collections were light red coloured and these were basically semi-wrinkled except for three collections which were smooth fruited. The rest were six dark red and two brown fruit coloured collections (Table 4.7).

The 11 triangular shaped collections were basically dark red coloured fruits (5), three light red, two light brown and only one brown coloured. The dark red coloured collections were separated into one semi-wrinkled and two each smooth and mixed fruit surface (Table 4.7).

All the eight collections with intermediate FP and elengotated fruit were red light coloured of which six were semi-wrinkled and two smooth fruit surface. While the eight collections with mixed FP and elongated fruits were separated into four light red, two orange red and one each dark red and brown coloured fruits, of which six had semi-wrinkled and one each smooth and mixed fruit surface (Table 4.7). The collections are further separated based on calyx annular constrication (CAC), fruit cross sectional corrugation (FCSC) and fruit shape at both pedicel attachment (FShPA) and blossom end (FShBE) in smaller groups which is detailed in Teble 4.7.

Table 4. 7: Flower and fruit characteristics of pepper collections based on pooled data of the two sites

Collection	Farmer/Institute	FP	FSh	FCMS	FS	NBF	CAC	FShPA	FShBE	FCSC	FL	FW	FWTh	FWt	NFr/P	Y/Pl
DME04	Same as previous	Erect	Elong	D. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	9.7	1.9	1.8	9.8	60.4	346
DDB02		Erect	Elong	L. red	Smth	Absent	Present	Trunc	Blunt	Mixed	8.3	2.2	1.9	16.9	37.2	314.5
DDK05		Erect	Elong	L. red	Smth	Mixed	Absent	Mixed	Pointed	Mixed	10	2.3	2.3	17.1	44.6	572.5
NRSG02		Erect	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	11.6	1.4	1.6	8.6	75.8	471
NRSG17		Erect	Elong	L. red	S-Wr	Mixed	Present	Obtuse	Pointed	S.Corr	9.2	1.9	1.8	11.1	58.8	273.5
NRSG18		Erect	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	10	1.6	1.5	8.1	43.2	239.5
NRSG19		Erect	Elong	L. red	S-Wr	Mixed	Present	Obtuse	Pointed	S.Corr	8.8	1.5	1.8	7.3	58.5	303.5
NRSG22		Erect	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	9.9	1.4	1.6	7.1	64.3	317.5
NRSF01		Erect	Elong	L. red	S-Wr	Mixed	Mixed	Trunc	Blunt	Inter	9.5	1.9	1.9	14.5	59.4	406.5
DDB04		Erect	Elong	L. red	Mixed	Mixed	Mixed	Obtuse	Blunt	S.Corr	8.9	2.1	2.1	13.9	42.4	320.5
DDB06		Erect	Elong	Brown	S-Wr	Absent	Mixed	Trunc	Blunt	S.Corr	8.6	2.2	1.9	13.3	39.2	352
DDB03		Erect	Elong	Brown	S-Wr	Mixed	Absent	Obtuse	Blunt	Inter	9.4	2.5	1.9	14.5	67.5	314
ANE04		Erect	Elong	L.brown	Smth	Absent	Present	Mixed	Blunt	S.Corr	9.2	2.5	2.1	14.7	48.9	409
NRSG05		Erect	Elong	L.brown	S-Wr	Mixed	Absent	Mixed	Pointed	S.Corr	10.8	1.4	1.5	7.9	61.8	397
NRSG12		Erect	Elong	L.brown	S-Wr	Mixed	Absent	Obtuse	Pointed	Mixed	10.5	1.8	1.8	9.7	64	369
ANE07		Erect	Triang	D. red	Smth	Mixed	Absent	Trunc	Blunt	Mixed	8	2.2	2	11.9	45.2	331.5
DME09		Erect	Triang	L. red	Mixed	Mixed	Absent	Mixed	Pointed	S.Corr	10	1.7	1.6	7.3	89.2	400
NRSG01		Erect	Mixed	Brown	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	10.5	1.4	1.6	8.3	87.3	444
NRSG21		Inter	Elong	D. red	Smth	Mixed	Absent	Trunc	Blunt	S.Corr	10.1	2.9	1.8	29.1	56.6	1111
NRSF02		Inter	Elong	D. red	S-Wr	Absent	Present	Trunc	Blunt	Inter	9.7	2.4	1.9	14.1	47.5	499

FP=Flower position CAC=Calyx annular constriction FCMS=Fruit color mature stage FSh=Fruit shape FShPA=Fruit shape pedicel attachment NBF=Neck at base of fruit FShBE=Fruit shape at blossom end FS= Fruit surface FCSC=Fruit cross sectional corrugation FL=Fruit length(cm) FW=Fruit width(cm) FWTh= Fruit wall thickness (mm) FWt= Fruit weight (g) TSS=Total soluble solids NFr/P=No of fruits per plant Y/Pl= Yield per plant Inter=Intermediate L.red=Light red D.red=Dark red O.red=Orange red Elong=Elongate Trunc=Truncate S.Wr=Sliahtly wrinkled Smth=Smooth S.Corr=Sliahtly corrugated

Table 4.7 Cont….

Collection	Farmer/Institute	FP	FSh	FCMS	FS	NBF	CAC	FShPA	FShBE	FCSC	FL	FW	FWTh	FWt	NFr/P	Y/Pl
NRSAF02	Same as previous	Inter	Elong	D. red	S-Wr	Absent	Present	Trunc	Blunt	S.Corr	10.7	2.8	2.4	23.9	40.4	607
NRSG04		Inter	Elong	D. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	10.9	2.2	1.7	8.6	65.6	338.5
NRSG27		Inter	Elong	D. red	S-Wr	Mixed	Mixed	Trunc	Blunt	Inter	9.9	2.6	2	17.6	41.6	500.5
DME06		Inter	Elong	L. red	Smth	Mixed	Present	Trunc	Pointed	S.Corr	8.2	2.1	2.2	14.3	31.8	310.5
NRSG24		Inter	Elong	L. red	Smth	Mixed	Absent	Mixed	Blunt	S.Corr	10.8	1.7	1.8	10.4	61.9	465.5
NRSAF08		Inter	Elong	L. red	Smth	Mixed	Present	Trunc	Blunt	S.Corr	10.3	2.9	2.4	25.3	31.4	605.5
NRSAF10		Inter	Elong	L. red	Smth	Mixed	Present	Trunc	Blunt	Mixed	10.6	3.1	2.4	27.1	25.3	441.5
Red Long		Inter	Elong	L. red	Smth	Mixed	Absent	Trunc	Blunt	Inter	9.5	3.2	2.5	25.2	31.8	482
DME05		Inter	Elong	L. red	S-Wr	Absent	Absent	Obtuse	Pointed	S.Corr	9.6	1.4	1.6	6.8	68.7	336
DDK03		Inter	Elong	L. red	S-Wr	Mixed	Present	Mixed	Pointed	S.Corr	10.4	1.9	1.9	12.2	55	415
DME01		Inter	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	Mixed	10.3	1.6	1.6	7.4	70.7	353
NRSAF06		Inter	Elong	L. red	S-Wr	Mixed	Present	Trunc	Blunt	Inter	10.9	3.1	2.3	26.3	39.3	851.5
NRSAF19		Inter	Elong	L. red	S-Wr	Mixed	Mixed	Trunc	Blunt	S.Corr	10.4	2.4	2.2	21.4	27.8	529
NRSG06		Inter	Elong	Brown	S-Wr	Mixed	Mixed	Obtuse	Pointed	S.Corr	9.1	1.8	1.7	10.1	48.8	291.5
NRSG14		Inter	Elong	Brown	S-Wr	Mixed	Absent	Trunc	Blunt	S.Corr	10.8	2	2	15.6	69.1	412.5
HD0123		Inter	Triang	D. red	S-Wr	Absent	Absent	Trunc	Blunt	Mixed	9.8	3.1	2.2	24.8	46.4	569.5
HD0134		Inter	Triang	D. red	Mixed	Absent	Absent	Trunc	Pointed	Inter	10.9	3.6	2.6	35.5	46.4	1139
Red short		Inter	Triang	D. red	Mixed	Mixed	Present	Trunc	Blunt	S.Corr	10.4	3.1	2.6	28.1	32.7	585
HD0031		Inter	Triang	L. red	Smth	Absent	Absent	Trunc	Pointed	Inter	11.7	3.1	2.6	36.7	37.7	861.5
NRSAF12		Inter	Triang	L. red	S-Wr	Absent	Mixed	Trunc	Blunt	Inter	10.5	3.1	2.3	26.4	36.2	602

FP=Flower position CAC=Calyx annular constriction FCMS=Fruit color mature stage FSh=Fruit shape FShPA=Fruit shape pedicel attachment NBF=Neck at base of fruit FShBE=Fruit shape at blossom end FS= Fruit surface FCSC=Fruit cross sectional corrugation FL=Fruit length(cm) FW=Fruit width(cm) FWTh= Fruit wall thickness (mm) FWt= Fruit weight (g) TSS=Total soluble solids NFr/P=No of fruits per plant Y/Pl= Yield per plant Inter=Intermediate L.red=Light red D.red=Dark red O.red=Orange red Elong=Elongate Trunc=Truncate S.Wr=Slightly wrinkled Smth=Smooth S.Corr=Slightly corrugated

Table 4.7 Cont….

Collection	Farmer/Institute	FP	FSh	FCMS	FS	NBF	CAC	FShPA	FShBE	FCSC	FL	FW	FWTh	FWt	NFr/P	Y/Pl
NRSAF14	Same as previous	Inter	Triang	L. red	Mixed	Absent	Mixed	Trunc	Blunt	S.Corr	9.8	2.2	1.8	16.3	66.5	755
HD0128	Same as previous	Inter	Mixed	L. red	Smth	Absent	Present	Trunc	Blunt	Mixed	9.5	2.6	2.1	21.8	30.5	433
NRSAF09		Inter	Mixed	L. red	Smth	Mixed	Present	Mixed	Blunt	Inter	11.5	3.1	2.3	30.2	35.1	559
NRSAF04		Inter	Mixed	L. red	S-Wr	Absent	Present	Trunc	Blunt	S.Corr	10.8	3	2.2	20.3	34.4	527
NRSAF07		Inter	Mixed	L. red	S-Wr	Mixed	Present	Trunc	Blunt	Inter	10.7	3	2.3	26.4	35.1	540.5
NRSAF11		Inter	Mixed	L. red	S-Wr	Mixed	Present	Trunc	Blunt	Inter	9.9	3.2	2.4	27.6	31.7	526.5
NRSAF18		Inter	Mixed	L. red	S-Wr	Mixed	Present	Mixed	Blunt	S.Corr	9.7	2.4	1.9	16	43.7	485
NRSAF20		Inter	Mixed	L. red	S-Wr	Mixed	Present	Mixed	Blunt	Mixed	9.6	2.2	1.8	13.9	53.2	496
HD0108		Inter	Mixed	Brown	Smth	Absent	Present	Mixed	Blunt	S.Corr	9.9	2.9	2.4	21.2	43.1	590
NRSAF01		Mixed	Elong	D. red	S-Wr	Absent	Present	Trunc	Blunt	Mixed	9.6	2.8	2	18.8	39.2	514
ANE03		Mixed	Elong	L. red	S-Wr	Mixed	Mixed	Trunc	Blunt	Mixed	8.8	2.2	1.9	13	40.6	383
DME12		Mixed	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	10.2	1.5	1.7	12.7	89.2	547.5
NRSG09		Mixed	Elong	L. red	S-Wr	Mixed	Absent	Obtuse	Pointed	S.Corr	11.2	1.6	1.7	12.9	79.3	571.5
NRSG15		Mixed	Elong	L. red	Mixed	Mixed	Absent	Obtuse	Pointed	Mixed	9	1.9	1.9	9.8	49.4	287.5
DDK02		Mixed	Elong	O. red	Smth	Mixed	Mixed	Mixed	Pointed	Mixed	9.6	2.5	1.9	16.9	40.5	441.5
ANE01		Mixed	Elong	O. red	S-Wr	Absent	Present	Trunc	Blunt	Mixed	9.6	2.6	2.1	12.5	44.2	359.5
NRSG03		Mixed	Elong	Brown	S-Wr	Mixed	Absent	Obtuse	Pointed	Inter	10.5	2.1	1.9	11.1	61.1	343.5
HD0083		Mixed	Triang	D. red	Smth	Absent	Absent	Trunc	Blunt	S.Corr	7.9	2.6	2	17.1	31.6	428
NRSF04		Mixed	Triang	D. red	Mixed	Mixed	Present	Trunc	Blunt	S.Corr	9.2	2.7	1.9	15.4	52.3	482
ANE08		Mixed	Mixed	D. red	Smth	Mixed	Mixed	Obtuse	Pointed	S.Corr	9.6	1.4	1.7	7	52.5	253

FP=Flower position CAC=Calyx annular constriction FCMS=Fruit color mature stage FSh=Fruit shape FShPA=Fruit shape pedicel attachment NBF=Neck at base of fruit FShBE=Fruit shape at blossom end FS= Fruit surface FCSC=Fruit cross sectional corrugation FL=Fruit length(cm) FW=Fruit width(cm) FWTh= Fruit wall thickness (mm) FWt= Fruit weight (g) TSS=Total soluble solids NFr/P=No of fruits per plant Y/Pl= Yield per plant Inter=Intermediate L.red=Light red D.red=Dark red O.red=Orange red Elong=Elongate Trunc=Truncate S.Wr=Slightly wrinkled Smth=Smooth S.Corr=Slightly corrugated

Plant height (PHt) categories were short (34-43.99 cm), medium (44-53.99 cm) and tall (54-68 cm). Majority of collection (78.3 %) were in the medium category and only 5% were tall plants. Collection were classified into those with thick stem (15-19 mm), medium (12-14.99 mm) and thin (7-11.99mm). Only 5% of the collections were with stem thichness in the range 7-11.99 mm, while majority were in the medium stem thickness category. Mature leaf length ranged from short (5-7.99 cm) to long (=> 11 cm), however, majority (76.7 %) of the collections were in the medium category which ranged 8-10.99 cm. Only 3.3% of the collections had wide life (=> 5.5 cm), while majority (75%) were in the medium category (4-5.49 cm) and 21.7 % were 2.5-3.99 cm (Table 4.8).

Phenological traits were categorized as early, medium and late. Majority of the collections (72.6%) were medium in days to germination (11-13 days), while both early (8-10 day) and late (14-16 days) germinating collections were each 13.7%. The early to flower collections (20%) were in the range 42-49.99 days and the late collections (15%) flowered 55-60 days, while the rest were medium. Majority of the collections (88.3%) of the collections were medium in days to flower (60-69.99) and 8.3 % were early fruiting (50-59.99 days), while late flowering (70-80 days) were only 3.3% (Table 4.8).

Variable quantitative fruit characteristics were observed (Table 4.8). Short fruit length (7-8.99 cm) represented 15% of the collections while long fruits (11-13 cm) were only 8.3 %, while the majority (76.7 %) were medium in fruit length. Variation in fruit width was more balanced where 18 % of the collections were with wide fruits (3-4 cm) and the slim fruits (1-1.99 cm) were 35% of the collection. The rest 47% were medium in fruit width (2-2.99). In revers to that 58.3 % of the collections had thin fruit wall thickness (1-1.99 mm), 40% medium (2-2.99 mm) and only 1.7% had thich wall thichness (3-4 mm). Fruit weight showed high variation. Collections with small fruits (4.5-14.99 g) were 53 %, medium (15-24.99g) 27 % and large fruits (=> 25 g) 20 % (Table 4.8).Both number of fruits and yield per plant showed balanced distribution among the categories. Collections with low number of fruits (19-38.99) and low yield (140-339.99 g) were 25% and 23.3 % respectively, while medium

number of fruits (39-58.99) and yield (340-539.99) were 45% and 48.3 % respectively, and collections with large number of fruits (=>59) and high yield (=> 540 g) were 30% and 28.3 % of the collections respectively (Table 4.8). Detailed descriptions of the 95 collections are in Tables 4.6 and 4.7.

Table 4.8: Percentage distribution of collections grouped in three categories for 13 quantitative traits**.**

	%		%		%		%
Pht (cm)	Coll	STh (mm)	Coll	MLL(cm)	Coll	MLW (cm)	Coll
34-43.99	16.7	7-11.99	5	5-7.99	8.3	2.5-3.99	21.7
44-53.99	78.3	12-14.99	68.3	8-10.99	76.7	4-5.49	75
54-68.00	5	15-19.00	26.7	=> 11	15	=>5.5	3.3

	%		%		%		%
DG	Coll	DFl	Coll	DFr	Coll	FL (cm)	Coll
8-10	13.7	42-49.99	20	50-59.99	8.3	7-8.99	15
11-13	72.6	50-54.99	65	60-69.99	88.3	9-10.99	76.7
14-16	13.7	55-60	15	70-80.00	3.3	11-13.00	8.3

	%		%		%		
FW (cm)	Coll	FWTh(mm)	Coll	FWt (g)	Coll	NFr/P	
1-1.99	35	1-1.99	58.3	4.5-14.99	53	19-38.99	25
2-2.99	47	2-2.99	40	15-24.99	27	39-58.99	45
3-4.00	18	3-4.00	1.7	=> 25	20	=>59	30

	%
Y/ P (g)	Coll
140-339.99	23.3
340-539.99	48.3
> =540	28.3

PHt= Plant height STh= Stem thickness LML=Mature leaf length LMW= Mature leaf length DG=Days to Germination DFl=Days to flowering DFr=Days to fruiting FL=Fruit length(cm) FW=Fruit width(cm) FWTh= Fruit wall thickness (mm) FWt= Fruit weight (g) NFr/P=No of fruits per plant Y/Pl= Yield per plant Coll= Collection

4.3.2. Variation of Quantitative traits

The analysis of variance of the quantitative traits (Table 4.9) showed that at Hamelmalo the difference among the 95 collections was significant for all traits except for DFl, DFr, PHt and TSS. In Asmara the difference was significant for all traits except for FL and Y/Pl. The analysis of the data when combined from the two sites showed that the major source of variation was due to genotype followed by location but genotype x location interaction was not significant. Genotype was significant for all traits except for TSS, while location was significant for all traits except for FL, FW and FWt. The genotype x location interaction was only significant for FWt, Y/Pl, TSS and DFl (Table 4.9). High coefficient of variation was observed on NFr/P, FWt and Y/Pl. At Hamelmalo it was 42.3%, 39.4% and 37.3% respectively and in Asmara it was 31.3% , 37.4% and 35% respectively. While when data of the two sites combined it was 43.5%, 39.9% 41.7% respectively (Table 4.9).

Table 4.9: Analysis of variance of quantitative tarits on genotypes, locations and genotype x location interaction for 60 collections.

Trait	Hamelmalo		Asmara		Combined of the two sites				
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Significance		
							G	L	G x L
CLL[H]	23.66***	0.3	-	-	-	-	-	-	-
CLW[H]	6.67***	0.1	-	-	-	-	-	-	-
DFl	53.58 [NS]	8.5	52.21**	10.2	52.99	9.5	**	**	*
DFr	62.96 [NS]	9.6	64.93***	7.9	64.2	8.9	***	**	NS
DG[H]	11.92***	1.0	-	-	-	-	-	-	-
FL	10.04***	14.0	9.90 [NS]	13.4	9.92	14.1	***	NS	NS
FW	2.24***	16.0	2.35***	27.7	2.31	23.2	***	NS	NS
FWTh	2.23***	13.7	1.75***	20.6	1.98	18.4	***	***	NS
FWt	16.06***	39.4	17.13***	37.4	16.57	39.9	***	NS	*
LML	9.85***	17.0	9.23***	18.0	9.61	17.3	***	***	NS
LMW	4.68***	18.6	4.18***	12.7	4.46	15.7	***	***	NS
NFr_P	60.22***	42.3	40.22***	31.3	50.3	43.5	***	***	NS
PHt	45.82 [NS]	15.8	48.29**	14.2	47.3	15.1	***	**	NS
STh	14.37*	19.4	13.6*	15.5	14.08	17.1	***	***	NS
TSS	5.34 [NS]	16.0	7.59**	15.3	6.42	16.8	NS	***	**
Y_Pl	633.7***	37.3	298.4 [NS]	35	475	41.7	***	***	***

CLL=Cotyledon leaf length CLW= Cotyledon leaf width DFl=Days to flowering DFr=Days to fruiting DG=Days to germination FL=Fruit length FW=Fruit width FWTh=Fruit wall thickness FWt=Fruit weight LML=Leaf mature length LMW=Leaf mature width NFr/P= Number of fruits per plant PHt=Plant height STh=Stem thickness TSS=Total soluble solids Y/Pl=Yield per plant.

[H] *= Trait recorded only at Hamelmalo.*

4.3.3. Variation among the collections

4.3.3.1. Quantitative traits

Based on principal components analysis (PCA) of the quantitative traits, the first three components explained 71% of the variation at Hamelmalo, 65% in Asmara and 74% combined of the two sites (Table 4.10). PC1was the most important component, which explained 44%, 35% and 46% of the variation at Hamelmalo, Asmara and combined of the two sites respectively. Both PC 2 and PC3 explained 17% and 10% at Hamelmalo, 18% an 12% in Asmara and 16% and 12% combined of the two sites respectively (Table 4.10).

The correlation between the components and morphological traits showed slight differences in the two sites and when the combined data of the two sites was considered (Table 4.11). Data of Hamelmalo and Asmara as well as combined of the two sites showed that PC1 mainly accounted for by fruit width (FW), fruit wall thickness (FWTh), fruit weight (FrWt) and number of fruits per plant (NFr/P). PC2 at Hamelmalo was accounted for mainly by days to flowering and days to fruiting. This corresponded to PC3 in Asmara and the combined data of the two sites. Similarly PC3 at Hamelmalo was accounted for mainly by plant height (PHt) and yield per plant (Y/P) as well as fruit length (FL) and number of fruits per plant (Table 4.11). It is noteworthy, that both fruit length and number of fruits per plant had no clear structure at Hamelmalo. NFr/P scored high in both PC1 and PC3, while fruit length scored high in both PC3 and PC4. Contribution of collections to five PCs based on the combined data of the two sites is in Table 4.12.

Table 4. 10: Latent roots and percentage variation of quantitative traits at Hamelmalo, Asmara and combined of the two sites.

PC	Hamelmalo			Asmara			Combined of the two sites		
	Root	%	Cum %	Root	%	Cum %	Root	%	Cum %
PC1	5.703	44	44	4.573	35	35	6.018	46	46
PC2	2.182	17	61	2.397	18	53	2.108	16	62
PC3	1.359	10	71	1.605	12	65	1.618	12	74
PC4	1.038	8	79	1.395	11	76	0.975	8	82
PC5	0.989	8	87	0.879	7	83	0.76	6	88

PC= Principal component *Cum= Cumulative*

Table 4.11: Eigen vectors of quantitative variable at Hamelmalo, Asmara and combined data of the two sites

Traits	Principal Components of quantitative traits														
	Hamelmalo					Asmara					Combined of the two sites				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
DFl	0.00	-0.69	-0.05	-0.03	0.01	0.06	-0.02	-0.69	0.02	0.15	0.03	0.00	-0.69	-0.05	0.03
DFr	0.05	-0.70	-0.01	-0.04	0.00	-0.04	0.00	-0.65	0.01	-0.12	0.01	0.01	-0.67	0.06	0.03
FL	-0.27	-0.15	0.47	0.59	-0.11	-0.35	-0.24	-0.15	-0.65	0.10	-0.07	-0.67	-0.17	-0.10	-0.24
FW	-0.43	0.06	-0.03	-0.19	0.05	-0.44	0.04	-0.03	0.04	-0.09	-0.46	-0.03	0.04	0.05	0.07
FWTh	-0.45	-0.02	0.03	-0.01	-0.05	-0.48	0.06	0.04	0.10	0.13	-0.49	-0.01	0.02	-0.08	0.02
FWt	-0.45	0.03	0.11	-0.01	0.01	-0.53	-0.01	0.03	-0.04	-0.05	-0.48	-0.20	0.02	0.06	-0.05
LML	-0.14	-0.07	0.10	-0.43	-0.05	-0.10	-0.14	-0.05	0.53	0.14	-0.10	0.06	-0.05	0.02	0.50
LMW	-0.14	-0.02	0.03	-0.48	-0.01	-0.16	-0.12	-0.04	0.47	-0.05	-0.12	0.14	0.00	0.06	0.51
NFr/P	0.49	0.02	0.42	-0.01	-0.03	0.28	-0.38	0.11	-0.22	0.06	0.44	-0.33	0.11	0.08	-0.02
PHt	0.06	0.06	0.53	-0.11	0.02	0.11	-0.55	0.00	0.09	-0.09	0.14	-0.39	0.12	-0.07	0.35
STh	0.12	-0.05	0.35	-0.43	-0.01	0.01	-0.51	-0.15	0.11	-0.07	0.16	-0.20	-0.05	-0.08	0.54
TSS	0.01	0.02	-0.05	-0.05	-0.99	-0.08	-0.09	0.03	-0.02	-0.91	0.03	-0.02	-0.02	0.96	0.00
Y/Pl	-0.19	0.02	0.42	-0.03	0.03	-0.22	-0.43	0.21	0.03	0.25	-0.21	-0.43	0.06	0.14	0.07

DFl=Days to flowering DFr= ruit length FW=Fruit width FWTh=Fruit wall thickness FWt=Fruit weight LML=Leaf mature length LMW=Leaf mature width NFr/P= Number of fruits per plant PHt=Plant height STh=Stem thicknDays to fruiting FL=Fess TSS=Total soluble solids Y/Pl=Yield per plant

Table 4.12: Contribution of the collection to the five PCs using the combined data of the two sites

Genotype	PC1	PC2	PC3	PC4	PC5
ANE01	1.203	-0.415	**-2.475**	**-1.956**	**-0.97**
ANE03	2.055	**2.472**	1.015	1.074	-0.399
ANE04	0.996	0.696	0.581	**-1.155**	-0.601
ANE07	2.154	1.146	-1.636	-2.2	0.468
ANE08	**4.064**	1.223	-1.773	**1.502**	-0.717
DDK02	0.361	0.675	-1.527	-0.904	0.737
DDK03	1.563	-0.563	1.885	-0.523	**-1.029**
DDK05	**-3.046**	0.241	**2.013**	**2.917**	-0.511
DME01	**2.831**	-0.165	0.712	**2.843**	0.836
DME04	**2.244**	0.17	0.729	-0.453	0.17
DME05	**2.428**	-0.276	-1.056	**1.715**	-0.36
DME06	1.149	**2.87**6	0.907	**-1.409**	**1.308**
DME09	**4.218**	-0.037	**4.371**	0.861	-0.773
DME12	1.714	-1.021	1.846	0.811	0.515
DDB02	1.941	**3.191**	-0.98	-0.711	0.151
DDB03	1.134	1.389	**2.815**	-0.08	0.005
DDB04	1.07	2.75	**1.979**	-0.077	0.756
DDB06	0.406	1.79	0.754	**-1.913**	**1.556**
NRSG01	**2.524**	**-3.337**	0.739	-0.454	0.794
NRSG02	1.967	**-3.019**	-0.137	-0.366	-0.253
NRSG03	1.965	0.043	-1.319	0.913	-0.518
NRSG04	**2.345**	-1.308	0.175	-0.393	-0.62
NRSG05	**3.008**	-1.625	-1.893	0.727	-0.423
NRSG06	1.987	0.438	-1.742	0.113	**0.972**
NRSG09	1.812	**-2.518**	0.909	0.603	-0.059
NRSG12	**2.181**	-1.761	-0.81	-0.324	-0.944
NRSG14	0.218	**-2.231**	-0.759	-0.105	0.021
NRSG15	1.327	0.751	**-2.721**	0.283	0.306
NRSG17	**3.562**	-0.042	1.483	**-1.935**	**-1.667**

Table 4.12 Cont…

NRSG18	**3.453**	0.39	-1.101	0.303	-0.507
NRSG19	**2.618**	0.278	0.447	0.519	0.69
NRSG21	-2.323	**-2.334**	0.821	-0.791	0.796
NRSG22	**2.456**	**-1.837**	-1.799	-0.871	0.882
NRSG24	0.848	-1.504	**-1.867**	0.204	0.738
NRSG27	-1.183	-0.238	-0.436	0.005	0.862
NRSF01	0.966	-0.168	0.17	0.837	0.85
NRSF02	-0.539	-0.26	-0.28	0.363	**0.909**
NRSF04	0.167	-0.194	0.48	-0.955	0.704
NRSAF01	-1.676	-0.107	-0.939	-0.46	-0.602
NRSAF02	**-3.557**	-0.509	-0.86	0.616	-0.063
NRSAF04	-2.683	-0.352	-1.176	-0.399	**1.508**
NRSAF06	**-4.271**	-1.729	0.058	1.614	-0.844
NRSAF07	**-3.506**	-0.175	-0.214	0.858	0.278
NRSAF08	-2.67	0.241	0.54	-0.643	**-1.991**
NRSAF09	**-3.365**	-0.482	-0.152	0.056	-1.19
NRSAF10	-2.592	1.657	**-2.262**	1.575	-0.877
NRSAF11	**-3.976**	0.655	-0.524	0.995	**1.655**
NRSAF12	**-3.445**	0.61	-0.552	0.532	0.14
NRSAF14	-0.501	**-1.811**	**2.464**	-0.811	0.233
NRSAF18	-0.864	-0.535	-1.738	-0.53	-0.076
NRSAF19	-2.188	1.157	0.407	0.484	-0.117
NRSAF20	-0.051	0.136	1.086	**3.267**	0.309
Red Long	-2.429	**1.965**	-1.047	0.519	**-1.669**
Red short	-2.874	1.167	-0.241	-0.168	**-1.905**
HD0031	**-3.259**	0.319	0.423	-1.266	**-2.595**
HD0083	-0.788	**3.039**	-0.572	0.233	**1.223**
HD0108	**-3.563**	-0.142	1.294	-0.144	0.684
HD0123	**-3.134**	0.35	1.85	-1.529	**1.938**
HD0128	-0.187	1.224	1.173	**-2.254**	**-1.381**
HD0134	**-6.267**	**-2.343**	0.461	-1.56	0.67

The Principal coordinate bi-plot results of the first two coordinates (Fig 4.3, 4.4 and 4.5) using data of the 16 quantitative traits showed that in both sites and with combined data from the two sites the collections were distributed into all the four quadrates. The spread pattern in the two research sites and combined data of the two sites was similar. All the breeding lines of HAC and NARI (except Group 1 A from NARI) and farmer varieties collected from Afabet located together the positive side of PC1 axis, while all farmer collections (with few exceptions in each site) collected from the remaining sub-regions were distributed in the negative area of the same PC. On the other hand the 16 quantitative variables spread into three quadrats. In these three quadrats, NFr/P solitarily located in quadrat 3, while Y/Pl, STh, PHt and FL; DFl and DFr and LML, LMW, FW, FWt and FWTh tend to be grouped together. The last group composed of CLL, CLW, TSS and DG tend to spread close to the centre. However, position of some individual varieties within the plot area showed slight differences depending on the data collected from the two sites and the pooled data.

Although no grouping differences of the collections between the bi-plots resulted from the data sets of the two experimental sites, the location of the collections and variables on the positive and negative sides of the PC 2 axis on the bi-plot of Asmara was reverse to the bi-plot of Hamelmalo (Fig. 4.3, 4.4 and 4.5).

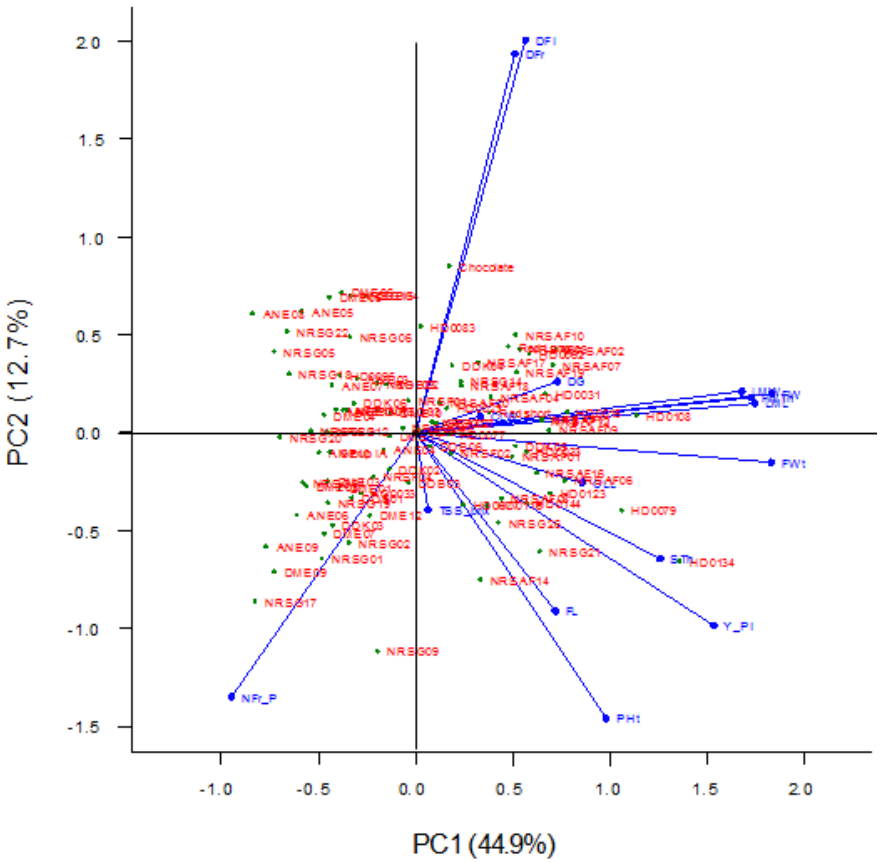

Figure 4. 3: Principal coordinate biplot of the first two PCs using quantitative data from Hamelmalo of the 95 collections

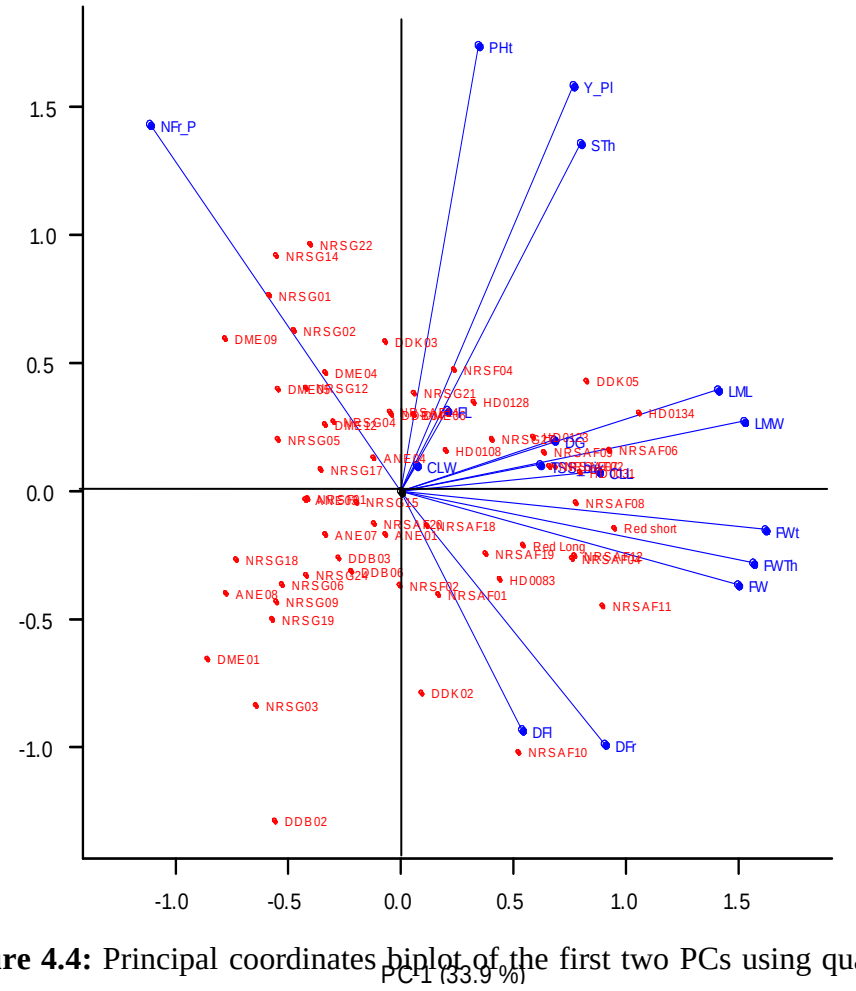

Figure 4.4: Principal coordinates biplot of the first two PCs using quantitative data from Asmara of the 60 collections

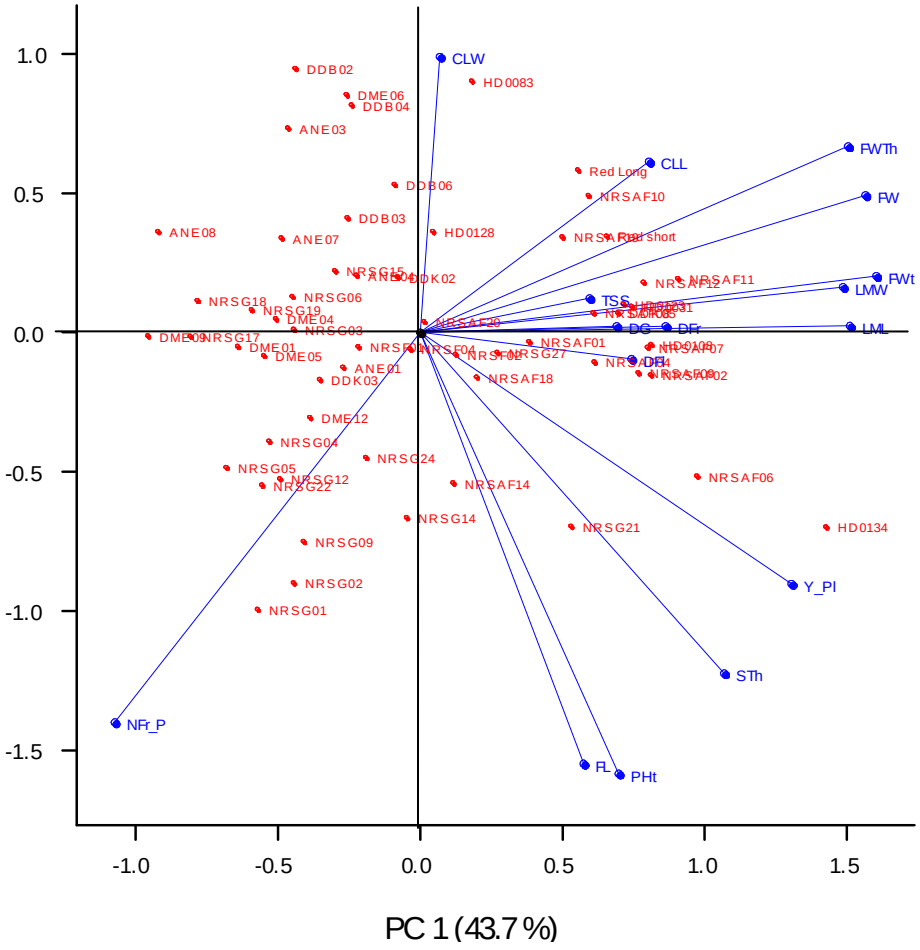

Figure 4.5: Principal coordinate biplot of the first two PCs using pooled quantitative data of the two sites for 60 collections

4.3.3.2. Qualitative traits

The principal component analysis of qualitative traits showed that 13 out of the 23 traits had low or no contributions in the variation among the varieties. Thus the analysis was conducted on the remaining 10 traits that scored 0.4 and above. In this analysis the first four components for the genotypes tested at Hamelmalo and five components for those tested in Asmara and the combined data were able to explain 69%, 70% and 75% of the variation respectively. At Hamelmalo the first three components were able to explain 59% of the variation of which 29% was explained by the first component. In Asmara the first three components explained 49% of which 22% was explained by the first component. While the combined data showed

55% of the variation explained by the first three components of which 28% was by the first component alone (Table 4.13). The correlation between the traits and components in Table 4.14 showed that at Hamelmalo and the combined data for PC1 was mainly related to fruit shape components (CAC, FShPA and FShBE) with lower relation to flower position (FP), while in Asmara it was accounted for mainly by CAC and FP. PC2 in the three cases was related to the green colour in both fruit and leaf (FCIS and LC). At Hamelmalo PC3 was related to fruit colour at mature stage (FCMS) and leaf shape (LSh), in Asmara it was related only to LSh, while with the combined data each of LSh and FCMS were related solely to PC4 and PC5 respectively. PC4 the last component at HAC was related to fruit cross sectional corrugation (FCSC) and plant growth habit (PGH), the two traits were related to PC3 in the combined data of the two sites. While in Asmara PC4 was related to FCMS, FShPA and PGH and PC5 was related to FCSC and FShBE.

The results of the principal coordinate bi-plot of the first two coordinates showed the general pattern of the vcollections distribution in the four quadrats was similar to the bi-plots of the quantitative traits in showing variability among the varieties in the two sites. However, slight difference in the grouping and distribution of the variables in the four quadrats was observed. Bi-plot of Hamelmalo showed that the 10 variables were distributed in three quadrats grouping FShPA and FShBE, CAC, FCSC and PGH, LC, FCIS and FCMS together, while LSh and FP were close to the last group but distant from each other (Fig. 4.6).

The bi-plot of Asmara showed that the variables distributed in the four quadrats and in wider angles from each other compared to Hamelmalo. It also showed that each PGH and LSh together and FP and FCMS occupied their own quadrat, while the rest remained in their respective grouping (Fig. 4.7). The bi-plot of the pooled data showed similar trend of the collections but slightly different distribution of the variables (Fig. 4.8).

Table 4.13: Latent roots and percentage variation of quantitative variables at Hamelmalo, Asmara sites and using combined data of the two sites.

PC	Hamelmalo			Asmara			Combined of the two sites		
	Root	%	Cum %	Root	%	Cum %	Root	%	Cum %
PC1	2.867	29	29	2.235	22	22	2.807	28	28
PC2	1.842	18	47	1.449	14	36	1.525	15	43
PC3	1.25	12	59	1.278	13	49	1.15	12	55
PC4	0.973	10	69	1.115	11	60	1.037	10	65
PC5				1.017	10	70	0.952	10	75

PC= Principal component *Cum= Cumulative*

Table 4.14: Eigen vectors of qualitative variable at Hamelmalo, Asmara and combined data of the two **sites**

Trait	Principal Components of the qualitative traits													
	Hamelmalo				Asmara					Combined				
	1	2	3	4	1	2	3	4	5	1	2	3	4	5
CAC	0.45	0.09	0.01	-0.14	-0.54	0.11	-0.06	-0.11	0.04	0.44	-0.15	0.03	-0.17	-0.26
FCIS	0.11	-0.60	-0.05	0.09	0.04	-0.54	-0.31	0.09	0.28	0.16	0.62	-0.08	-0.17	-0.06
FCMS	-0.02	-0.33	-0.59	0.20	0.47	0.13	0.31	-0.58	0.05	0.10	-0.08	-0.03	-0.01	0.91
FCSC	0.25	0.04	-0.01	-0.73	-0.19	0.02	0.13	0.01	0.56	0.20	-0.19	0.42	0.29	-0.10
FP	-0.36	-0.14	-0.27	-0.14	0.61	0.05	-0.19	0.06	-0.01	-0.36	0.04	0.22	-0.17	0.23
FShBE	0.50	-0.13	-0.06	0.03	0.14	-0.02	-0.06	-0.04	0.74	0.53	0.15	0.02	0.09	0.19
FShPA	0.51	-0.11	-0.03	0.04	-0.17	0.10	-0.16	-0.57	0.12	0.56	0.03	0.06	-0.05	0.07
LC	-0.02	-0.60	0.06	-0.19	0.05	-0.76	0.20	-0.07	-0.12	-0.09	0.72	0.08	0.16	0.02
LSh	-0.08	-0.32	0.76	0.13	-0.08	-0.05	0.83	0.12	0.09	-0.03	0.02	-0.02	0.88	0.01
PGH	0.28	0.13	-0.01	0.57	0.17	0.28	0.06	0.54	0.15	0.04	-0.08	-0.87	0.10	-0.02

CAC=Calyx annular constriction FCIS= Fruit color at intermediate stage FCMS=Fruit color at mature stage FCSC=Fruit cross sectional corrigation FP=Flower position FShBE=Fruit shape at blossom end FShPA=Fruit shape at pedicel attachment LC=Leaf color LSh=Leaf shape PGH=Plant growth habit

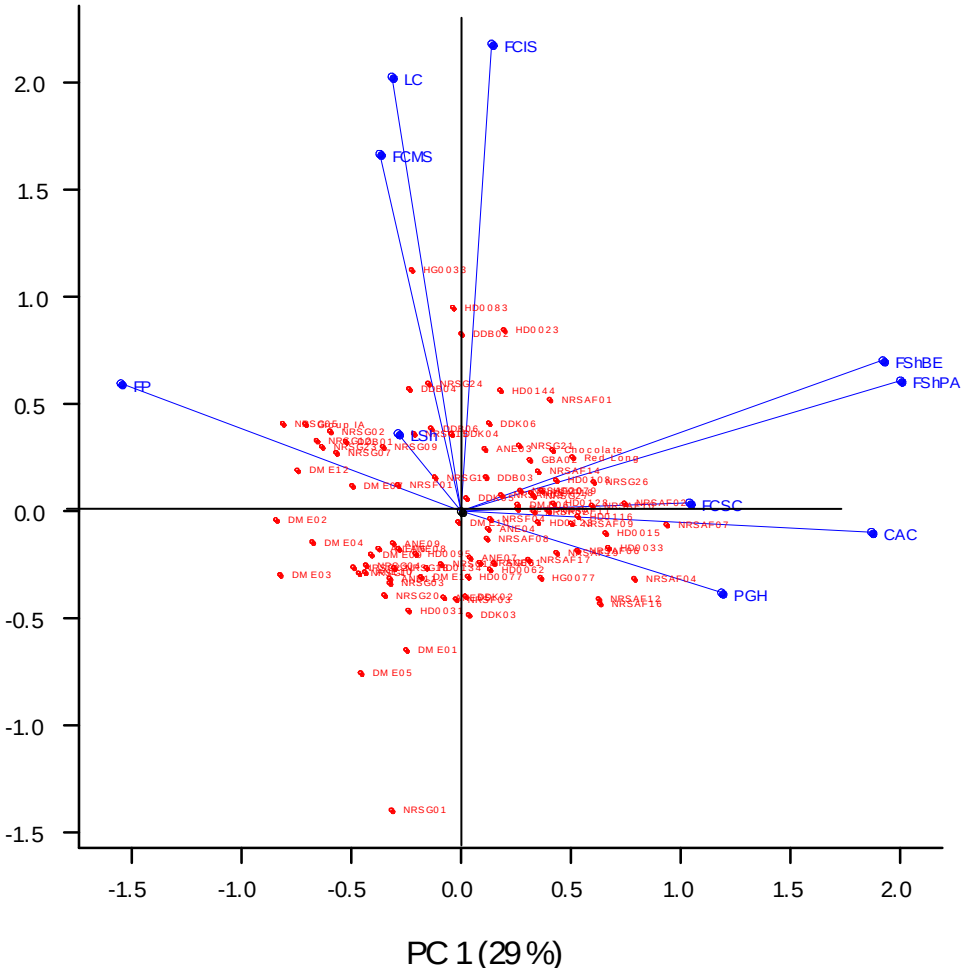

Figure 4.6: Principal coordinate biplot of the first two PCs using qualitative data from Hamelmalo of the 95 collections

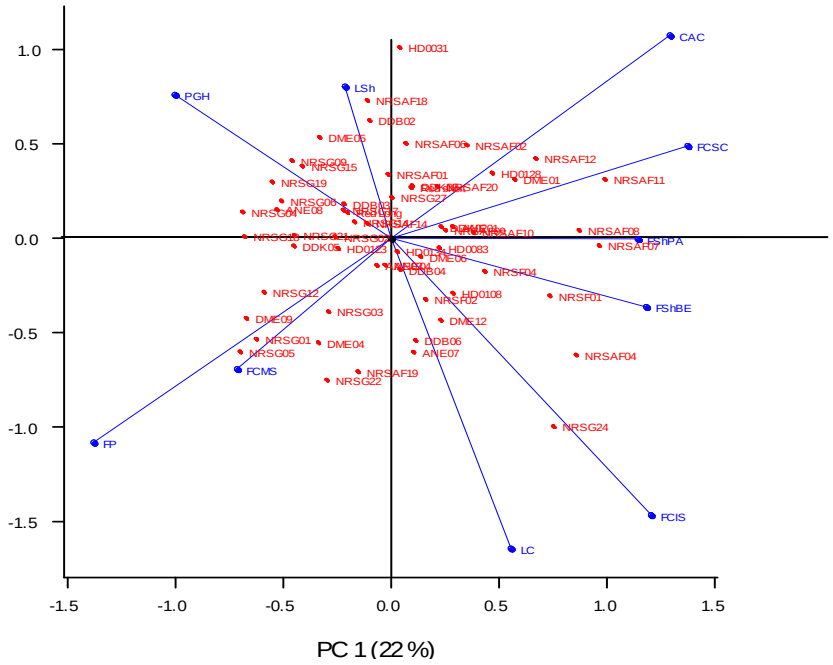

Figure 4.7: Principal coordinate biplot of the first two PCs using qualitative data from Asmara of the 60 collections

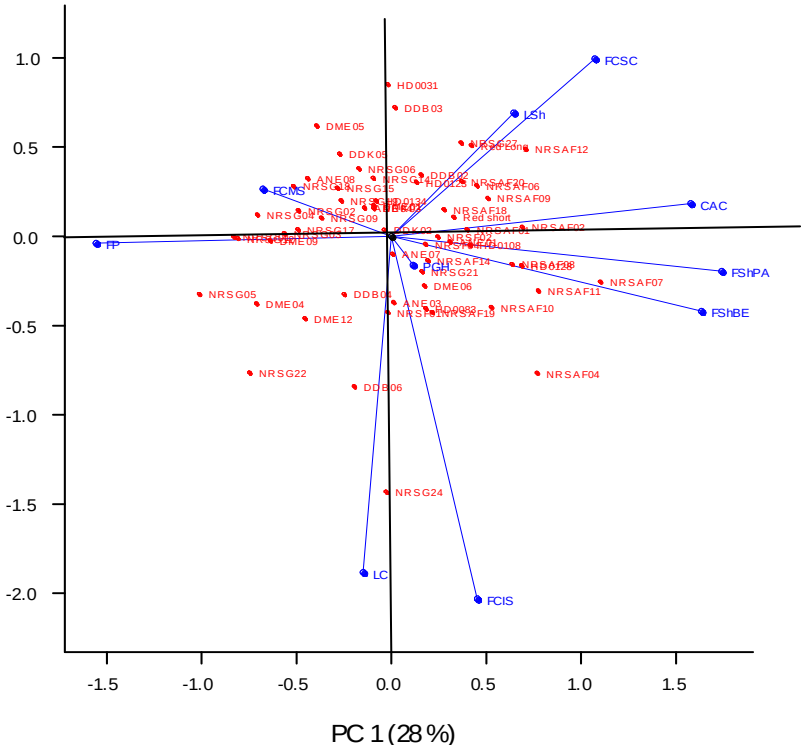

Figure 4.8: Principal coordinate biplot of the first two PCs using combined qualitative data of the two sites for the 60 collections

4.3.4. Classification of collections

Quantitative traits

Grouping of the collections was at 82 % similarity coefficient and sub-clustering at 87% for the two sites separately and combined data. Based on the aforementioned coefficients the 95 collections tested at Hamelmalo were grouped into three main clusters while those tested in Asmara and combined data of the two sites the 60 collection were grouped into four main clusters.

At Hamelmalo site Cluster I was the largest with a total number of 41 collections (43.2% of the total population). All farmer varieties collected from Elabered, Anseba region (except ANE03 and ANE04) , Mendefera, Debub region (except DME06 and DME10) and Gindae, Northern Red Sea region (except NRSG14, NRSG21, NRSG24, NRSG26 and NRSG27) were inferred to this cluster. This cluster was farther divided at 87% similarity coefficient to sub-clusters A, B &C (Fig 4.9). Sub-cluster A was composed of six collections, three from Elabered and one each from Dekemhare, Gindae and Afabet. Furthermore, Sub-cluster C was composed of three collections (one each from Elabered, Mendefera and Gindae). The large sub-cluster (B) was composed of 33 collections, but divided into four smaller groups. The most prevalent feature of this sub-cluster is that it includes 14 collections from Gindae and 8 from Mendefera. The collections from Gindae were distributed in three smaller groups of five, seven and two, while collections from Mendefera were distributed into four groups; two groups had three each and another two groups had one collection each. The rest 11 collections in this sub-cluster are four from Elabered, two from each Dbarwa and HAC and one each from Foro, Dekemhare and NARI (Fig 4.9).

Cluster II was composed of 24 collections and no further clustering was found at 87% similarity coefficient level. However, three smaller groups of 6, 14 and 4 collections composed this cluster. The main feature of this cluster was it is an

admixture of farmer variety collection from all sub-regions and it included five collections from HAC and one from NARI. However, grouping of the collection was not totally based on geographic relationtionship except that four of the five HAC collections were in one group (Fig 4.9).

A total of 29 collections were inferred to cluster III divided into two sub-clusters (E and F). Sub-cluster 'F' is composed of only two collections (HD0134 and NRSG21), while sub-cluster 'E' was composed of 27 collections divided into two groups. The first group included 16 collections, 12 of these are farmer varieties collected from Afabet (Northern Red Sea region), two from Gindae and one each from Dekemhare and HAC. The second group included 11 collections of which eight are from HAC, two from NARI (Red-long and Red-short) in addition to one collection from Afabet. The main feature observed in this cluster is that majourity of farmer varieties from Afabet and breeding lines from institutions are the main constituent, however, each of the farmer varieties and breeding lines tend to group separately from each other (Fig 4.9).

Based on the 82% similarity coefficient, the 60 collections that were tested in Asmara site were grouped into four clusters. Cluster I was composed of 16 collections all of farmer varieteis. At 87% similarity coeeficient cluster I was divided into two sub-clusters (A and B). Sub-cluster 'A' was composed of six collections, two each from Elabered (ANE01 and ANE07) and Afabet (NRSAF01 and NRSAF18) and one collection each from Gindae (NGSG15) and Dekemhare (DDK02). Sub-cluster'B' was composed of 11 collections of which six are from Gindae, two from Foro and one from each Elabered, Mendefera and Dbarwa (Fig 4.10).

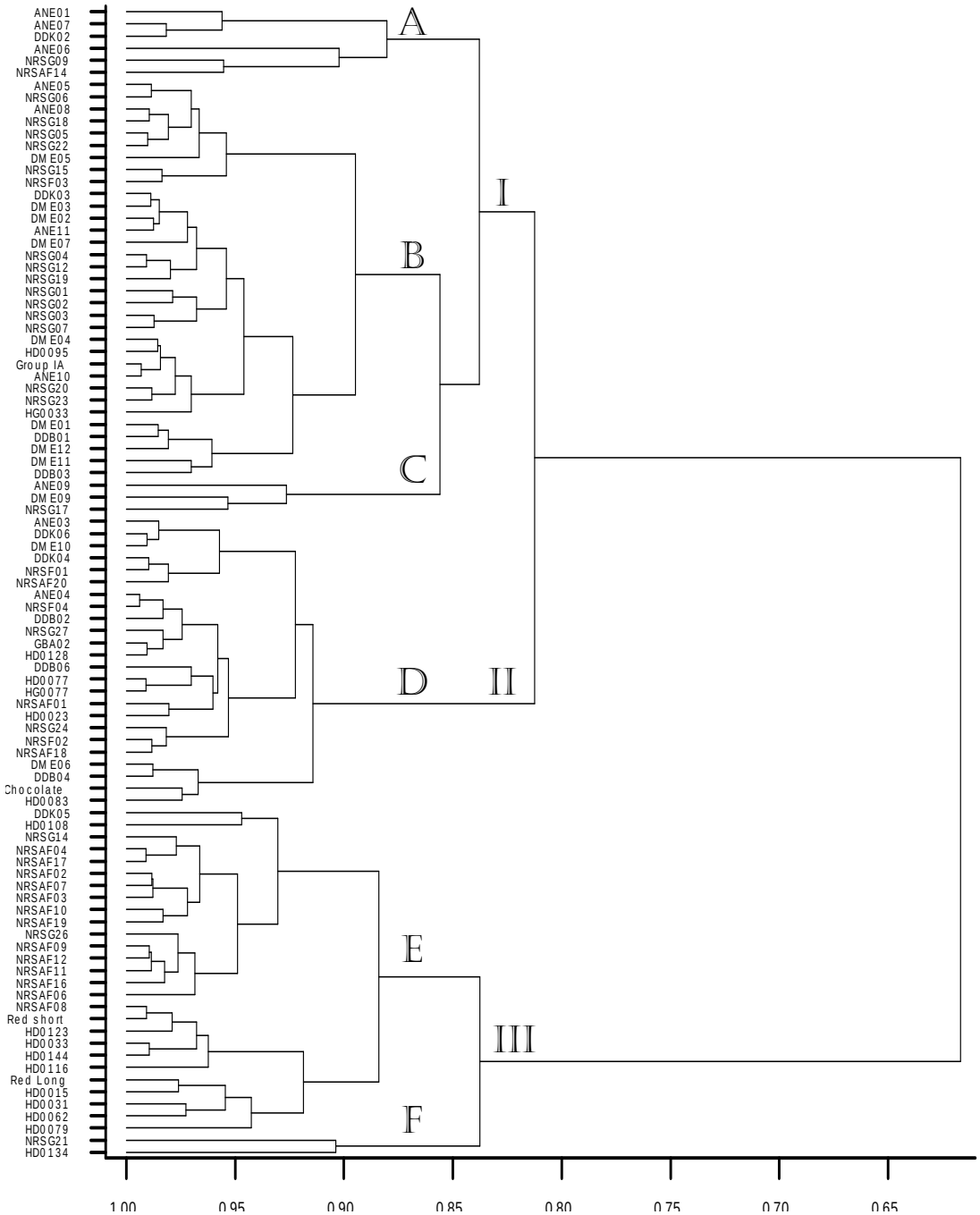

Figure 4.9: Clustering of the 95 collections tested at Hamelmalo using quantitative traits

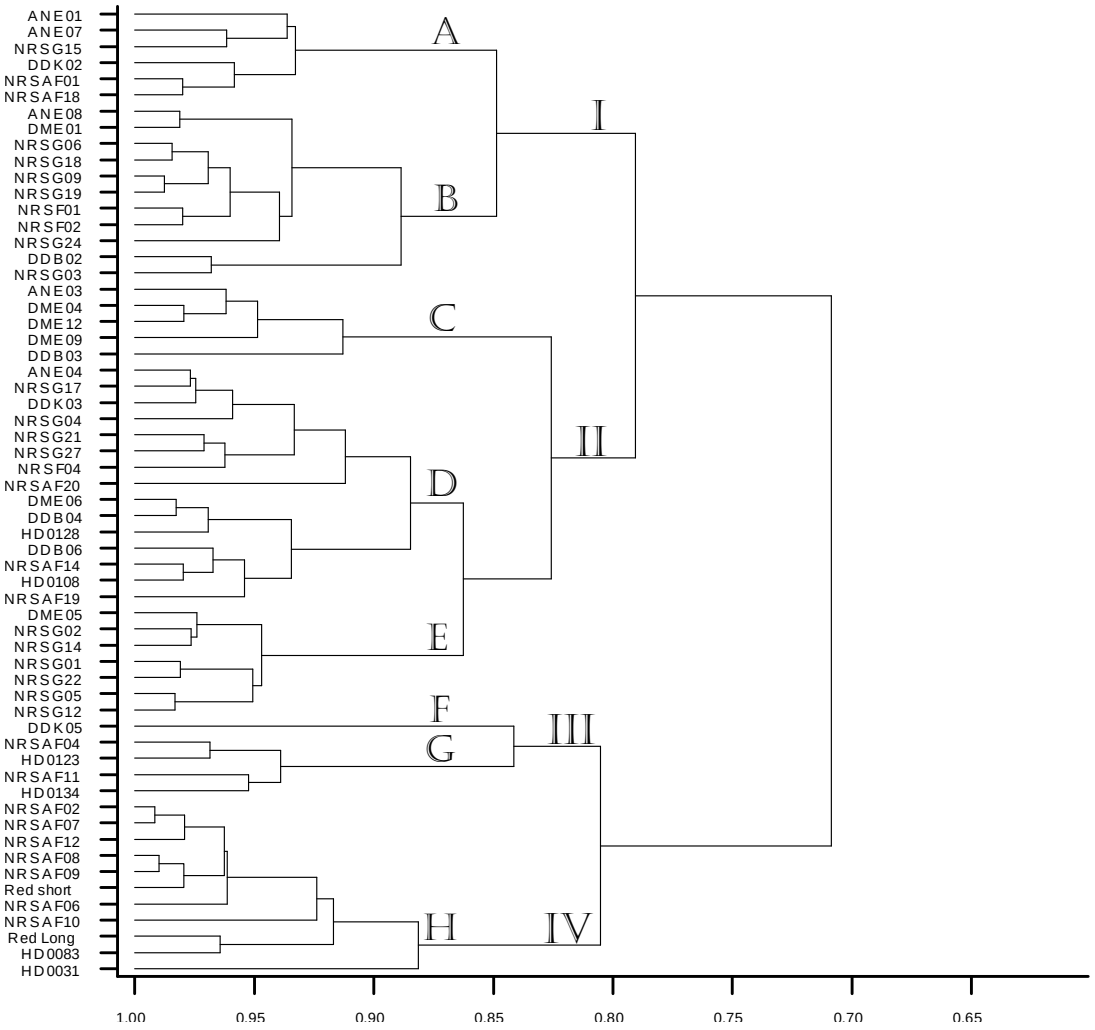

Figure 4. 10: Clustering of the 60 collections tested at Asmara using quantitative traits

Cluster II with 27 collections was the largest and grouped into three sub-clusters. The major feature of this cluster is that all populations except collections from NARI are represented, five out of six collections from Mendefera and 10 out of 17 from Gindae collections are inferred to this cluster. This cluster was further sub-clustered into three (C,D and E). Sub-cluster 'C' included three collections from Mendefera and one from each Elabered and Dbarwa. Sub-cluster 'D' was composed of four collections from Gindae, three from Afabet , two each from HAC and Dbarwa and

one collection each from Elabered, Mendefera and Dekemhare. Sub-cluster ‘E’ was composed of five collections from Gindae and one collection from Mendefera (Fig 4.10).

Cluster III included collection DDK05 solitary in sub-cluster ‘F’ and two collections each from Afabet and HAC in sub-cluster ‘G’. Cluster IV included 11 collections seven of them from Afabet. The rest four are two each from NARI and HAC (Fig 4.10).

Based on 82% similarity coefficient using combined data of the two sites the 60 collections are grouped into four clusters and further clustering at 87% similarity coefficient resulted in seven sub-clusters (Fig 4.11).

Cluster I was composed of two sub-clusters and included 20 collections. Sub-cluster A with 15 collections was the largest and composed of four collections from Elabered, three from Dbarwa and two collections each from Foro and Afabet and one collection each from Dekemhare and Gindae, Mendefera and HAC. The second sub-cluster in this group was sub-cluster B which included five collections one each from Dekemhare, Dbarwa and Mendefera of the Debub region in addition to one each from Afabet and HAC (Fig 4.11). The main feature of this cluster was it is an admixture of farmer variety collections from all regions in addition to two collections from HAC.

Cluster II was composed of 20 collections with no sub-clustering. The main feature of this cluster is that 14 out of the 20 members are farmer variety collections from Gindae. Sub-cluster C included four collections from Gindae, two from Mendefera and one each from Elabered, Foro and Afabet. In sub-cluster E all collections except one (DME12) are from Gindae.

Cluster III was the smallest one and composed of only two collections (DME09 and NRSG17).

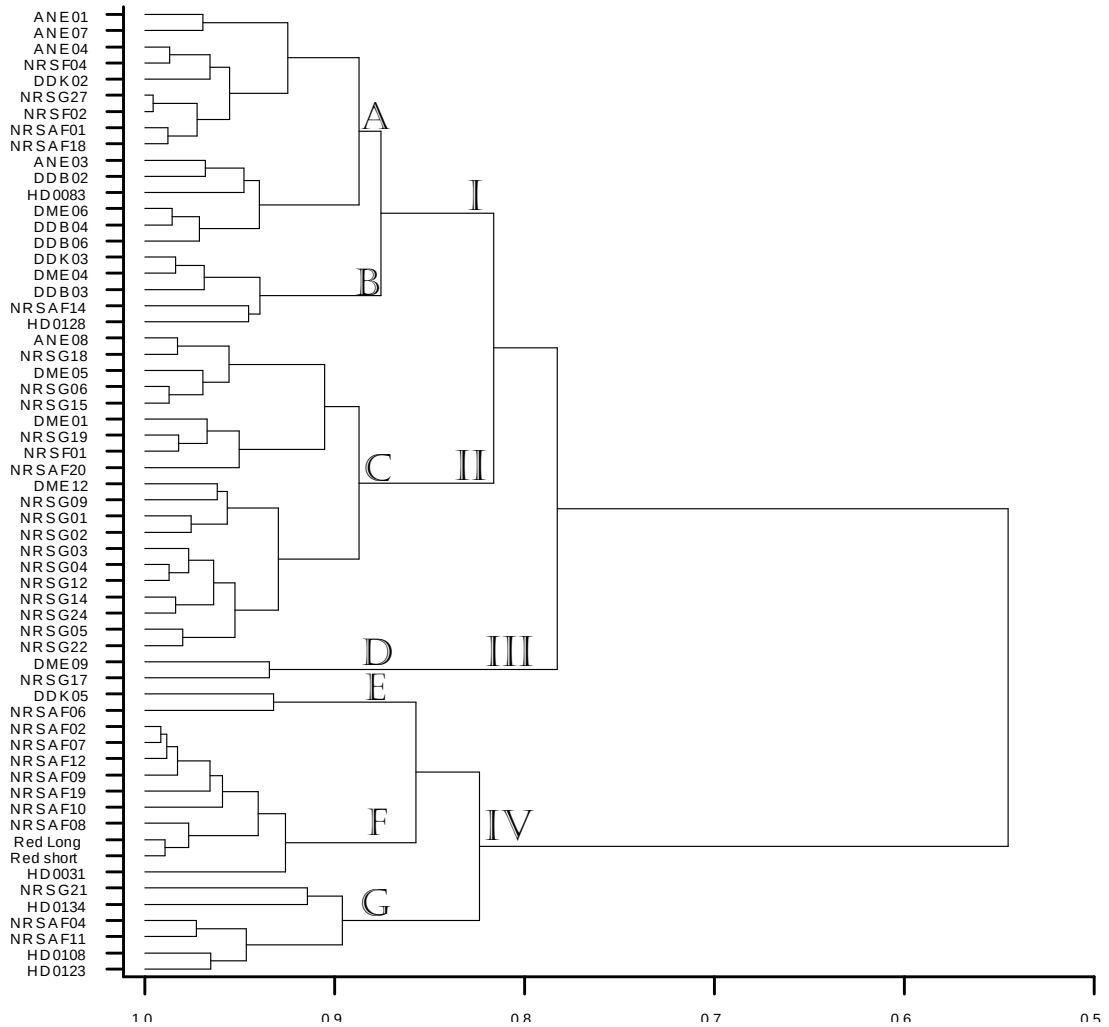

Figure 4. 11: Clustering of the 60 collections using the combined quantitative data of the two sites

Cluster IV included 18 collections grouped into three sub-clusters. Sub-cluster E was the smallest composed of two collections, one each from Dekemhare (DDK05) and Afabet (NRSAF06). Sub-cluster F is the largest in this cluster and was composed of 10 collections of which seven members are farmer varieties from Afabet. This is in addition to two collections from NARI and one from HAC. The six members in sub-cluster G are three from HAC, two from Afabet and one from Gindae. The main future of this cluster is that first except two (DDK05 and NRSG21) all the collections are farmer varieties collected from sub-region Afabet and breeding lines collected from HAC and NARI.

Qualitative traits

Cluster analysis based on qualitative traits was conducted using the same criteria used for the quantitative data. However, similarity coefficient at 75% for main clusters and 80% for sub-clustering was followed. Based on this criteria the collections at Hamelmalo and Asmera as well as clustering using combined data of the two sites formed five main clusters each. (Figs 4.12, 4.13 and 4.14).

At Hamelmalo 53% (50) of the collections inferred to cluster I which was composed of three sub-clusters (A, B and C) (Fig 4.12). Sub-cluster A included 19 members, four each from Afabet and HAC, three from Dekemhare and two from NARI. This is in addition to two collections each from Elabered and Foro and one each from Gindae and Mendefera. Sub-cluster 'B' included 11 members and only one (NRSG27) was from Gindae, while the rest were six from Afabet and four from HAC. Out of the 20 collections grouped in sub-cluster C, seven were from Afabet, three each from HAC and Gindae, two each from Dekemhare and Anseba and one each from NARI (Red-short) and Mendefera (DME06) along with one collection from Akurdat (GBA02). The main feature in this cluster is that all collections from Dekemhare and 16 collections from Afabet (out of 18 collections), 11 collections from HAC (out of 17 collections) and 3 collections from NARI (out of 4) are members of this cluster. This is to say similar to the quantitative traits farmer varieties collected from Afabet are clustering with breeding lines of HAC and NARI; however, unlike the quantitative traits more farmer variety collections from different sub-regions have joined this cluster (Fig 4.12).

Cluster II was the smallest and composed of two sub-clusters. Sub-cluster 'D' was formed of two collections from Elabered (ANE07 and 09), and one each from Gindae (NRSG15) and Dbarwa (DDB03). Sub-cluster E was composed of two collections from HAC (HD0031 and HD0134) and one from Mendefera (DME11).

Cluster III was composed of 18 collections in two sub-clusters. All except three in this cluster belong to farmer varieties collections from Mendefera, Gindae and and Anseba. Sub-cluster F included six collections from Mendefera, four from Gindae

and three from Elabered. The sub-cluster G was composed of two from Gindae and one each from Mendefera, Dbarwa and Foro (Fig 4.12).

Cluster IV included 12 members out of which seven are from Gindae and the rest two are from HAC and one each from Mendefera, Foro and NARI. No further clustering was observed at 80% similarity coefficient level for both Clusters IV.

Cluster V was composed of eight members in two sub-clusters (I and J). Sub-cluster I was composed of three farmer variety collection from Dbarwa and three breeding line collections from HAC, while sub-cluster J was composed of two collections from Gindae (Fig 4.12)**.**

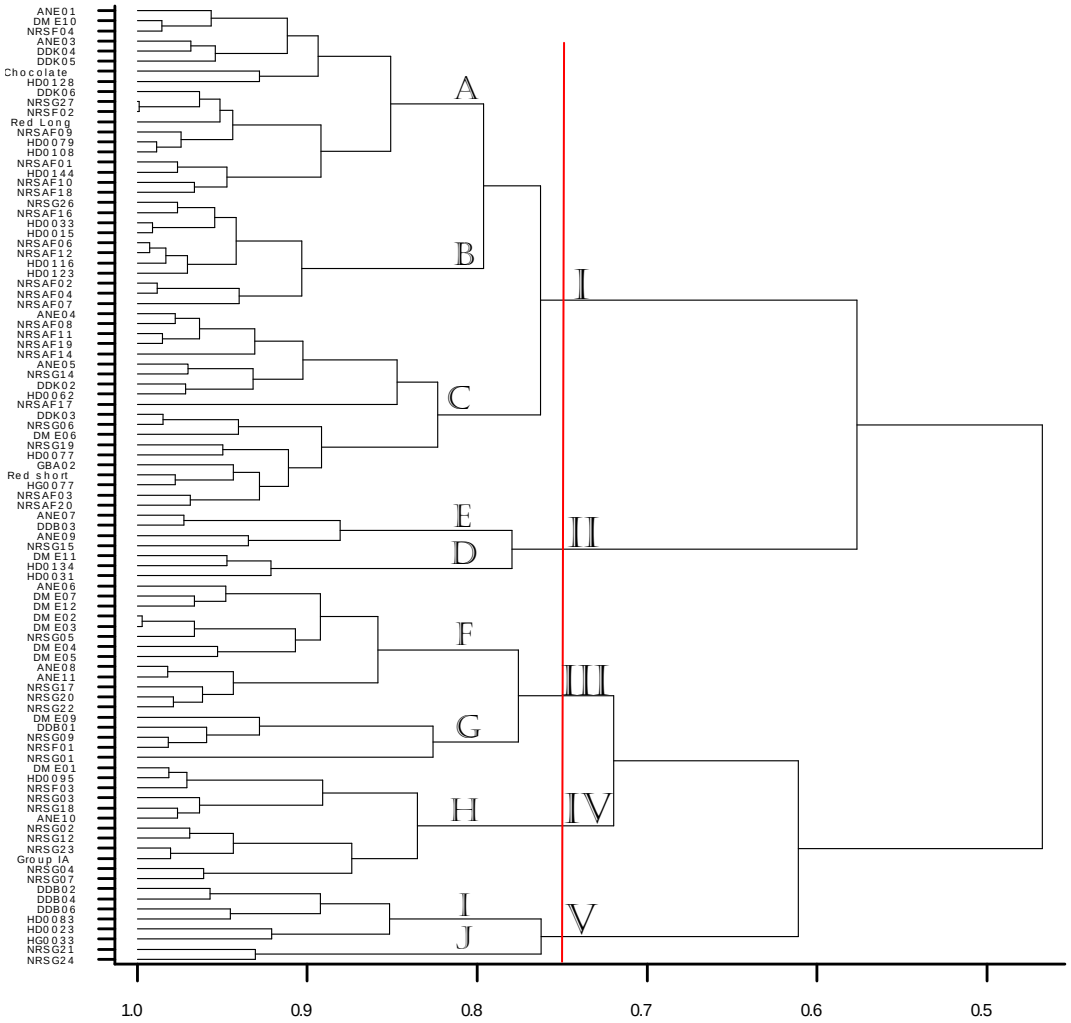

Figure 4.12: Clustering of the 95 collections tested at Hamelmalo using qualitative traits

In Asmara (Fig 4.13) cluster I included 17 collections distributed into three sub-clusters. Sub-cluster A with 10 collections was the largest in this cluster. It included three collections from Mendefera, two each from Elabered and Dekemhare and one each from Foro (NRSF02), Dbarwa (DDB04) and NRSAF14 from Afabet. Four out of five in sub-cluster B are from Afabet. The fifth member was from Foro. Sub-cluster C was composed of NRSG24 from Gindae and NRSAF04 from Afabet. The main feature in this cluster was an admixture of farmer variety collections from all sub-regions (Fig 4.13).

Only one collection each from Foro (NRSF04), Red-short from NARI and HD00108 from HAC were grouped in Cluster II.

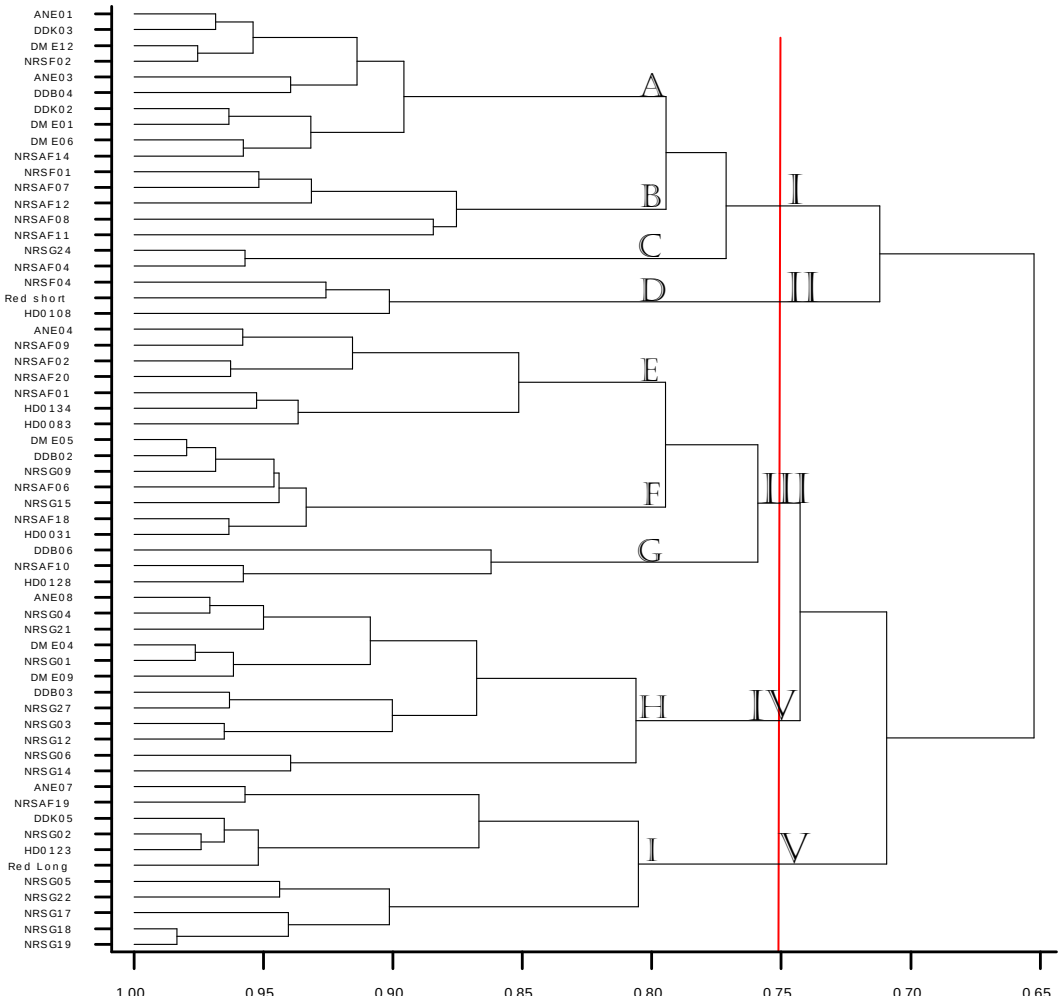

Figure 4. 13: Clustering of the 60 collections tested at Asmara using qualitative traits

Cluster III was composed of a total of 17 collections distributed in three sub-clusters. Sub-cluster D included four collections from Afabet, two from HAC and one from Elabered. Sub-cluster E included two collections each from Gindae and Afabet and one each from HAC, Mendefera and Dbarwa. While, sub-cluster F was composed of three collections; one each from Afabet, Dbarwa.

Both clusters IV and V had no sub-clusters at the set similarity coefficient. Collections from Gindae are the major constituent of the two clusters. In cluster IV, eight out of the 12 are from Gindae. In cluster V, seven out of the 11 collection are from Gindae (Fig 4.13).

Compared to clustering base on the quantitave traits of both sites and qualitative traits at Hamelmalo the main feature observed in Fig 4.13 were that first only 50% of farmer varieties collected from Afabet were grouped with breeding lines of HAC, while the rest mainly moved to the admixed cluster I. Secondly, NARI collections (Red-short and Red-long) no longer belong to this group; Red-short moved with HD0108 of HAC in addition to NRSF to form cluster II, while Red-long and HD0123 formed a small bundle in cluster V with DDK05 that used previously to cluster near to them.

Clustering of collections using the combined data resulted in five main clusters. Clusters I consisted of two sub-clusters (Fig 4.14). Cluster I was an admixture of collections representing almost all sub-regions and included 24. Sub-cluster ‘A’ composed of 16 and farmer variety collections from Afabet (five collections) were the largest group. The remaing were two each from Elabered, Dekemhare and Dbarwa, and one each from Mendefera, Foro, NARI and HAC. While sub-cluster ‘B’ had eight members, two each from Foro and HAC and one each from Elabered, Dbarwa, Gindae and Afabet.

Cluster II included 6 members; these are two from HAC and one each from NARI, Gindae, Dbarwa and Mendefera. While Cluster III was composed of 10 members of which eight are from Afabet; the remaining two are from HAC and Gindae. Both

clusters II and III were closely related and showed the close relationship among farmer varieties of Afabet and collection of HAC and NARI.

Clusters IV and V were very close to each other that were difficult to separate at the 75% similarity coefficient. Cluster IV included 16 members. Collections from Gindae with 13 members were the major constituents of this cluster. The cluster also included one each from Mendefera, Elabered and Dekemhare. Cluster V was a small one composed of three collections from Mendefera and one from Gindae. (Fig 4.14).

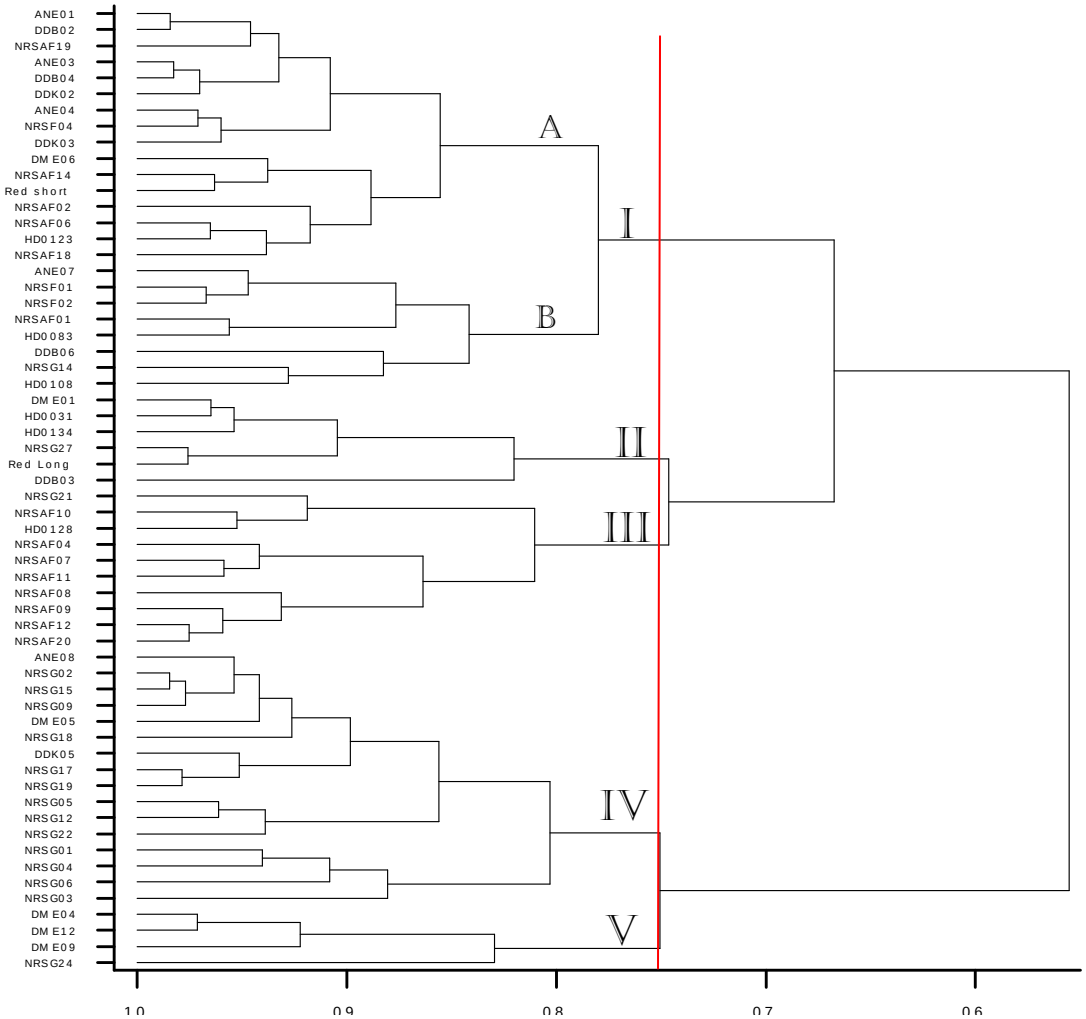

Figure 4. 14: Clustering of the 60 collections tested using the combined data of the two sites.

4.3.5. Evaluation of the performance of the collections

The performance of the collections was evaluated based on yield and four fruit quality parameters. Based on the selection index value of five; the best 30 collections that scored a value of three and above are listed in Table 4.15. These included 14 from HAC, 10 from Afabet, two each from NARI and Gindae and one each from Mendefera and Gash-Barka. The 30 collections were grouped into 8 ranks. The first in rank included one collection (HD0079) which is characterized by medium fruiting, long wide fruit with thick fruit wall and average yield per plant of 1132g. The second in rank included three collections (HD0134, HD0031 and NRSAF16) that were characterized by medium fruiting, medium to long fruit, wide fruit, medium fruit wall thickness and yield ranging 861.5 to 1139 g. The third in rank included two characterized by early fruiting, medium fruit length, wide fruit with medium to thick fruit wall thickness and an average yield of 693 to 985 g. The fourth rank included three collections characterized by very early to early fruiting, medium fruit length and width, thin to medium fruit wall thickness and an average yield of 822 to 1110.5 g. The fifth rank included one collection (NRSAF06) which was characterized by medium in days to fruiting, medium fruit length, wide fruit, medium fruit wall thickness and an average yield of 851.5 g. The last three in rank included six, seven and seven collections respectively as described in Table 4.14.

Table 4.15: Ranks of the best 30 collections based on yield, days to fruiting and three fruit quality parameters for dry consumed pepper in Eritrea. (Season 2013)

Collection	DFr	FL (cm)	FW (cm)	FWTh (mm)	Y/Pl (g)	Selection index	Rank
HD0079	60.3	13.4	3.5	3.1	1132	4.8	1
HD0134	68.7	10.9	3.6	2.6	1139	4.2	2
NRSAF16	64.3	12.1	3.0	2.7	993	4.2	2
HD0031	63.0	11.7	3.1	2.6	861	4.2	2
HD0144	61.3	10.3	3.2	2.7	985	4.0	3
HD0015	62.0	10.2	3.6	3.1	693	4.0	3
HG0077	59.7	10.7	2.6	2.3	950	3.8	4
NRSG21	63.0	10.1	2.9	1.8	1110	3.8	4
HD0116	56.3	11.5	2.4	2.4	822	3.8	4
NRSAF06	68.8	10.9	3.1	2.3	851	3.6	5
NRSG26	59.7	12.0	2.8	2.5	849	3.4	6
NRSAF17	67.7	11.0	2.9	2.3	776	3.4	6
HD0062	67.0	10.8	2.8	2.8	738	3.4	6
NRSAF03	66.3	9.4	3.1	2.5	682	3.4	6
HD0033	61.0	8.9	3.4	2.5	673	3.4	6
NRSAF09	65.5	11.5	3.1	2.3	559	3.4	6
NRSAF08	62.5	10.3	2.9	2.4	605	3.2	7
DME11	64.3	11.0	1.7	2.2	650	3.2	7
NRSAF12	66.0	10.5	3.1	2.3	602	3.2	7
HD0077	62.0	10.4	2.6	2.4	576	3.2	7
HD0123	61.2	9.8	3.1	2.2	569	3.2	7
NRSAF07	68.2	10.7	3	2.3	540	3.2	7
NRSAF04	68.0	10.8	3	2.2	527	3.2	7
GBA02	61.7	8.2	3.1	2.2	631	3.0	8
NRSAF02	69.3	10.7	2.8	2.4	607	3.0	8
HD0108	64.7	9.9	2.9	2.4	590	3.0	8
Red Long	66.8	9.5	3.2	2.5	482	3.0	8
Group IA	59.7	11.2	1.5	2.1	468	3.0	8
HG0033	57.3	11	1.7	2.1	466	3.0	8
HD0128	58.8	9.5	2.6	2.1	433	3.0	8

4.3.6. Correlation of yield and yield components

Yield per plant, fruit weight and number fruits per plant are components that determine total yield. These components correlate to each other and with other plant characteristics. Correlation analysis of the combined data from the two sites (Table 4.16) showed that yield per plant was positively correlated with plant height, stem thickness, fruit length, fruit width, fruit wall thickness, fruit weight and number of fruits. The highest positive correlation was with stem thickness (0.504) followed by fruit wall thickness (0.484), while the lowest correlation was with days to germination (0.115). All the positive correlations were significant. Yield per plant was negatively and significantly correlated with both days to fruiting and TSS.

Table 4.16: Correlation among yield and yield contributing characteristics using combined mean data of the two sites.

	DFl	DFr	PHt	STh	FL	FW	FWTh	FWht	TSS	NoF/P
DFl										
DFr	0.78*									
PHt	-0.15*	-0.11*								
STh	-0.01 ^{NS}	-0.04 ^{NS}	0.57*							
FL	0.01 ^{NS}	-0.03 ^{NS}	0.28*	0.27*						
FW	0.03 ^{NS}	0.06 ^{NS}	0.21*	0.33*	0.21*					
FWTh	0.17*	0.07 ^{NS}	0.01 ^{NS}	0.23*	0.24*	0.51*				
FWht	0.03 ^{NS}	0.06 ^{NS}	0.22*	0.30*	0.37*	0.76*	0.61*			
TSS	-0.18*	0.02 ^{NS}	0.15*	-0.06 ^{NS}	-0.03 ^{NS}	0.10 ^{NS}	-0.34*	0.09 ^{NS}		
NoF/P	-0.15*	-0.29*	0.34*	0.32*	0.16*	-0.32*	-0.10 *	-0.30*	-0.23*	
Y/P	-0.01 ^{NS}	-0.15*	0.40*	0.50*	0.35*	0.37*	0.48*	0.43*	-0.32*	0.38*

DFl=Days to flowering DFr=Days to fruiting FL=Fruit length FW=Fruit width FWTh=Fruit wall thickness FWt=Fruit weight NFr/P= Number of fruits per plant PHt=Plant height STh=Stem thickness TSS=Total soluble solids Y/Pl=Yield per plant.
*^{NS}= Not significant *= Significant at 0.05*

Number of fruits per plant was positively and significantly correlated with plant height, stem thickness and fruit length, however, it was negatively and significantly correlated with the rest of characters. Fruit weight positively and significantly correlated with all characteristics except number of fruits per plant (negatively correlated) and both days to flowering and days to fruiting (not significant). Remarkably high positive correlations were recorded between DFl and DFr (0.778) and FWht with both FW (0.763) and FWTh (0.612). While high negative correlation were recorded between Y/P and TSS (-0.320) and NoFr/P with both FW (-0.316) and FWht (0.304) (Table 4.16).

4.4. Discussion

4.4.1. Diversity of the collections

The distributions of characters of the different quantitative and qualitative traits and the performance of the collections (Tables 4.5 - 4.8) Results of this study showed considerable diversity of collections with respect to phenology, plant and fruit characteristics.

The first step of any meaningful breeding programme is to identify crop plants that exhibit exploitable variation for the trait(s) of interest (Aremu, 2012). The ranges in performance of the collections (Tables 4.6 and 4.7), variations of the quantitative data (Figs. 4.3-4.5) and selection index (Table 4.15) showed the existence of collections recorded high values that can be exploited as a source for the respective traits in future breeding. A group composed of four collections, viz HD0134 and HD0079 from HAC, NRSG 21 from Gindae and NRSAF16 of Afabet showed high potential in yield that ranged from 1139 g/plant (HD0134) to 993g/plant (NRSAF16). This group also showed high values for yield related traits such as fruit length, fruit width, fruit wall thickness and fruit weight as well as plant hight and stem thickness in addition to medium number of fruits per plant. A second group composed of NRSG09, NRSG17 and NRSG01 from Gindae, DME09 from Mendefera and ANE09 from Elabered showed high recorded in number fruits per plant that ranged (118 to 63.7) and was early in phonological characteristic; days to

germination, flowering and fruit set in addition to relatively higher TSS compared to the other groups. This group can be exploited in adding earliness and increasing number of fruits to elite genotypes. A third group composed of four collections viz NRSAF 12 and NRSAF 14 from Afabet, DDK05 from Dekemhare and HD0108 from HAC was medium in almost all traits and recorded a yield per plant range 755 (NRSAF 14) to 572 (DDK05). This group can be useful in selecting superior individuals for further improvement.

In some traits, there was limited variation based on the skewed distribution of the collections. In others, the collections were evenly distributed across the different characters of the trait (Table 4.5). The even distribution reflects the variability within the Eritrean pepper germplasm. In addition to that many traits showed within collection variation resulted in mixed of characters. Due to the high outcross that ranges in pepper 0.5-91% (do Rego, et.al., 2012; Bosland, 1996) and possibility of interspecies cross in pepper it is logical to observe this variation, specially most of the pepper growers in Eritrea are small scale farmers who cultivate small lands adjacent to each other and save their seed from selected plants in the field or the crop after harvest.

The lowest CV% was recorded on phonological attributes such as DFl and DFr. Similar results were reported by Ballina-Gómez et al. (2013). The principal coordinate biplots also showed similar distribution of traits. CAC, FCMS, FSh, FShBA and NFPA are traits that recorded the highest CV% of qualitative characteristics. For CAC even a much higher level of coefficient of variation (576.9%) was reported by Ballina-Gómez et al. (2013) which indicated the importance of these traits for discriminating genotypes.

The high variation recorded on yield components and fruit characteristics (Tables 4.5, 4.7 and 4.8) reflected the intensity of selection for yield and fruit quality parameters.This is similar to the situation of New Mexico pepper landraces described by Votava et al. (2005) which were heterogeneous and survived a long period of selection by farmers for various traits and adapted to local conditions as a result of natural selection.

4.4.2. Variation among collections

The variation explained by the five principal components of the quantitative traits (Table 4.10) was somewhat similar to the results obtained in previous studies in pepper and other crops as described by Occhiuto et al. (2014) , Nsabiyera et al. (2013), Del et al. (2007), Beyene et al. (2005) and Naugeer (2009). The variation was slightly lower when compared to those reported by Aruah et al., (2010). However, the variation was much higher than other studies conducted previously on pepper and other crops (Bozokalfa et al., 2009; Furat & Uzun, 2010).

The variation explained by the first five components (four at Hamelmalo) from the ten qualitative traits (Table 4.13) was much lower at Hamelmalo and Asmara and slightly lower for the combined data compared to the results of Aruah et al. (2010) who evaluated variation in cucumber (74.94% from the first three components). Del et al. (2007) reported that 82% of the variation was found in pepper, however, the results are similar since they accepted a characteristic value of 0.75 for the fifth component. On the other hand similar results were reported for the first three components (57%) and lower percentage (21%) for the first component.

Occhiuto et al., (2014) found fruit characteristics as the most efficient in the differentiation of the accessions. In the current study both quantitative and qualitative fruit characteristics were the most important contributors in explaining the variation among the genotypes. These are fruit width, fruit wall thickness, fruit weight and number of fruits per plant of quantitative traits and fruit shape at blossom end, fruit shape at pedicel attachment and fruit colour at intermediate stage. However, for qualitative traits this was true for Hamelmalo and combined data, while in Asmara fruit shape at both pedicel attachment and blossom end were less important compared to fruit colour at mature stage.

Other characteristics that were important contributors for differentiating among genotypes are phonological attributes (DFl and DFr) and vegetative growth (PHt and STh) of the quantitative and CAC, LC and FP of the qualitative variables. These results were similar to the results reported by Nsabiyera et al. (2013), Cankaya et al.

(2010); and Del et.al. (2007) for the quantitative traits. While for qualitative traits they were similar to the findings of Del et.al. (2007) regarding flower position, leaf and fruit colour.

4.4.2. Classification of collections

Cluster analysis of the quantitative traits (Fig. 9) showed at 82% similarity coefficient for the 95 collections tested at HAC clustered into 3 major groups and at 87% of the collections formed 6 sub-clusters. However, the 60 collections tested in Asmara (Fig.10) were clustered into four major clusters and eight sub-clusters. Similarly the combined data (Fig. 11) showed that the 60 collections were clustered into four main clusters and nine sub-clusters. This confirms the high variability of the Eritrean pepper collections. Considering clustering of the collections using the combined data of the two sites, all collections except four in the list of best 30 collections for dry pepper (Table 4.15) are members of Cluster IV. The result of the PCA showed that all the collections inferred to this cluster had a high contribution to PC1 which is related to three yield contributing traits (fruit weight, fruit width and fruit wall thickness (Table 4.11 and 4.12). Most collections also had medium to high contribution to PC2 which is related to yield/plant, plant height, and fruit length. Except for fruit length de Rego et al. (2011) reported all the previous traits to be correlated with yield. On the other hand, some of the collections in this cluster contributed highly to PC4 which is related to phonological traits, while other had a high contribution to PC5 which is related to TSS and leaf characteristics (Table 4.11 and 4.12). Thus, this cluster can be useful for selecting collections for improving yield and yield related fruit characteristics as well as earliness and TSS. Triangular fruit shape and light red mature fruit colour were dominant characters to elongate fruit shape and dark red or brown fruit colour. Calyx annular constriction with few exceptions present. Fruit surface was smooth or semi-wrinkled, while fruit cross sectional corrugation was intermediate or semi-corrigated. Cluster III composed from two collections and positively contributed to PC1 which is related to Number of fruits/plant and PC3 which is related to earliness. The contribution of the majority of collections inferred to Cluster II was basically to PC1 and PC2 related to number of

fruits and plant height; but also a considerable number of collections in this Cluster had contribution to PC4 and PC5. Yadeta, et al. (2011), found a positive, non significant correlation between plant height and yield/plant, but the direct and indirect effect of plant height on yield was high. In studying Turkish red pepper, Cankaya et al. (2010), suggested utilization of plant height for increasing yield/palnt. Similarly Maga et al. (2013), reported indirect effect of plant height on yield. Although Cluster II was lower compared to Cluster IV in the mean performance of the interrelated traits, plant height, stem thickness and fruit length, which are closely related to yield/plant. This could be due to differences in number of collections inferred to each cluster. Consequently, Cluster II may be useful as a source of genes for number of fruit and plant height. Absence of calyx annular constriction, elongate fruit and semi-corrigate fruit cross sectional corrugation were the dominant fruit characters in Cluster. In addition to that light red fruit colour was dominant, however, considerable number of collections were with brown or dark red fruits. Cluster I was mainly good contributor to PC3 which is related to cotyledon leaf length and width and days to germination; and PC5 which is related to TSS and leaf length and width. Thus, members of this Cluster can be useful as a source of individuals for selection or source of genes related to the aforementioned traits. Collections in this cluster characterized by elongate fruit shape with smooth or semi-wrinkled fruit surface and semi-corrigated or intermediate cross sectional corrugation. Dark red fruit colour was dominant however, collections with light red, orange red, brown and light brown fruit colours existed indicating rich fruit colour array.

A similarity coefficient of 75% for major clusters and 80% for sub-clusters was used for the 10 qualitative characters. These grouped both the 95 collections tested at Hamelmalo and the 60 collections tested in Asmara into five main clusters and nine sub-clusters each, while analysis of the combined data of the two sites with the same criteria grouped the 60 collections into three major clusters and six sub-clusters. These showed that quantitative traits were more variable compared to the qualitative, may be due to qualitative traits being less affected by environmental conditions.

Some difference was observed in inferring collections to clusters from the quantitative and qualitative data of the same collections (Figs 4.12-4.14). Similar results were reported by Del et al. (2007). The majority of the materials used in this study were farmer varieties who grow pepper under conditions that allow high out-cross, thus the heterogeneity observed within collection of the current study was expected. This might have contributed to the lower variation in qualitative traits. However, the major population structure pattern was similar with both quantitative and qualitative data sets, except that the structure resulting from the quantitative data was clearer.

Majority of the collections collected from the same sub-region partially tend to cluster together in large or small groups. However, clustering of collections was not completely influenced by geographic and agro-climatic factors. Factors such as seed exchange among farmers and common ancestry seem to have contribution. For instance materials of Afabet, HAC and NARI are from three different agro-climatic regions and geographically distant from each other, but clustered together. Similarly, Gindae and Mendefera or Elabered and Gindae are distantly located in three different administrative and agro-ecological regions (Table 4.3), however, materials obtained from Gindae were clustered with those obtained from Mendefera and Elabered. Seed exchange or common ancestry could be the best reason to explain the grouping of materials from the three sub-regions. Similar condition was concluded as seed movement by Baral and Bosland (2002b) in studying pepper germplasm from Nepal.

The seed exchange and common ancestry can better be elaborated by the total or partial clustering of materials from Afabet, HAC and NARI in the same group. In Afabet pepper is produced by small holding farmers in two villages (Naro-Ans and Kubkub) along the Mogae seasonal river. A distance of 8 km separates the two villages. Land size allotted for pepper in the two villages range from 150 m^2 up to maximum of 2 ha (Table 3.7). Due to difficult access roads, the two villages are somewhat isolated from each other and more isolated from the other pepper producing areas. Collections from HAC and NARI are breeding lines resulting from mass selection in two separate breeding programs. Seed from diverse local sources

was used in the two breeding programs. Later NARI released some of its breeding lines to farmers. Therefore, either seed from NARI found its way to Afabet and crossed with other collections while some of it moved to HAC, or seed from Afabet found its way to the two breeding programs suggesting common ancestry and seed movement as the most probable reasons for clustering the three sub-populations together.

Majority of farmer variety collections from Gindae and Mendefera tend to cluster together or closer to each other, especially when quantitative data was used. On the other hand in both Hamelmalo and Asmara; cluster II was observed to be a kind of admixture that included collections from almost all sources (Figs 4.9 and 4.10). Since Gindae and Mendefera are very old pepper producing areas, seed movement in the two places is widely known. Pepper farmers in Eritrea usually use their saved seed that is passed from one generation to the next, however, they also look for reliable source of germplasm from other farmers within the village or far places. Therefore, the admixture cluster and individuals appeared away from their group possibly indicated that seed exchange have taken place among farmers from different areas (Baral & Bosland, 2002b) which is a common practice in Eritrea, or it could be due to common origin or ancestry of the collections (Naujeer, 2009).

4.4.3. Variability among genotypes

The principal coordinate biplots (Figs 4.3-4.5) displayed more than 48% of the variation existed among the genotypes based on the quantitative data of the two sites. This could be explained by the first two PCs. It supports the results of the analysis of variance.

For quantitative traits it showed that the genotypes were significantly different for 12 out of 16 variables in the two locations. Moreover, the combined data analysis of the two sites showed that the difference among genotypes was significant for all variables except for TSS, however, location had significant effect on TSS which was in agreement with Geleta and Labuschagne (2006) who found influence of environment on TSS. The highest coefficient of variation in the two sites was

observed on yield components such as NFrP, FWt and Y/P. This was similar to the results reported by Nsabiyera et al., (2013) in pepper and Naujeer (2009) in eggplant.

4.4.4. Correlation among the pepper traits

Plant characters may be interrelated to each other. Estabilishing the relationship can be useful for breeding to achieve desired selection in shorter time. The use of this tool was studied in many crops. In pepper Naik (2009) reported that correlation analysis helps the breeder to take decision on the choice of the character as selection criterion. Determining the relationship between characters affecting optimum output is very important for increasing yield components in pepper genotypes (Cankaya et al., 2010). Balkaya et al. (2011) found that correlation among plant characteristics could advance breeding practices. On the other hand variability among the genotypes under study is pre requisite for breeding. Breeding programme solely depends on magnitude of variability for the characters which need to be improved (Naik, 2009). The collections in the current study have shown significant differences for almost all the growth and yield characteristics suggesting high diversity that can be useful for selection by breeders.

The significant differences among collections had variable effects on yield components and on each other. Days to flowering and fruiting were highly and positively correlated to each other, however, both had negative impact on yield/plant and number of fruits/plant (Table 4.16). The association between days to flowering and days to fruiting expressed as days to maturity and days to flowers with yield/plant and number of fruits per plant was similar to that found by Yedeta et al. (2011), who suggested possibility of identifying early genotypes without waiting maturity. Similarly, the negative impact of longer days to flowering on yield and number of fruits can be useful in identifying potential high yielding varieties. In the current study 55% of the collections had mean in the range 50-55 days with minimum and max of 39 and 75 days respectively. This is lower than that reported by Nsabiyera and Sseruwagi (2012), who had genotypes exceeded 80 days.

Plant height and stem thickness are the strongly positively associated with yield components. These traits were highly and positively correlated to each other and to yield per plant and number of fruits per plant. This results are similar to that reported by (Cankaya et al., 2010) who suggested that plant height should be utilized in increasing yield per plant in red pepper. In the current study 5% of the genotypes were tall plants in the range of 54 to 68 cm with maximum of 69.5 that suggest the local genotypes can contribute to selecting high yielding genotypes.

4.5. Conclusions

The Eritrean pepper germplasm was found to be diverse with respect to quantitative and qualitative morphological traits. The collections were variable for all the 39 traits studied except for five qualitative traits. The variability included fruit shape, colour, size, pericarp thickness, TSS and other phonological, cotyledon and vegetative growth characteristics. This study also identified at least four promising collections (HD0134, HD0079, NRSG21 and NRSAF16) for dry consumption. In addition the study identified groups that can be used for selecting superior individuals or as a source of desired genes to be used in future peppers improvement programs. Plant height, stem thickness, fruit length and other traits can be potentially useful for selecting high yielding genotypes in breeding process.

CHAPTER FIVE

MOLECULAR CHARACTERIZATION OF ERITREAN PEPPER GENOTYPES AS REVEALED BY SSR MARKERS

Abstract

Pepper (*Capsicum spp.*) is one of the most important vegetable crops and the most widely used spice worldwide including Eritrea. Diversity studies are an essential step for crop breeding and improvement. The objectives of the study was to determine the molecular diversity of local Eritrean pepper collected from farmers and research institutions and to evaluate the relatedness of the Eritrean pepper with accessions obtained from five other countries. A total of 150 seed collections were evaluated using 28 SSR markers. The results showed that cultivars maintained *in situ* by farmers were heterogeneous. Diversity parameters indicated extensive genetic variation among the Eritrea genotypes. The 28 markers revealed a total of 352 alleles with an average of 13 alleles per marker. Mean Polymorphic Information Content was 0.62 and, mean Observed Heterozygosity was 0.41. The analysis of molecular variance showed only 10% variation was among populations, 30% among individuals within populations and 60% within individuals. This can be explained by the high mean number of effective migrants (2.25) that ranged from 1.01 to 10.45 among populations indicating movement of germplasm among farmers in different geographic and agro-ecological regions. A Neighbour joining clustering and the model based clustering (Structure) classified the collections into 3 groups. However, in the model based clustering; increasing the number of populations to 4 (K=4) caused majority of non-Eritrean genotypes to fall in a separate cluster suggesting availability of potentially rich diversity within the Eritrean populations justified by the large number of private alleles observed.

5.1 Introduction

The study of genetic variation among individuals, groups of individuals or populations is a pre-requisite for plant breeding. Knowledge about germplasm diversity and genetic relationships among breeding materials informs crop

improvement strategies. It allows exploration of variability in available traits of interest within the population under study (Mohammadi & Prasanna, 2003). Molecular markers are useful tools for diversity studies, and are usually more reliable than morphological descriptors. In recent years, molecular markers have been used intensively in molecular characterization of different plant species. However, few studies attempted to characterize a broad selection of cultivated *C. annuum* genetic diversity and each of these used a relatively small number (<150) of mostly anonymous markers (Hill et al., 2013). Random amplified polymorphic DNA (RAPD) was one of the most popular markers used. Baral and Bosland (2002b), Sanatombi et al. (2010), Bhadragoudar and Patil (2011), Akbar et al., (2010) used RAPDs for diversity studies of germplasm from Nepal, India and Pakistan. RAPDs were also used for varietal identification and genetic purity of genotypes from Turkey (Ilbi, 2003) and for characterizing and comparing genetic structure of landraces and wild populations (Votava et al., 2005 and Oyama et al., 2006). Similarly, Amplified Fragment Length Polymorphism (AFLP) markers were useful to reveal genetic diversity among pepper genotypes from Ethiopia compared to germplasm from other countries (Geleta et al., 2005) and from Turkey (Aktas et al., 2009).

Simple sequence repeats (SSR) markers also called Microsatellites are tandem repeated motifs of 1-6 nucleotides abundant in most eukaryotic and prokaryotic genomes (Kalia et al., 2011). More recently these markers have become more widely used in genetic studies of pepper and other plants. The information value of microsatellites is high compared to AFLPs and RAPDs (Lee et al., 2004). SSR markers are genome specific, abundant, highly polymorphic, co-dominant and are easily detected (Ijaz, 2011). They have been widely used in many plant species including cereals, vegetables and fruits for genetic diversity studies, population genetics and evolutionary studies, genome analysis, gene mapping and marker-assisted selection (Kalia et al., 2011). Compared to single nucleotide polymorphisms (SNP) markers, which can only be transferred to different mapping populations within the same species, SSRs exhibit multiple alleles, are cost-effective and transferable and will continue to play an important role in different genetic studies in

many minor plant species (Wang et.al., 2009). The use of SSR markers in genetic studies of capsicum varied from constructing several pepper linkage maps (Lee et al., 2004, Barchi 2007 and Mimura, et al., 2012), complement tests of distinctiveness (Kwon et al., 2005), genetic diversity and structure (Aguilar-Meléndez et al., 2009, Rodrigues & Tam 2010, Tilahun et al., 2013, Rai et al., 2013; Dhaliwal et al., 2014) and genetic relationship in *Capsicum* cultivars (Patel et al., 2011). In the current study 28 SSR markers obtained from the Asian Vegetable Research and Development Centre (AVRDC) were used. The objective was to characterize local Eritrean pepper germplasm collected from farmers and research institutions.

5.2 Materials and Methods

5.2.1 Plant material

A total of 129 seed samples were collected from farmers and institutions in Eritrea in 2012 (Table 4.1). Thereafter, an additional 17 accessions from AVRDC, one pepper and three tomato accessions from Kenyan Agriculture and Livestock Research Organization (KALRO) were added (Table 5.1). AVRDC materials were selected to represent, 1) Ethiopia where germplasm exchange took place across the border for a long period, 2) Italy from where many varieties were introduced to Eritrea, 3) India which is probably a source of the first peppers supplied to Eritrea and 4) Mexico which is considered the centre of diversity in addition to improved varieties developed in AVRDC (Table 5.1).

Due to variation observed within sample collections during the survey and seed collection as well as morphological characterization, three individual plants from each seed collection were collected as a sampling strategy for genotyping. However, as a result of seed viability constraint, in some cases only one or two plants from each were used, therefore a total of 407 individual plants were genotyped.

Table 5.1: Reference genotypes collected from AVRDC and KALRO

Genotype	Source	Country of Origin	Nomenclature
C05465	AVRDC	Italy	*Capsicum annuum*
C00766	AVRDC	Italy	*Capsicum annuum*
C01188	AVRDC	Italy	*Capsicum annuum*
C01408	AVRDC	Italy	*Capsicum annuum*
C02264	AVRDC	Ethiopia	*Capsicum annuum*
C05558	AVRDC	Ethiopia	*Capsicum annuum*
C03573	AVRDC	India	*Capsicum annuum*
C03007	AVRDC	India	*Capsicum annuum*
C02847	AVRDC	Mexico	*Capsicum annuum*
C02280	AVRDC	Mexico	*Capsicum annuum*
C002392a	AVRDC	Mexico	*Capsicum annuum*
C002392b	AVRDC	Mexico	*Capsicum annuum*
C00226	AVRDC	Mexico	*Capsicum annuum*
TC06847A	AVRDC	Ethiopia	*Capsicum annuum*
AVPP0105	AVRDC	AVRDC	*Capsicum annuum*
AVPP0303	AVRDC	AVRDC	*Capsicum annuum*
AVPP9813	AVRDC	AVRDC	*Capsicum annuum*
GBK-034707	KARLO	Kenya	*NA*
GBK-034712	KARLO	Kenya	Lycopersicon esculentum
GBK-034777	KARLO	Kenya	Lycopersicon esculentum
GBK-043392	KARLO	Kenya	Lycopersicon esculentum

5.2.2 DNA extraction

The Mace et al. (2003) protocol with some modifications was used for DNA extraction. Leaf samples of 30 to 50 mg stored at -80 ^{o}C were cut into small pieces and placed in 12x8 strip tubes together with 2 stainless steel balls and 450 µL of preheated (65°C) extraction buffer (100 mM Tris-HCl [pH 8], 1.4 M NaCl, 20 mM EDTA, CTAB [2-3% w/v], DDT [0.03-3% v/v]) was added to each sample and

secured with 8-strip caps. Samples were thereafter ground using Geno/Grinder 2010 (Spex Sampleprep) at 1500 strokes/min for 8 min and incubated in 65^{o}C water bath for 20 min. A volume of 450 µL of chloroform-isoamylalcohol (24:1) was added to each sample, mixed by several inversions and centrifuged at 3000 rpm for 15 min (Alegra 25R Centrifuge, Beckman Coulter). A fixed volume of 400 µL of aqueous layer was transferred to new strip tubes and 0.7 vol isopropanol (stored at –20°C) was added to each sample and mixed before being incubated at -20 °C for 1-2 hours or overnight. After incubation centrifugation was done at 2500 rpm for 15 min. The supernatant was decanted from each sample and the pellet was air-dried for 30 min. An amount of 200 µL low-salt TE (10 mM Tris, 0.1 mM EDTA [pH 8]) and 3 µL RNase A (10 mg/mL) was added to each sample before being incubated at 37°C for 30 min. Thereafter, 200 µL of chloroform-isoamylalcohol (24:1) was added to each sample, inverted twice to mix and centrifuged at 3000 rpm for 10 min. The aqueous layer was transferred to a fresh 96 strip-well plate, 315µL ethanol-acetate solution (30mL EtOH, 1.5mL 3 M NaOAc [pH 5.2]) was added to each sample, placed at –20°C for 1-2 hours or overnight and centrifuged at 3000 rpm for 10 min. Supernatant was decanted from each sample and pellets washed with 70% EtOH, centrifuged at 2500 for 10 min, supernatant was decant from each sample, the pellet was air-dried for 1 hour and suspended in 100 µL low-salt TE (50 µL if the pellet was small).

The amount and quality of genomic DNA were determined using Nano drop and on 1% agarose gel electrophoresis.

5.2.3 Genotyping

Gradient PCR was used to optimize conditions for the 44 fluorescent-labelled SSR markers obtained from AVRDC. A gradient of 11 temperatures from 50 to 60 ^{o}C was tested on two samples. All markers amplified SSR loci except five that were therefore excluded from the study. Of the remaining 39 markers, only 36 markers were used for amplification. The PCR conditions were initial denaturation at 95^{o}C for 5 minutes followed by 35 cycles of 94^{o}C for 30 seconds, annealing for 1 minute (Table 5.5) and extension at 72 ^{o}C for 1 minute and final extension at 72 ^{o}C for 20

min. PCR products were examined on 2% agarose gels. Sets of 4 markers each with a different colour labels, were co-loaded and genotyped using the Genetic analyzer ABI 3730, (Applied Biosystems). Alleles were called using GeneMapper version 4.1 (Applied Biosystems). Out of the 36 markers eight were excluded because of unreliable peaks (AVRDC-PP17 and AVRDC-PP68) or having more than 10% missing data (AVRDC-PP83, AVRDC-PP86, AVRDC-PP117, AVRDC-PP135, AVRDC-PP137 and AVRDC-PP160) while the remaining 28 markers proceeded to statistical analysis (Table 5.5).

5.2.4 Data Analysis

PowerMarker (Liu, 2001-2004) was used to determine gene diversity, heterozygosity, polymorphic information content, number of alleles in each marker and allele frequency. Genetic distance, Analysis of molecular variance (AMOVA), correlation, genetic dissimilarity and number of effective emigrants was calculated using GeneAlex (Peakall and Smouse, 2012). Darwin (Perrier and Jacquemoud-Collet, 2006) was used for clustering the genotypes in a dendrogram or tree. STRUCTURE version 2.3.4 (Pritchard et.al, 2012) was used for a model-based clustering for inferring population structure using genotype data. For inferring, number of populations (K) was set from 1 to 15 and the program ran 20 replicates for each K value at 10,000 burning speed and 50,000 Markov Chain Monte Carlo (MCMC) cycles. This was used for identifying the range of the true K value around 3. In addition, the program was run with setting K value 1 to 6 and a replicate of 20 for each K at burning in 500,000 repeats and MCMC replication of 750,000. K value was inferred using the L(K) method which is determined by identifying the point where the plateau starts (Rosenberg et al. 2001) and confirmed by an ad hoc value (ΔK) calculated using the below formula (Evanno et al., 2005).

L(K) = an average of 20 values of Ln P(D),

L'(K) = L(K)n – L(K)n-1,

L''(K) = L'(K)n – L'(K)n-1 and

$\Delta K = [L''(K)]/Stdv.$

Percentage of ancestry of the different collection areas to the three clusters was determined by counting the number of individuals scored 0.60 or greater membership proportion in a given cluster divided to the total number of the collection area. Then average membership was calculated by summing up the proportions of all individuals and divideding it by the number of individuals (Prichard et al., 2000).

Table 5.2: Characteristics of the 28 markers used for the study

S.N.	Name	Forward primer	Reverse primer	Amplicon length	SSR Motif	Ann. T. ^{o}C	Linkage group
1	AVRDC-PP24	AAAGCATGAAATCACCCTCC	CGGCAAGAAGATGAAAGTCA	126	(AT)18	52	NotKn
2	AGi096	GGGAAGAGAAATTGTGAAAGCA	ATGCCAACAATGGCATCCTA	160	[CAT]7	58	NotKn
3	AGi101	TGAGGAGACAAACTTCAACTGG	GATGAGGACAAAACCAAGGACT	181	[TCA]14	58	NotKn
4	AGi121	AACACGCCAAGAAAATCATC	TGGAGACCTGAGCCATTG	162	[CA]15	55	NotKn
5	AVRDC-PP120	CGAATCAGCAAGGAGATCAA	TCAGCAGAAGCCATAATTGG	378	(TAA)12	55	NotKn
6	AVRDC-PP121	GCGGCCTTTTGATTCATAC	AACACCAGTGCTTGTCGTGT	219	(AT)10	55	NotKn
7	AVRDC-PP126	GCAGTTGATATCGCCTCCAT	TGCACATTTCGAATCTAGGG	393	(AT)9	55	NotKn
8	AVRDC-PP128	ATCGATCCAGAGGTGAATCC	TGTACTTCCATCCTCCACAA	233	(TA)12	58	NotKn
9	AVRDC-PP129	AAGAGCTTCACGGGATCACT	CAGCCATTTCTGCTGTAGGA	374	(TCT)8	55	NotKn
10	AVRDC-PP133	TCAGTGGTGGTGTTGGAGTT	CAACATGCATCCAGCTTCTT	301	(AT)9	58	NotKn
11	AVRDC-PP144	TCCTCAGACACAAAATCCCA	CGGGGATTGCTTAGTTGTTT	182	(CA)14	58	NotKn
12	AVRDC-PP146	AGCAGAATTTTTCCACCCTG	GCATTGATGGTGAAGATTGG	208	(CT)17	55	NotKn
13	AVRDC-PP147	TTTCGCCAAGACTTGTTCTG	AAACGTGACCAACAACCTCA	261	(CT)11	55	NotKn
14	AVRDC-PP155	GGAGACAAACTTCAACTGGTCA	GCAGATGCAGCAACAGATTT	162, 158	(CAT)9, (TCA)13	55	NotKn

NotKn= Not known

Table 5.2 Cont…

S.N.	Name	Forward primer	Reverse primer	Amplicon length	SSR Motif	Ann. T. ^{o}C	Linkage group
15	AVRDC-PP19	GGGTGTCAAGAAATCACACG	AGATACGTATGTGGCCTCTGT	119	(AT)12	55	NotKn
16	AVRDC-PP37	GCACGAGGAAGACTTGACAG	TGTGCATAGGTGCAGATTGA	150	(AT)11	55	NotKn
17	AVRDC-PP49	AGGGTTTGACACTGGGAAAG	CGAGCTCGATGAGGATGAAC	140	(AGC)8	55	NotKn
18	AVRDC-PP5	GCATCAACCAGCAGCATACTA	TTTGTTTCGTGAAGTGCTCC	180	(TA)11	55	NotKn
19	AVRDC-PP67	TATTCCTTCTTCACCCCTCC	GAAAGAGGCGCTAACTGGAC	197	(AT)13	55	NotKn
20	AVRDC-PP87	AGCAGCAACTCTAACCACCA	CAGATGAGCCAGTGAGCATT	238	(AAC)10	55	NotKn
21	AVRDC-PP88	AGTAGCTCCATCGCCAGTTT	TCGAAAGACAACTCCATCGT	114	(CAA)8	55	NotKn
22	AVRDC-PP95	CGTCTTTCACTTGTCTTTTGTTC	AGTGGGTTCACTGACTTGGG	90	(CTT)3(CAT)9	55	NotKn
23	CA526211	AAGTGTCAAGGAAGGGGACA	CCTAACCACCCCCAAAAGTT	243	(AGT)14A(GAA)9	55	NotKn
24	CA519548	TCTCTCTCTACATCTCTCCGTTG	TGTCGTTCGTCGACGTACTC	233	(CTT)12	55	5
25	CA524065	GGAAACTAAACACACTTTCTCTCTC	ACTGGACGCCAGTTTGATTC	196	(CT)14(CA)9GA(CA)4GA(CA)4	55	11
26	BM59622	ACGCCAAGAAAATCATCTCC	CCATTGCTGAAGAAAATGGG	147	(CA)15	55	3
27	CAMS-855	TCGAACAAATGGGTCATGTG	GATGAGGGTCCTGTGCTACC	176-220	(ATT)5T(TTA)7	55	2
28	GPMS 169	TCGTATTGGCTTGTGATTTACCG	TTGAATCGAATACCCGCAGGAG	205	(CT)17(CA)5A21	55	9

NotKn= Not known

5.3 Results

5.3.1 Allelic analysis

Collections from different sub-regions and institutions varied in total number of alleles, mean number of alleles per locus and number of private alleles. The highest number of alleles per population (Na) and highest mean number of alleles per locus (MNa) was found in Gindae (228 and 8.14 respectively) followed by Mendefera (190 and 6.96), while the lowest numbers were found in population KALRO1 (63 and 2.25). The AVRDC Population had the highest number (20) of private alleles, followed by Gindae (18) and Mendefera (17), while the lowest number of private allele were 1 (found in KALRO1) and two alleles (found in Elabered, Dubarwa and Akurdat). The populations showed high % polymorphic loci. It was 100% in seven populations, while in the remaining five populations it ranged from 82.14 in KALRO1 to 96.43 in Dubarwa (Table 5.3).

Table 5. 3: Allelic distribution within collections of different sub-regions and institutions

Population	N	Na	Na Freq. >= 5%	MNa	NPA	% PL
Elabered	32	139	87	4.96	2	100.00
Dekemhare	18	148	114	5.29	3	100.00
Mendefera	36	190	105	6.79	17	100.00
Dubarwa	18	111	90	3.96	2	96.43
Gindae	82	228	104	8.14	18	100.00
Akurdat	7	94	94	3.36	2	89.29
Afabet	58	187	104	6.68	15	100.00
NARI	14	110	88	3.93	3	89.29
HAC	83	169	95	6.04	13	100.00
AVRDC	48	182	115	6.50	20	100.00
KALRO1	3	63	63	2.25	1	82.14
KALRO2	8	92	92	3.29	9	92.86

***N**= Population size **Na**= Total number of alleles per populations **MNa**= Mean number of alleles per marker **NPA**=Number of private alleles per population **% PL**= % polymorphic loci .*

total of 352 alleles were detected. The number of alleles per locus (Na) ranged from 6 in AVRDC-PP49 and AVRDC-PP129 to 48 alleles in AVRDC-PP147. Thirteen markers revealed 6 to 10 alleles and 6 revealed 15 to 48 alleles. Average number of

alleles per marker was 13. Mean major allele frequency (MAF) ranged from 0.17 (AVRDC-PP67) to 0.93 (AVRDC-PP129) with an average of 0.48 (Table 5.4). Mean polymorphic information content (PIC) ranged from 0.13 for AVRDC-PP129 to 0.89 for AVRDC-PP67 with an average of 0.62. Only 3 markers (AVRDC-PP129, AVRDC-PP146 and CA519548) of the 28 had PIC value of less than 0.5, while 10 markers had 0.7 or greater PIC value (Table 5.4).

Table 5.4: Genetic diversity in the collections revealed by the 28 SSR markers

Marker	Na	MAF	GD	Ho	PIC
AVRDC-PP95	19	0.58	0.62	0.46	0.59
AVRDC-PP128	10	0.35	0.77	0.53	0.74
AGi101	12	0.30	0.80	0.57	0.78
AVRDC-PP49	6	0.46	0.67	0.60	0.61
AGi121	11	0.48	0.70	0.35	0.66
CAMS-855	9	0.56	0.62	0.36	0.58
CA526211	11	0.29	0.79	0.54	0.76
AVRDC-PP133	10	0.48	0.68	0.24	0.64
AVRDC-PP121	12	0.65	0.55	0.51	0.53
BM59622	11	0.42	0.71	0.37	0.66
AVRDC-PP37	9	0.45	0.72	0.41	0.69
AVRDC-PP87	13	0.52	0.67	0.31	0.64
CA524065	7	0.49	0.68	0.25	0.64
GPMS-169	8	0.39	0.74	0.36	0.70
AVRDC-PP126	14	0.38	0.79	0.43	0.76
CA519548	7	0.81	0.33	0.13	0.31
AGI096	12	0.37	0.72	0.31	0.68
AVRDC-PP24	15	0.65	0.55	0.27	0.52
AVRDC-PP129	6	0.93	0.13	0.04	0.13
AVRDC-PP146	7	0.85	0.26	0.10	0.24
AVRDC-PP155	15	0.30	0.79	0.68	0.76
AVRDC-PP88	13	0.39	0.74	0.52	0.70
AVRDC-PP5	8	0.65	0.54	0.54	0.51
AVRDC-PP19	7	0.54	0.58	0.34	0.50
AVRDC-PP144	9	0.38	0.68	0.42	0.63
AVRDC-PP67	20	0.17	0.89	0.51	0.89
AVRDC-PP120	23	0.26	0.81	0.46	0.78
AVRDC-PP147	48	0.32	0.79	0.87	0.76
Mean	13	0.48	0.65	0.41	0.62

Na=Nuber of alleles MAF= Major allele frequency GD= Gene diversity
Ho= Observed heterozygosity PIC= Polymorphic information content

Table 5.5 shows partitioning of the molecular variance of the collections under study, 10% of the variation occurred among the collection areas, while 30 and 60 % occurred among collections and within collections. The variation in all three partitions was highly significant (P< 0.001).

Table 5.5: Summary AMOVA showing the variability patterns of the collection relative to populations

Source	df	SS	MS	Est. Var.	%	P
Among collection areas	11	791.529	71.957	0.950	10%	0.001
Among collections	395	4438.146	11.236	2.779	30%	0.001
Within collections	406	2310.500	5.677	5.677	60%	0.001
Total	812	7540.174		9.406	100%	

5.3.2 Similarility within and between collections of different areas

Genetic distance among collections from different areas was determined using Nei genetic distance (Nei 1972) matrix (Table 5.6). The KALRO collections that included 3 peppers (KALRO1) and 8 tomatoes (KALRO2) were the most distant compared to almost all other collections. The outgroup collection, KALRO2, with mean distance of 0.83 and ranging from 0.70 – 0.95 showed the highest genetic distance, followed by KALRO1 with mean 0.39 and ranged from 0.270 – 0.825. Among the Eritrean collection areas Mendefera with mean genetic distance 0.36 was the most distant to KALRO2 (0.95). It showed also a relatively high genetic distance with all other collections except Gindae (0.12) and Dekemhare (0.11). Similarly Akurdat with mean genetic distance 0.346 was the most distant to KALRO1 (0.46) and showed a relatively high genetic distance with all other collections except Afabet (0.13), HAC (0.17) and NARI (0.20). Afabet with the lowest mean genetic distance (0.22) showed also the lowest genetic distance with almost all collections ranging from 0.06-0.70. Low dissimilarity was observed in three groups; these are Elabered, Dbarwa and Gindae (0.07, 0.09 and 0.11), Dekemhare, Mendefera and Gindae (0.11, 0.07 and 0.12) and Afabet, NARI and HAC (0.08, 0.06 and 0.01). The last group showed also the lowest distant to AVRDC (0.15, 0.16 and 0.13) among the Eritrean collections (Table 5.6).

Table 5. 6: Pairwise Matrix of Nei Genetic Distance among the collections

Elb	Dk	Men	Db	Gin	Ak	Af	NARI	HAC	AVRDC	KALRO1	KALRO2		Mean †
0.00												Elb	0.29
0.18	0.00											Dk	0.27
0.31	0.11	0.00										Men	**0.36**
0.07	0.24	0.34	0.00									Db	0.31
0.09	0.09	0.12	0.11	0.00								Gin	**0.23**
0.41	0.25	0.31	0.45	0.26	0.00							Ak	**0.35**
0.20	0.18	0.30	0.20	0.14	0.13	0.00						Af	**0.22**
0.23	0.21	0.34	0.26	0.21	0.2	0.08	0.00					NARI	0.26
0.22	0.22	0.40	0.27	0.22	0.17	0.06	0.07	0.00				HAC	0.25
0.21	0.23	0.30	0.26	0.20	0.27	0.15	0.16	0.13	0.00			AVRDC	0.27
0.31	0.35	0.46	0.34	0.32	0.46	0.3	0.35	0.29	0.27	0.00		KALRO1	0.39
0.94	0.91	0.95	0.86	0.81	0.91	0.70	0.76	0.71	0.77	0.83	0.00	KALRO2	**0.83**

Elb=Elabered Dk=Dekemhare Men=Mendefera Db=Dubarwa Gin=Gindae Ak=Akurdat Af=Afabet

† Mean distance of a population was determined by dividing the sum of all pairwise distances of a particular population divided by 11 which is the number of populations paired with the concerned population.

5.3.3 Cluster analysis of the collections

The neiboughr joining clustering method using dissimilarity distance matrix, (Fig. 5.1) showed grouping of the collections into three main clusters. Materials collected from farmers in Eritrea separated into two clusters. Cluster 1 was mainly composed of two sub-clusters. Sub-cluster 1 was the largest and composed of collections from Gindae, Elabered and Dubarwa in addition to collections from Afabet and few from Mendefera and Dekemhare. Sub-cluster 2 was smaller and composed collections from AVRDC, four from HAC and two from NARI, in addition to farmer collection from Elabered (4), Dkemhare (4), Gindae (2) and Mendefera (2). AVRDC materials in this sub-cluster included 5 Ethiopian, 3 Italian, 2 Mexican and 2 improved varieties. Likewise, Cluster 2 was composed of two sub-clusters of which sub-cluster 1 was the largest and included collections from Gindae, Mendefera and Dekemhare, in addition to 10 from Afabet and 3 from Akurdat. KALRO2 (the outgroup) formed a distinct group in sub-cluster 2 that included four improved AVRDC varieties and one from each Mexico and Ethiopia in addition to one genotype from KALRO1 and a few local genotypes (Fig 5.1).

Cluster 3 comprised four sub-clusters. The first sub-cluster was the major sub-cluster composed of collections from HAC, NARI and Afabet, in addition to few collections from AVRDC, KALRO1 and Dekemhare. The second sub-cluster comprised 11 collections from Afabet, two from NARI and each one from Gindae and Elabered. The third sub-cluster is composed of 23 collections from AVRDC and only two local genotypes from Dubarwa and Dekenhare. The AVRDC germplasm in this sub-cluster included all the Indian and the majority of the Italian accessions. HAC also formed a small sub-cluster composed of five collections (Fig 5.1).

The ad hoc value ΔK ran from Structure showed high likelihood value for existence of three clusters (Figure 5.2) as previously inferred the neighbour joining tree (Figure 5.1). The assignment of collections into the three clusters was slightly different in the

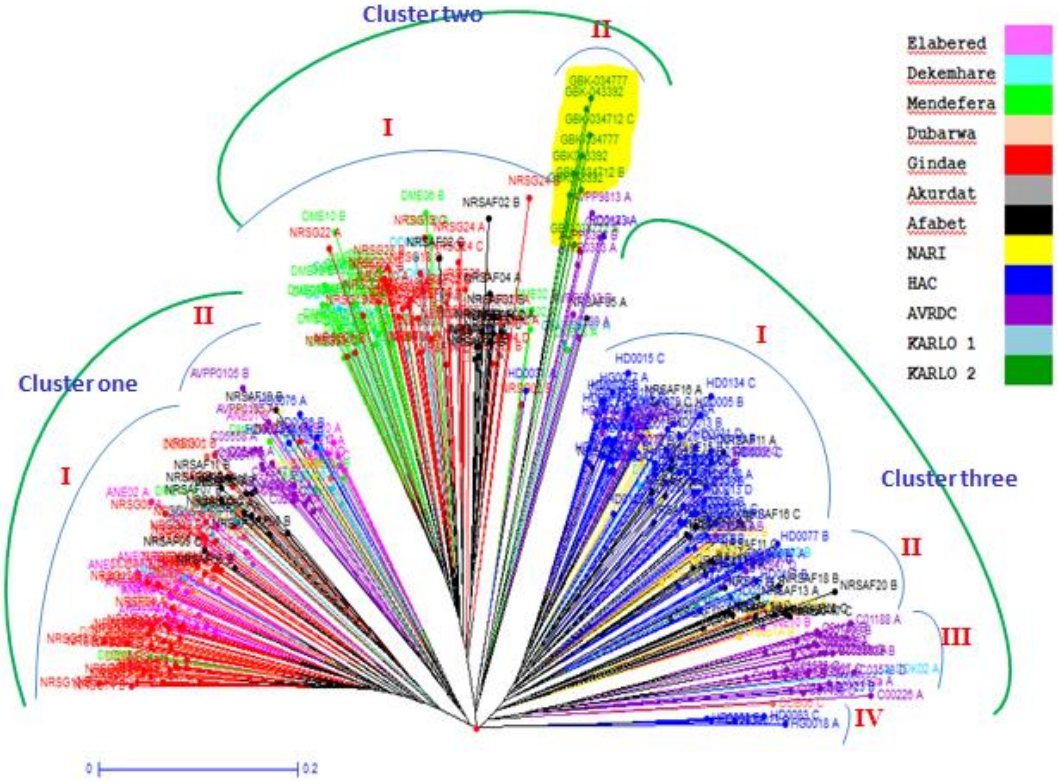

Figure 5.1: Neighbour joining tree showing clustering of the collections

tree constructed from dissimilarity matrix using DarWin in which only 62.5 % of AVRDC collections inferred into cluster 3 compared to 90% according to the model based method (Figure 5.2). Figure 5.2 shows that at least 90% of the genotypes from the three institutions viz HAC (97%), NARI (93.5%) and AVRDC (90%) inferred together in cluster 3. Only 3% from HAC and 8% each from NARI and AVRDC inferred in cluster 1. According to the model based structure, collections from farmers in Afabet were the only group that had most collections (64%) inferred in cluster 3 while 13% and 22% were inferred to clusters 1 and 2 respectively. Most of the materials from Dbarwa and Elabered (85 and 80% respectively) inferred to cluster 1,while 15 and 19% from the previous areas were inferred to cluster 3. Majority of collections from Gindae and Akurdat inferred to clusters 1 and 2 and cluster 2 and 3 respectively. Only 7% of collections from Gindae inferred to cluster 3 and 1% from Akurdat inferred to cluster 1. Half of the Dekemhare collections was inferred in cluster 2, while the remaining 50% was equally divided between cluster 1 and 3 (Table 5.2). Cluster 1 consisted of 86 collections from sub-regions Gindae

(39), Elabered (22) and Dbarwa (15) and few collections from Mendefera (4), Dekemhare (2), Afabet (3) and AVRDC (1). An admixture group of 36 collections was also found in this group. Cluster 2, included 96 collections, basically composed of materials collected from Gindae (37) and Mendefra (26), in addition to 12 from

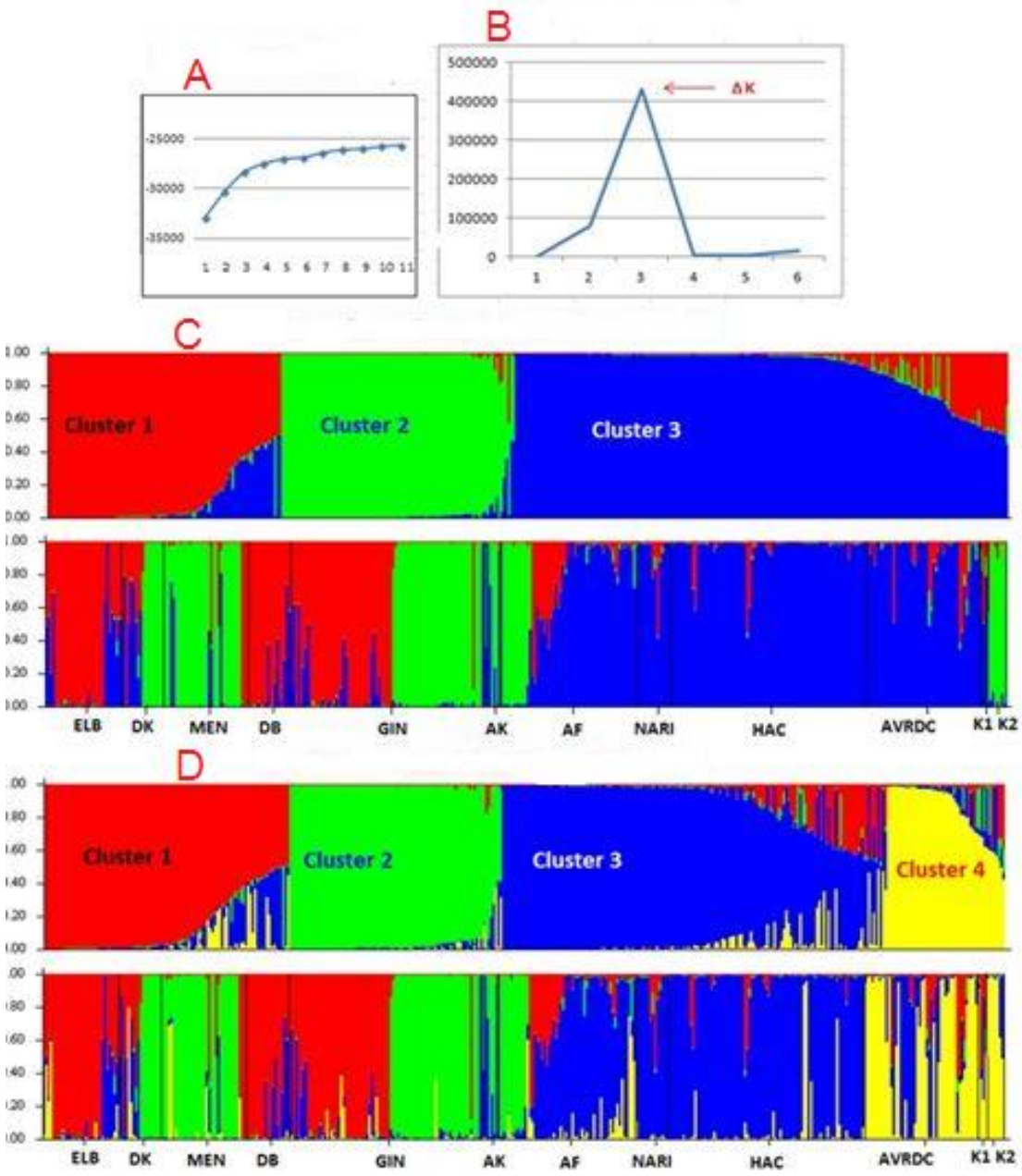

Figure 5.2: Model-based clustering of the collections using 28 SSR markers showing the number of clusters by the plateau method (A), the delta K method (B) , inferred 3 clusters (C) and inferred 4 clusters (D)

Afabet, 9 from Dekemhare and 4 from Akurdat. This cluster also contained all the 8 tomato outgroup genotypes. The admixed group of this cluster was very small compared to the other two clusters. Cluster 3 was the largest and comprised 188 collections and an admixed group of 36 individuals. This cluster comprised genotypes from the 11 collection areas, however, the major constituents are materials from HAC, AVRDC, NARI and sub-region Afabet. Of the total number of materials collected from HAC, 96% (80) inferred to this cluster. Similarly, 93% (13) from NARI and 88% (42) from AVRDC and 60% (35) from Afabet inferred to this cluster. Other collections included 3 each from Elabered, Dekemhara, Gindae, Akurdat and Mendefera, 2 from KALRO1 and 1 from Dbarwa.

Expanding the number of clusters to 4 resulted in moving 63% of AVRDC, 51% of KALRO1 and 82% of KALRO2 collections to form its own cluster. Ancestry of the AVRDC and KALRO1 collections in cluster 1 remained almost similar, 9% for AVRDC and 24% for KALRO1 compared to the previous 8% and 21% respectively, while for KALRO2 it declined from 7% to 4%. However, both AVRDC and KALRO1 maintained strong ancestry in cluster 3 (27% and 24% respectively), while for KALRO2 the average proportion was lower (11%). Apart from this, the remaining populations clustered the same in the three clusters (Table 5.7). Raising the number of clusters beyond 4 showed no remarkable change.

Table 5.7: Proportion of each collection area in each of the 3 or 4 inferred clusters

Collection area	Number of individuals	Inferred clusters 3			Inferred clusters 4			
		1	2	3	1	2	3	4
Elb	32	0.80	0.01	0.19	0.80	0.01	0.14	0.06
Dk	18	0.25	0.50	0.26	0.24	0.50	0.20	0.06
Men	36	0.12	0.79	0.09	0.12	0.79	0.03	0.06
Db	18	0.85	0.01	0.15	0.83	0.01	0.01	0.16
Gin	82	0.48	0.45	0.07	0.48	0.44	0.06	0.03
Ak	7	0.01	0.52	0.47	0.01	0.50	0.48	0.01
Af	58	0.13	0.22	0.64	0.13	0.22	0.57	0.09
NARI	14	0.08	0.01	0.91	0.09	0.02	0.88	0.02
HAC	83	0.03	0.01	0.97	0.03	0.01	0.91	0.06
AVRDC	48	0.08	0.02	0.90	0.09	0.01	0.27	0.63
KALRO1	3	0.21	0.09	0.70	0.24	0.02	0.24	0.51
KALRO2	8	0.07	0.89	0.04	0.04	0.02	0.11	0.82

Elb=Elabered Dk=Dekemhare Men=Mendefera Db=Dubarwa
Gin=Gindae Ak=Akurdat Af=Afabet

Based on membership proportion of 0.60 or greater, percentage of collections assigned to each cluster and their ancestry varied from given collection area to another. Frequency of collections from Elabered, Dbarwa and Gindae assigned to cluster 1 was 69%, 83% and 48% at an average membership proportion of 0.98, 0.94 and 0.94 respectively. The remaining collections were assigned to either cluster 2 or 3 or have parents from each (Table 5.18). Frequency of collections from Mendefera, Dekemhare, Gindae, Akurdat and KALRO2 assigned to cluster 2 was 75%, 50%, 45%, 57% and 87.5% at membership proportion of 0.99, 0.97, 0.98, 0.84 and 0.94 respectively. Similar to the above the remaining collections were assigned to either cluster 1 or 3 or have one parent from each. Frequency of collections from Afabet, NARI, HAC, AVRDC and KALRO1 was 60%, 93%, 96%, 88% and 66% at membership proportion of 0.92, 0.95, 0.98 0.96 and 0.80 respectively with the remaining of collections in each population assigned to cluster 1 or 2 or have one parent from each (Table 5.8).

Average number of effective individual migrants (Nm) for the total population was relatively high (2.25). The pairwise population Nm (Table 9) ranged from 0.98 between Dbarwa and Akurdat to 10.45 between Dekemhare and Gindae (excluding the tomato, KALRO2). Overall, gene flow among collection areas in the same cluster was very high compared to among collection areas in different clusters (Table 5.9)

Table 5.8: Percentage of collections ancestry from the given collection areas into the inferred 3 clusters

Pop	N	% Ind in C1 MP > 60%	Ave. MP in C1	Ave. MP in C2	Ave. MP in C3	% Ind in C2 MP > 60%	Ave. MP in C1	Ave. MP in C2	Ave. MP in C3	% Ind in C3 MP > 60%	Ave. MP in C1	Ave. MP in C2	Ave. MP in C3	% Ind in MP >40 & <60%	Ave. MP in C1	Ave. MP in C2	Ave. MP in C3
ElB	32	69	0.975	0.005	0.021	0	0.000	0.000	0.000	9	0.224	0.01	0.766	22	0.489	0.022	0.489
DK	18	11	0.83	0.08	0.1	50	0.023	0.972	0.004	17	0.225	0.01	0.765	22	0.465	0.009	0.527
Me	36	11	0.9	0.008	0.097	75	0.005	0.991	0.004	8	0.029	0.23	0.744	6	0.264	0.501	0.235
DB	18	83	0.935	0.004	0.060	0	0.000	0.000	0.000	6	0.258	0.004	0.738	11	0.496	0.010	0.495
Gin	82	48	0.940	0.007	0.053	45	0.009	0.981	0.01	3.7	0.252	0.01	0.742	3.7	0.543	0.007	0.450
AK	7	0	0.000	0.000	0.000	57	0.004	0.842	0.155	43	0.023	0.09	0.885	0	0.000	0.000	0.000
AF	58	5	0.770	0.005	0.225	21	0.007	0.966	0.027	60	0.061	0.02	0.918	14	0.403	0.069	0.528
NARI	14	0	0.000	0.000	0.000	0	0.000	0.000	0.000	93	0.037	0.01	0.948	7	0.566	0.015	0.418
HAC	83	0	0.000	0.000	0.000	0	0.000	0.000	0.000	96	0.014	0.01	0.981	4	0.449	0.017	0.534
AVRDC	48	2	0.61	0.04	0.35	0	0.000	0.000	0.000	88	0.030	0.01	0.957	10	0.410	0.065	0.525
KALRO1	3	0	0.000	0.000	0.000	0	0.000	0.000	0.000	66.7	0.066	0.139	0.796	33.3	0.491	0.004	0.505
KALRO2	8	0	0.000	0.000	0.000	87.5	0.023	0.938	0.039	0	0.000	0.000	0.000	12.5	0.426	0.552	0.022

Ind= Individuals MP=Membership proportion C= Cluster

Table 5. 9: Pairwise population Nm values based on Fst values

Elb	Dk	Men	Db	Gin	Ak	Af	NARI	HAC	AVRDC	KALRO1	KALRO2	
0.00												Elb
2.83	0.00											Dk
1.41	**6.39**	0.00										Men
9.78	2.21	1.36	0.00									Db
5.28	**10.45**	**4.61**	**5.48**	0.00								Gin
1.01	2.46	1.60	**0.98**	2.21	0.00							Ak
2.17	3.06	1.58	2.29	3.52	4.91	0.00						Af
1.78	2.65	1.35	1.63	2.45	2.39	**8.03**	0.00					NARI
1.73	2.14	1.11	1.50	2.02	2.77	**8.00**	**8.14**	0.00				HAC
2.27	2.78	1.75	1.99	2.79	2.13	3.50	3.40	3.56	0.00			AVRDC
1.86	2.26	1.26	1.69	2.41	1.02	2.28	1.47	2.01	3.74	0.00		KALRO1
0.49	**0.65**	**0.57**	**0.53**	**0.68**	**0.51**	**0.66**	**0.54**	**0.57**	**0.69**	**0.58**	0.00	KALRO2

Elb=Elabered Dk=Dekemhare Men=Mendefera Db=Dubarwa Gin=Gindae Ak=Akurdat Af=Afabet

5.4 Discussion

5.4.1 Genetic diversity

Genetic diversity parameters showed that the set of SSR markers used in this study were similar or more informative compared to markers used in many previous studies in pepper and other crops. Of the amplified markers 97.2% were polymorphic and only one marker (AVRDC PP117) was monomorphic. This polymorphism rate was similar to the results reported by Oh et al. (2012) in pepper and QI-Lun et al. (2008) in maize who used 22 and 45 SSR markers and obtained 100% and 96.3% polymorphism respectively. However, it was higher compared to many previous studies in pepper by Dhaliwal et al. (2014) who used 50 markers, Rai et al. (2013) using 103 markers and Kwon et al. (2005) using 316 markers who found 54%, 24.3% and 8.5% of polymorphism respectively. In other crops, Munoz-Falcón et al. (2011) used 17 genomic SSRs in eggplant and obtained 82.3% polymorphism and Sajib et al. (2012) used 24 SSR markers in rice, and obtained 37.5% polymorphism.

Diversity indicators obtained from the 28 markers of the current study, viz allele richness, polymorphic information content (PIC), and Observed heterozygosity (H_o) were similar or much higher compared to many previous studies using SSR and other markers in pepper and other crops. Total number of alleles (352) and average number of alleles per marker (13) obtained in this study were slightly higher compared to that reported by González-Pérez et al. (2014) who analyzed 39 markers and obtained a total of 381 alleles and an average of 9.8 alleles per locus, but much higher compared to Dhaliwal et al. (2014) and Oh et al. (2012), who reported average number of alleles per marker and total number of alleles in pepper to be 75 and 2.78 and 29 and 3.22 respectively. Similarly, Sow et al. (2014) reported in rice 178, and 9.89. However the results were lower compared to average number of alleles (18.21) reported in pepper by Nicolai et al. (2013) and total number and average number of alleles reported in grape (499 and 22.68) by Emanuelli et al. (2013). These differences could be due to the set of samples used in the current study was smaller compared to the last two reports (González-Pérez et al. 2014).

Mean and range of PIC recorded by the current study was 0.62 and 0.13-0.89 respectively. This was similar to previous reports of SSR markers in pepper (González-Pérez et al., 2014; Dhaliwal et al., 2014; Rai et al., 2013; and Lee et al., 2004) and in rice (Sow et al., 2013). However, the results of the current study are higher than reports in pepper (Oh et al., 2012 and Hanáček et al., 2009). PIC values take into account the number of alleles and their distribution, thus determine informativeness of markers (González-Pérez et al. 2014). In the current study only three markers showed PIC value less than 0.5 indicating the set of SSR markers used were highly informative. Average observed heterozygosity (Ho) of the current study was 0.41. This was comparable to the reports of Oh et al. (2012) in pepper and QI-Lun et al. (2008) in maize, and higher than SSR in pepper (Nicholaï et al., 2013 and Ibiza et al., 2012) and rice (Sow et al., 2014). Ibiza et al. (2012) justified the low Ho obtained in their study by tendency of *Capsicum* species to self-pollinate. However, since out-cross in *Capsicum* is very high and pepper in Eritrea is mainly produced by small holding farmers who grow different varieties in close proximity to each other which favours cross-pollination; the high Ho obtained in the current study is justified.

Variation among populations was relatively low (10%) and most variation in the total population was within individuals (60%) and among individuals within populations (30%). This was similar to the 12% among populations reported by Tesfamichael et al. (2014) studying Eritrean sorghum landraces but in contrast to the 31% and 57% within individuals and among respectively reported in the same study. The results also are in contrast with the findings of Backes et al. (2009) who reported within field variation in Eritrean barley to be 97.3 %. There is no documentation on when pepper was introduced it Eritrea, but oral reports indicate that it has been grown for a very long time. Selection of superior genotypes, carrying it over generations and acquiring seeds from trusted farmers within the village or distant places are common practices of pepper growers in Eritrea. This gives a chance for variation to be selected and fixed which may explain the relative high heterozygosity and availability of rare alleles (Table 5.3) leading to existence of significant amount of diversity within Eritrean pepper germplasm. This was in contrast to the deficiency of

heterozygosity (Fis=0.7533) reported by Rodrigues and Tam (2010) for *Capsicum frutescens* varieties cultivated in isolation within the island ecosystem of Borneo, Malaysia.

5.4.2 Pair wise Genetic dissimilarity among collection areas

Generally, the results of the current study show low dissimilarity among collections compared to the results of Geleta et al. (2005) who compared Ethiopian pepper genotypes with each other and with genotypes from AVRDC and other countries using AFLP markers. In the current study, two groups appeared to have low genetic distance (Table 5.6) and high average number of effective individual migrants (Table 5.9). The first group is composed of collections form Afabet, HAC and NARI. The genetic distance between collections from Afabet and HAC was 0.056, Afabet and NARI was 0.079 and HAC and NARI was 0.071. This group was closer to the AVRDC collections than to the rest of the Eritrean collections. Compared to the other collections, this group also showed closest distance with the KALRO1 and the outgroup KALRO2 (tomato) collections. The most probable reason for the close genetic distance among collections from the three areas (Afabet, HAC and NARI) is that breeding lines from NARI are from local sources and served as source of seed for Afabet. Later on, breeding program started at HAC and benefited from the available local sources. This is evident from the high Nm levels among the three collection areas that ranged from 7.976 between Afabet and HAC to 8.144 between NARI and HAC (Table 5.9). The second group is composed of collection of Gindae, Dbarwa, Medefera, Dekemhare and Elabered, with genetic distance ranging from 0.086 between Gindae and Dekemhare to 0.117 between Gindae and Mendefera. This group had higher distance with AVRDC ranging 0.20-0.302 with Gindae and Mendefera respectively. Compared to the previous group, this group was also highly dissimilar with both KALRO1 and KALRO2. The close distance in this group could be due to intensive seed exchange among farmers in these areas. The high level of immigration observed among these collection areas expressed by the Nm is evidence for that. The highest Nm was 10.499 between Dekemhare and Gindae followed by Nm= 9.777 between Elabered and Dubarwa (Table 5.9).

5.4.3 Cluster analysis

Based on the Neighbor-joining clustering and the model- based program (STRUCTURE), the collections under study were grouped into three clusters, and increasing the number of clusters in STRUCTURE program resulted in forming a separate cluster for the non-Eritrean collections. This is in contrast to similar study conducted in Nepal where all the local accessions clustered in a single cluster (Baral & Bosland, 2002b).

González-Pérez et al. (2014) reported that grouping of genotypes from Spain was according to fruit shape and size which was not in agreement with the current study. Red long, Red short, Gahteilay, Chocolate, Group1A and Group1B are breeding lines from NARI. The first four are of triangular shaped and for dry consumption while the last two are with elongated slim fruits and used for fresh consumption. Red long, Gahteilay and chocolate are similar in fruit shape and size but differ in fruit colour. Due to the differences in fruit characteristics, it was expected that individuals for the dry consumption group would cluster together and those of fresh consumption group cluster separately or with other genotypes of similar characters. However, one variant of Red long (Red long A) and Group 1A clustered in the same sub-cluster while, the rest two variants (Red long B & C), Chocolate and Group 1B A clustered in another sub-cluster, and Gahteilay and Red short grouped in a separate sub-cluster. A similar condition was observed with HAC genotypes. HD0083A &B and HD0023B (Dry) grouped with HG0018A (Fresh) in a sub cluster far away from all other HAC genotypes. This indicates that fruit size, shape and colour had no effect in genotype clustering in the current study. The first reason could be due to SSR markers measuring genetic variation mainly in non-coding area, which has a minor impact on phenotypic characters (Kwon et al., 2005). Secondly, association between marker loci and quantitative trait loci (QTLs) is necessary for correlation between molecular and phenotypic characters (Burstin and Charcosset, 1997). Thus it is possible that the 28 markers could not capture the regions of these characters. However, since each of the NARI and HAC breeding lines are a result of mass selection, out crossing may

have happened so that genotypes of each group shared common gene pool to cluster together.

Most of the breeding lines and large number of farmer varieties from the sub-region Afabet grouped together in cluster 3. The reason could be that breeding lines in both NARI and HAC are as a result of mass selection from an original local seed source in two separate breeding programs. Afabet is a new pepper production area, farmers in this area acquired seeds from different sources, therefore lines from NARI that have been released to farmers may be found in this area and crossed with genotypes from other sources. A breeding program started at HAC using local seed sources and continued to select superior genotypes to meet market requirements. Later on, selection by farmers led to genotypes with common ancestry to be selected. Majority of the AVRDC genotypes inferred in this cluster primarily into a separate sub-cluster. Similarly, the individuals from Afabet clustered in a separate sub-cluster similar to genotypes from HAC and NARI which clustered also in smaller groups indicating of variability within the cluster (Figure 1).

Baral and Bosland (2002b), found that clustering of pepper accessions collected from diverse geographical and ecological features in Nepal did not cluster according to geographical regions. In studying *C. annuum* accessions from 89 countries Nicolaï et al. (2013) also found that geographic origin was not clearly visible in clustering genotypes. González-Pérez et al. (2014) also found partial influence of geographic origin in clustering Spanish pepper. A similar condition was observed in the current study, geographic and agro-climatic factors seem to have some influence but were not preponderant in clustering the collections from farmers. Sub-region Gindae is one of the oldest pepper growing areas. Collections from Gindae have been collected from three distinct areas, viz a midland area (*Gindae* town and *Dongolo*), a lowland area (*Damas*) and the coast. Majority of the collections from *Damas* clustered in cluster 1 with genotypes from Elabered (Midland) and Dbarwa, (highland). The three areas are distantly located in three different regions. Similarly, collections from the midland and coastal areas were inferred to cluster 2 with collections from Mendefera and Dekemhare of the Debub region. The two sub-regions are highland areas located close to each other but relatively away from Gindae. Seed exchange among these regions in the two clusters is a common practice. Therefore, acquiring seed from

common ancestry or movement of seed among the areas could be the main reason of this close relationship. The Nm values (Table 5.9) and ancestry levels of collections from these areas (Table 5.8) supports the influence of seed exchange and common ancestry factors for clustering of these materials. Clustering of collections from Mendefera and Dekemhare could be partially due to influence of geographic factors.

Afabet is an isolated area which is difficult to access. Commercial pepper production in Afabet started about 15-17 years ago. Materials from Afabet were collected from two closely distanted villages (Kibkub and Naro-Ans). All collections from Kibkub except one (NRSAF19 B) were inferred to cluster 3, while collections from Naro-Ans were equally divided into clusters 1 and 2, except two (NRSAF11 A and NRSAF11 C) which joined cluster 3. Infering the collections of Afabet into three clusters indicates they are from three ancestries. However, the relationship among these collections is better explained by the findings of Votava et al. (2005). In Afabet 85% of the farmers prefer to save and use their own seed and they grow pepper in small plots usually adjacent to each other (Table 3.7). Thus the collections that cluster together have either a relatively narrow gene pool or they are derived from the same sources, and now share sufficient genetic characteristics to cluster together due to growing in close proximity (Votava et al., 2005). The case of NRSAF19 B, NRSAF11 A and NRSAF11 C is mostly a migration situation.

One genotype (NRSG28) from the coastal area of Gindae inferred to cluster 3 at 98.9% ancestry level; this is a breeding line from the Red-long of NARI that inferred to the same cluster at ancestry level of 99%. Gahteilay, another breeding line of Red-long was also inferred to the same cluster at similar ancestry level. This indicates that the 28 SSR markers used in this study were specific enough to group closely related genotypes together at high ancestry level.

The genomes of tomato and pepper are similar, but a typical number of common types of chromosomal rearrangements differentiate the genomes of tomato and pepper (Livingstone et al., 1999). Dias et al. (2013) reported that a dendogram generated using data from 26 ISSR primers used for evaluating four pepper species and tomato as outgroup separated tomato from the pepper species. However, although *C. annuum, C. fruitiscence* and *C. chinense* belong to the same complex and *C. baccatum* to a different complex, the same dendogram clustered *C. chinense*

away from its complex , while *C. baccatum* clustered with the annuum complex. In the current study, although mean genetic distance between tomato and the other pepper populations was much greater (0.829) compared to 0.29 among the pepper populations, the eight tomato genotypes did not cluster separately from pepper, instead inferred with the pepper genotypes to cluster 2 but appeared distinct within sub-cluster II (Figure 1). The reason could be due to low number of genotypes representing the tomato so it is maintained as distinct group within the closest cluster (Nicolaï et al., 2013) or the set of 28 SSR markers were not specific enough to distinguish tomato as a separate species.

Due to the seed system and selection methods followed by farmers, high heterogeneity within cultivars is common in Eritrea for major crops. Backes et al. (2009) reported high heterogeneity within small fields of barley and described it as striking results compared to modern barley fields. Similarly Asgedom et al. (2011) found non-uniformity within tomato varieties maintained by farmers in Eritrea and mentioned it unusual for true to type varieties. In Eritrea pepper is mainly produced by small holding farmers in land size as small as 0.015 ha who usually keep their own seed by selecting the best plants in the field (Table 3.7). In the current study three individual plants were sampled from each seed sample and each analysed separately. The three individual plants clustered in different or the same cluster but none of them was identical to each other indicating heteroginity within genotype. Since pepper is a self-pollinating crop with high outcross, the high heterogeneity observed within the collections is expected. This was similarly found with Hanáček et al. (2009) in pepper, QI-Lun et al. (2008) in maize who analysed 3 and 12 samples of each cultivar or landrace respectively.

Both plateau and delta K methods and the neighbour joining clustering proved the existence of three clusters. However, Prichard et al. (2012) indicated that the aim should be targeting the smallest value of K that can capture the best structure. In the current study raising the number of clusters to 4 moved majority of the AVRDC and KALRO genotypes into a separate cluster leaving the first two clusters to be almost purely for Eritrean genotypes and the third cluster mainly for Eritrean germplasm but with some of the reference materials. This indicates the richness of genetic diversity in Eritrean pepper.

5.5 Conclusion

There is a considerable amount of variation among Eritrean pepper genotypes confirmed by the diversity parameters and clustering of collections. Absence of systematic introduction of improved varieties for long period, and continuous selection by geographically distributed farmers for more than a century may have resulted in divergent gene pools different from those observed in the reference materials, and also existence of considerable amount of private alleles unique to Eritrean collections. These alleles may be a useful source for some characteristics. Thus, future strategy for pepper breeding in Eritrea could rely on local Eritrean germplasms\ unless specific traits, not locally available.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Study of current status and future opportunities is important for designing a sound strategy for improving a crop. Thus, this study precedes the morphological and molecular characterization of Eritrean pepper so that it gives a picture of the existing conditions of the crop and its opportunity in future. A participatory rural appraisal method that included collection of secondary data, interviewing experts, focus group discussions and a formal household survey were used in this study. The results showed a great potential for pepper in Eritrea, however, constraints need to be addressed. Constraints are unavailability of improved and quality seed, inputs and support services, insect pests and diseases, small acreage, unfavourable land tenure system, improper marketing chain, poor extension service and persistent drought that affect the availability of water. Although, unavailability of improved seed was among the major constraints of pepper production, however, the results also showed that a wide range of peppers have been grown in Eritrea for a long period. These peppers are traditionally passed from generation to the next and exchanged among farmers giving chance to diversity to exist. Exploiting these diversity in improving the existing genotypes could have a significant contribution to addressing some of the major challenges in pepper production.

Understanding genetic diversity of a crop is a key step for exploiting genetic resources in breeding programs and conserving them for future utilization for the highly dynamic agriculture. The complex taxonomic status of the genus Capsicum to which pepper belongs is known for its interspecies crossability thus leading to high phenotypic variability among and within the species. Eritrean farmers have been selecting and saving their own seed and transferring it from generation to the next suggesting availability of considerable amount of variability within these germplasm. However, this diversity has never been evaluated. On the other hand to date, the gene bank in Eritrea includes no accessions of pepper conserved. Thus the materials collected from farmers and institutions could provide an opportunity to conserve valuable genetic resources. Both morphological and molecular techniques were used

for evaluating the magnitude of this diversity. Planting materials were collected from farmers in 24 villages distributed in four administrative regions and breeding lines from research institutions. Production centres, geographical distribution and agro-climatic representation were considered in collecting the materials.

Morphological characterization using 16 quantitative and 23 qualitative descriptors for capsicum was ran for evaluating 95 farmer varieties and breeding lines in two different locations. Of 95 collections 60 were tested in two sites, while the remaining 35 were evaluated only at Hamelmalo. The analysis of variance, factor analysis and clustering of the genotypes revealed the availability of morphologically distinct groups and sub-groups that are characterized by variability within the groups and sub-groups. No identical varieties were observed indicating to high variability of the materials. Cluster IV was characterized by high average yield and fruit characteristics switable for dry pepper consumption, while Cluster one was characterized by fruit characteristics more relevant for fresh consumption.

The results showed that high variability among genotypes exist for different fruit size, shape, colour, wall thickness characteristics, phonological attributes related to earliness of germination, flowering and fruiting, in addition to characteristics related to seedling stage and vegetative growth. Variability in all these traits has its agricultural and economic importance through selecting improved genotypes that can respond to the needs of farmers for high yielding and desired quality and satisfy consumer preferences. Ranking based on yield, days to flowering and three fruit quality characters, viz fruit length, fruit width and fruit wall thickness; 30 genotypes. These can be used as starting point for further selection and improvement. Yield per plant showed significant positive correlation with the closely related plant characteristics; plant high and stem thickness. Since higher plant population requires compact plant growth, this relationship is important for deciding plant population in the field. Similarly, yield per plant was negatively correlated with total soluble solids (TSS) which is important in selection for certain quality levels.

In the molecular analysis, a total of 150 seed collections including 129 from the local germplasm, 17 reference materials from AVRDC accessions representing four

countries and one pepper from Kenya were used. In addition to that three tomato accessions were added to serve as an out-group. This helped revealing the population structure of the Eritrean germplasm and its relatedness to materials from the centre of origin in Mexico and other countries that may be source of some introduced germplasm such as Italy and India and Ethiopia that shared germplasm exchange with Eritrea for long period. Since seed saving methods used by farmers in Eritrea, growing pepper in small plots in close proximity to each other and the variable out-crossing nature of pepper are factors in favour variability within variety. Three plants from each seed sample were used for evaluating the variation. Clustering of the genotypes using neighbour joining and the model based Structure data generated using 28 SSR markers confirmed that the local pepper germplasm used for this study is categorised into three groups and each group is farther divided into smaller sub-clusters indicating to the high variability of the germplasm. The clusters separated breeding lines collected from institutions and materials collected from farmers, except that materials collected from sub-region Afabet were clustered with those breeding lines of institutions suggesting common ancestry. The model based clustering also showed that increasing the number of inferred clusters into four moved most of the reference materials into a separate cluster leaving the three clusters for the Eritrean genotypes. These revealed weak ancestry of the reference materials with clusters 1 and 2 (mainly farmer varieties) and relatively strong with cluster 3 (breeding lines) confirming existence of rich genetic resource different from those reference materials. Genetic diversity parameters determine the level of variability within a population. In this study an average number of alleles of 13 and maximum of 48, average polymorphic information content of 6.2 and maximum of 8.9 and average observed heterozygosity of 4.1 was revealed by the 28 markers, indicates rich genetic diversity within the Eritrean germplasm. In addition to that the allelic distribution pattern confirmed the genetic richness with the number of private alleles revealed in the Eritrean germplasm, specially in Mendefera (17), Gindae (18), Afabet (15) and HAC (13). These are potential for obtaining unique genes for specific characters that can be utilized through breeding.

Based on the results it can be concluded that the results of this study confirmed through both morphological characterization and molecular analysis the pepper genetic resources available in Eritrea is variable enough to support reliable improvement program of the Eritrean Peppe. The following broad issues are recommended:

- Government intervension is recommended for addressing pepper production constraints. This are improved and quality seed, agricultural inputs such as pesticides, fertilizers and tools, marketing methods, land related regulations and extension services.
- The results proved availability of divergent pepper gene pool in Eritrea. Thus future pepper breeding program should utlize the local germplasm.
- Conservation and utilization of the collections made in the current study is recommended.
- Collection and genetic characterization of germplasm from the areas not reached by the current study is recommended.
- Several promising collections were identified from the morphological characterization. These are recommended for further evaluation that may lead to new improved varieties.

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APPENDICES

Appendix 1: List of the visited offices and documents or data collected

S.N.	Office visited	Documents or information collected
1.	Planning and statistics division, MoA Headquarters	- Agricultural achievements 1992-2009 - Production data for the last 10 years - Agricultural policy and strategy - Constraints and issues in crop and horticulture development
2.	Agricultural Extension Department,	Horticulture survey document (conducted 2008)
3.	Department of Customs	Report of export data
4.	National statistics office	Data of pepper consumption
5.	Department of Foreign Trade, Ministry of Trade and Industry	Documents related to export of agricultural commodities
6.	National Agricultural Research Institute	- Consultancy report on horticulture research 2004-2005 - Consultancy report on horticulture research 2005-2007 - Annual reports 2005-2011 - 5 seed samples to be utilized in the morphological and molecular characterization experiments
7.	MoA, Debub regional office	- Annual reports 2005-2011 - List of horticulture farmers in the zoba - Rainfall data 2000-2011
8.	MoA, Northern Red Sea regional office	- List of horticulture farmers in different sub-zobas
9.	MoA, Anseba regional office	Annual reports

Appendix 2: Key informants interviewed in different offices

Place	Number of interviewed experts
MoA headquarters	3
National Agricultural Research Institute	3
Southern region	10
Anseba region	2
Northern Red Sea region	5
Deseret Locust Control Organization*	1
Eritrean Sugar Corporation*	1
Total	**25**

* Pathologist and entomologist served in MoA for very long time and recently moved to other organizations.

Appendix 3: Check List for Key Informants Interview

1. What is the history of pepper growing in this zoba/sub-zoba?

2. Pepper growing places, production, cultivated area, yield per ha in this Zoba/sub-zoba.

3. In your zoba/sub-zoba, how many farmers are involved in horticulture production and how many of them involved in pepper production?

4. What is the status of pepper growing? Increasing/Decreasing

5. How do you evaluate the suitability of conditions in this zoba/sub-zoba for pepper production? Climate, Soil, availability of irrigation water, transportation facilities, availability of market, ….etc.

6. What are the most common pepper types growing in this area? (Green/Dry) What is the percentage of each?

7. What are the major varieties cultivated in this area and their origin?

8. What are the sources of seed used in this area?

9. What is the seed system followed by farmers? Do normally farmers keep their own seed?

10. What are the major problems and opportunities of pepper growing?

11. What is the common marketing chain of pepper?

12. How do you estimate profitability of green/dry pepper growing?

13. Do you think locally produced pepper can substitute the export pepper? How?

Appendix 4: Check list for group discussions

1. For how long do you think pepper have been grown in your area?

2. What is the current status of pepper growing in your area? Increasing/Decreasing

3. What is the reason for the increase/decrease?

4. What are pepper cultivars grown in your area and what are their major characteristics?

5. What are the main varieties grown in your area?

6. Do you have varieties grown in this area for long period?

7. What are the major constraints of pepper production in your area and what are the possible solutions?

8. What is the rank of pepper compared to other major horticultural crops?

9. How many times do you grow pepper per year? What are the seasons?

10. How many ha or Tsimdi you allocate for the following crops?

Tomato	Onion
Pepper	Other
Potato	

11. What are the cropping calendars for pepper?

12. What is the irrigation requirement of pepper in your area?

Times of irrigation _____

Stages of irrigation _____

13. What are the expenditures of pepper production?

Labor _____

Inputs _____

Transportation _____

14. What is the gross income obtained from pepper? _____ NKF/ha/season

15. What is the yield obtained per unit area? _____ Kg/ha or tsmdi

16. Out of the produce what percent do use for home consumption? _____

Appendix 5: Questionnaire for conducting household survey on pepper

Name of enumerator: _____Date: _____

1. Location

   Region: _____Sub-region: _____

   Village: _____Specific place: _____

   Nearest town: _____

   Distance to Nearest town: _____ Km. OR _____ hrs walk

2. Farmer (Must be head of houshold)

   Full name:_____Age:_____Gender: M [ ] F [ ] Marital status: Married: [ ] Unmarried: [ ] Divorced: [ ] Widowed: [ ]

   Education:

   Illiterate [ ] No formal education but read & write [ ] Primary school [ ] Junior school: [ ] Secondary school [ ] Other (Specify):_____

   Number of years in pepper production: _____

3. Household

3.1. Family characteristics

   Family size: Male: _____ Female: _____ Total: _____

   Number of working persons: Male: _____Female: _____ Total: _____

   Number of children in school: Male: _____Female: _____ Total: _____

3.2. Major income generating activity

   Vegetable crops [ ] Fruit crops [ ] Fruit and vegetable crops [ ]

   Cereals [ ] Cereals and vegetables [ ] Animal production [ ]

   Horticulture & Animal production (specify type and number of animals) [ ]

S.N.	Type of animal	Quantity	S.N.	Type of animal	Quantity
1			5		
2			6		
3			7		
4			8		

Other activity (specify): _____

_____

_____

4. Farm

4.1. Area

Total farm area (ha): _____Total area allocated for pepper (ha): _____

Area allocated for Green pepper (ha): _____

Area allocated for Dry pepper (ha): _____

4.2. What are the major crops in your farm and area allocated for each crop?

S.N.	Crop	Area allocated in ha	S.N.	Crop	Area allocated in ha
1	Tomato		10		
2	Potato		11		
3	Onion		12		
4	Cabbage		13		
5	Zucchini		14		
6	Carrot		15		
7	Swiss chard		16		
8			17		
9			18		

5. Cultural practices

5.1. Planting method

Direct seeding in the permanent field: [ ] Sowing in nursery and transplanting: [ ]

5.2. If you are using transplanting method, what is the source of your seedlings?

Produce your own seedlings: [ ] Purchase from other farmers: [ ]

Sometimes produce my own seedlings and sometimes purchase it form other : [ ]

Other (specify):_____

5.3. If you are sowing in nursery or purchasing seedlings from other farmers at what age you transplant the seedlings? _____ days after emergence.

5.4. How many times do you grow pepper per year?

One time: [ ] Two times: [ ]

Other (specify):_____

5.5. What are the pepper growing seasons in your region?

Season	Sowing in nursery	Land preparation	Transplanting	First harvest
First				
Second				
Third				

5.6. Land preparation

Time: _____

Number of ploughs :_____

Ploughing method: Animal driven: [ ] Tractor: [ ] By hand: [ ]

What type of ridging do you use for growing pepper?

Basins: [ ] Narrow ridges: [ ] Flat ridge: [ ]

Other (specify):_____

5.7. Plant population and staking

Do you apply specific spacing between rows and plants in row?

Yes: [ ] No: [ ] If yes specify:

Spacing between rows (cm): _____Spacing between plants in row (cm): _____

Do you apply staking on plants? Yes: [ ] No: [ ]

If yes specify:

Type: _____

Time of application: _____

5.8. Fertilizers

Do you apply organic fertilizers? Yes: [ ] No: [ ]

If yes specify:

Type: _____

Amount per ha:_____

Time of application: _____

Do you apply non-organic fertilizer? Yes: [ ] No: [ ]

If yes specify

Type: Urea: [ ] DAP: [ ] Urea and DAP: [ ]Other (Specify):_____

Amount per ha:_____

_____

Application method: Broadcast [ ] Side dressing [ ] Other (specify)

_____

_____

_____

Time of application: _____

_____

_____

_____

_____

_____

5.9. Irrigation

Do you grow pepper as irrigated or rain fed crop?

Rain fed: [ ] Irrigated: [ ] Rain fed & Irrigated: [ ]

if you irrigate specify

Source of water: Bore hole: [ ] Dam: [ ]

River: [ ] Other (specify):_____

Irrigation method: Flood: [ ] Ridge: [ ] Drip: [ ]

Other (specify): _____

How do you deliver the water to plots?

Pumped: [ ] Diversion canal: [ ]

Other (specify): _____

What is the frequency for irrigating pepper field during the dry season? _____

_____

What is the frequency for irrigating pepper field during the rainy season? _____

_____

If you use motor pump who own it?

I have my own motor pump: [ ] I share motor pump with ____ farmers: [ ]

I share central motor pump with community or village: [ ]

Other (specify):

_____

_____

If you use your own motor pump specify How many motor pumps you own:_____

What is/are the brand and horse power of your motor pump: _____

_____

_____

_____

5.10. How do you describe insect pests problem in your pepper crop for the current and last seasons

No problem [ ] Low [ ] Medium [ ]

Severe [ ]

5.11. How do you describe diseases problem in your pepper crop for the current

and last seasons

No problem [ ] Low [ ] Medium [ ]

Severe [ ]

5.12. What are the most common pests of pepper in your field

Insects	Diseases
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

5.13. What are the methods you apply for controlling insect pests and diseases?

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

5.14. How do you describe weed problem in your pepper crop for the current and last seasons?

No problem [ ] Low [ ] Medium [ ]

Severe [ ]

5.15. If you have weed problem, what control method or methods do you apply ?

Hand weeding: [ ] Cultivation: [ ] Chemical control: [ ]

Other (specify): _____

For each control method you apply specify the below details:

Hand weeding:

Type of tool used: _____

Number of weeding times:_____

Time of weeding: _____

_____

_____

_____

Cultivation:

Number of cultivations:_____

Time of cultivation:_____

_____

_____

_____

Cultivation method: Hand tools [ ] Animal driven [ ] Tractor driven [ ]

Chemical control:

Type of chemical: _____

Time of application: _____

_____

Application method:

Knapsack sprayer: [ ] Motorized sprayer: [ ] Other (specify): _____

If you use knapsack or motorized sprayer do you own it?

Yes:[ ] No: [ ]

6. Inputs

6.1. Seed

Source of seed

Keep your own seed: [ ] Get from MoA: [ ] Purchase from other farmers: [ ]

Other sources (Specify):_____

_____

_____

Price (mention the package unit):

_____

When you purchase seed how do you select the variety and quality of seed?

_____

_____

_____

_____

_____

_____

_____

_____

Do you know the name of pepper varieties you grow? Yes [ ] No [ ] if yes list down the varieties you are growing now or you have grown before.

_____

_____

_____

_____

_____

_____

_____

6.2. Fertilizer

Organic fertilizer

Source:_____

Availability: Easily available: [ ] Not easily available: [ ]

Price per truck or other (specify):_____

Mineral fertilizer

Source: MoA: [ ] Other (specify):_____

Availability: Easily available: [ ] Not easily available: [ ]

Price per KG: Urea:_____DAP: _____Other :_____

6.3. Pesticides:

Fungicide Source: MoA: [ ] Other (specify):_____

Availability: Easily available: [ ] Not easily available: [ ]

Price per KG: _____

Insecticide

Source: MoA: [ ] Other (specify):_____

Availability: Easily available: [ ] Not easily available: [ ]

Price per liter: _____

6.4. What type of pepper usually you grow?

Green pepper [ ] Dry pepper [ ] Green and Dry peppers [ ]

6.5. If your purpose is producing both green and dry peppers, how do you grow them?

Each of the green and dry pepper is produced as separate crop. [ ]

First green pepper is harvested then the crop is allowed to mature and harvested as dry [ ]

7. Labour requirement

7.1. Labour source:

Family labour [ ] Labour exchange [ ] Hired [ ] Other (specify):_____

7.2. Number of permanent labour per season of pepper: _____

7.3. Number of casual labour for pepper and the reason for hiring them:

_____

_____

_____

7.4. Cost of hired labour (nakfa per man/day): _____

8. Harvest and post-harvest

8.1. How do you decide the harvesting stage of each dry and green pepper?

Green pepper_____

_____

_____

Dry pepper_____

_____

_____

8.2. What is yield per ha in your land?

Green pepper _____ quintals Dry pepper _____ quintals

If you are producing both green and dry peppers from the same field _____ quintals green peppers + _____ quintals dry peppers per ha.

8.3. For dry pepper how do dry your crop?

_____

_____

_____

_____

_____

_____

8.4. For green pepper do you store your harvest or take it directly to the market?

If you store it after harvest

Where do you store it? _____

For how long do you store it? _____

8.5. Where do you sell your crop?

Middle men collect it from the farm [ ]

I sell it in _____

_____

8.6. What is the distance to the market? _____ K.m.

_____ hours/walk

8.7. What is the selling price per Kg. in the last three seasons?

Green pepper: _____

Dry pepper: _____

8.8. Compared to other crops do you think growing green pepper is profitable? Yes [ ] No [ ]

8.9. Compared to other crops do you think growing dry pepper is profitable? Yes [ ] No [ ]

8.10. In the future are you going to continue growing pepper or not? Why

_____

_____

_____

General Observations (This are additional information or explanation of items in the questionnaire)

_____

_____

_____

Appendix 6: Seed Collection Form

Full Name:_____

Zoba:_____ Sub-Zoba:_____

Village:_____

Name of pepper variety: _____

Source of seed:_____

Number of years the seed used by the farmer:_____

Remarks:_____

_____

_____

_____

_____

_____

_____

Appendix 7: Field layout at Hamelmal

Block 1					
Plot No	Treatment	Plot No	Treatment	Plot No	Treatment
1001	11	1035	34	1069	45
1002	38	1036	32	1070	33
1003	91	1037	29	1071	30
1004	56	1038	24	1072	122
1005	28	1039	17	1073	54
1006	35	1040	6	1074	110
1007	64	1041	100	1075	47
1008	52	1042	27	1076	16
1009	76	1043	71	1077	127
1010	36	1044	31	1078	117
1011	83	1045	19	1079	13
1012	85	1046	128	1080	115
1013	14	1047	121	1081	129
1014	43	1048	124	1082	8
1015	1	1049	66	1083	57
1016	41	1050	68	1084	104
1017	90	1051	7	1085	39
1018	120	1052	61	1086	51
1019	79	1053	113	1087	70
1020	78	1054	3	1088	106
1021	53	1055	26	1089	15
1022	4	1056	69	1090	46
1023	77	1057	62	1091	111
1024	125	1058	59	1092	89
1025	86	1059	96	1093	101
1026	21	1060	63	1094	37
1027	40	1061	74	1095	109
1028	50	1062	81	1096	60
1029	12	1063	84	1097	22
1030	73	1064	87	1098	9
1031	95	1065	75	1099	49
1032	102	1066	88	1100	48
1033	112	1067	18	1101	25
1034	5	1068	20	1102	55

Block 2					
Plot No	Treatment	Plot No	Treatment	Plot No	Treatment
2001	60	2035	48	2069	12
2002	56	2036	32	2070	31
2003	8	2037	29	2071	1
2004	43	2038	120	2072	124
2005	22	2039	81	2073	115
2006	117	2040	59	2074	68
2007	73	2041	45	2075	78
2008	129	2042	25	2076	27
2009	6	2043	100	2077	109
2010	15	2044	125	2078	3
2011	36	2045	50	2079	61
2012	11	2046	13	2080	75
2013	89	2047	106	2081	37
2014	96	2048	18	2082	76
2015	113	2049	110	2083	70
2016	88	2050	71	2084	21
2017	64	2051	57	2085	46
2018	121	2052	111	2086	104
2019	24	2053	55	2087	20
2020	49	2054	95	2088	7
2021	9	2055	91	2089	66
2022	101	2056	30	2090	26
2023	51	2057	128	2091	63
2024	52	2058	74	2092	28
2025	54	2059	47	2093	39
2026	122	2060	4	2094	69
2027	19	2061	83	2095	84
2028	17	2062	41	2096	102
2029	14	2063	112	2097	90
2030	62	2064	77	2098	33
2031	79	2065	127	2099	86
2032	35	2066	34	2100	5
2033	85	2067	38	2101	87
2034	40	2068	16	2102	53

Block 3					
Plot No	Treatment	Plot No	Treatment	Plot No	Treatment
3001	57	3035	26	3069	56
3002	50	3036	104	3070	46
3003	122	3037	6	3071	87
3004	81	3038	124	3072	22
3005	90	3039	63	3073	68
3006	120	3040	74	3074	49
3007	43	3041	52	3075	96
3008	29	3042	14	3076	109
3009	54	3043	15	3077	37
3010	100	3044	33	3078	115
3011	89	3045	79	3079	40
3012	7	3046	102	3080	70
3013	3	3047	53	3081	112
3014	73	3048	12	3082	95
3015	24	3049	13	3083	35
3016	71	3050	106	3084	4
3017	66	3051	38	3085	59
3018	34	3052	11	3086	86
3019	28	3053	110	3087	111
3020	129	3054	62	3088	51
3021	84	3055	83	3089	45
3022	91	3056	19	3090	48
3023	16	3057	101	3091	78
3024	128	3058	39	3092	5
3025	60	3059	69	3093	121
3026	36	3060	117	3094	113
3027	76	3061	127	3095	21
3028	9	3062	55	3096	18
3029	25	3063	8	3097	47
3030	27	3064	88	3098	77
3031	1	3065	41	3099	75
3032	30	3066	64	3100	17
3033	61	3067	125	3101	85
3034	32	3068	20	3102	31

Appendix 8: Field layout at Asmara

Block 1					
Plot No	**Treatment**	**Plot No**	**Treatment**	**Plot No**	**Treatment**
1001	11	1021	16	1041	64
1002	38	1022	122	1042	75
1003	76	1023	4	1043	27
1004	47	1024	31	1044	45
1005	79	1025	60	1045	86
1006	35	1026	32	1046	19
1007	69	1027	21	1047	121
1008	52	1028	40	1048	37
1009	68	1029	50	1049	57
1010	55	1030	12	1050	34
1011	30	1031	85	1051	39
1012	111	1032	51	1052	7
1013	8	1033	48	1053	115
1014	14	1034	88	1054	71
1015	43	1035	62	1055	3
1016	1	1036	90	1056	61
1017	101	1037	87	1057	78
1018	20	1038	81	1058	74
1019	120	1039	24	1059	54
1020	36	1040	73	1060	77

Block 2					
Plot No	**Treatment**	**Plot No**	**Treatment**	**Plot No**	**Treatment**
2001	78	2021	101	2041	4
2002	50	2022	12	2042	122
2003	75	2023	38	2043	52
2004	1	2024	16	2044	55
2005	85	2025	3	2045	24
2006	71	2026	32	2046	64
2007	27	2027	86	2047	73
2008	62	2028	69	2048	115
2009	68	2029	54	2049	74
2010	19	2030	57	2050	61
2011	60	2031	8	2051	77
2012	79	2032	120	2052	20
2013	87	2033	31	2053	43
2014	81	2034	34	2054	30
2015	40	2035	88	2055	11
2016	37	2036	35	2056	47
2017	48	2037	14	2057	121
2018	111	2038	76	2058	39
2019	90	2039	45	2059	36
2020	21	2040	51	2060	7

Block 3					
Plot No	**Treatment**	**Plot No**	**Treatment**	**Plot No**	**Treatment**
3001	122	3021	19	3041	75
3002	64	3022	73	3042	76
3003	30	3023	78	3043	77
3004	90	3024	61	3044	35
3005	20	3025	71	3045	3
3006	81	3026	37	3046	120
3007	48	3027	87	3047	86
3008	4	3028	47	3048	32
3009	14	3029	45	3049	21
3010	24	3030	79	3050	55
3011	50	3031	36	3051	27
3012	74	3032	16	3052	57
3013	43	3033	69	3053	12
3014	7	3034	111	3054	68
3015	39	3035	52	3055	34
3016	38	3036	101	3056	88
3017	54	3037	51	3057	1
3018	31	3038	40	3058	85
3019	115	3039	60	3059	8
3020	11	3040	62	3060	121