GENETIC ANALYSIS OF POTATO (*Solanum* species) GENOTYPES USING MORPHOLOGICAL AND MOLECULAR MARKERS

by

TESFAYE ABEBE DESTA

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Department of Plant Breeding

Faculty of Natural and Agricultural Sciences,

University of Free State, Bloemfontein

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Promotor: Dr. C.D. Viljoen

Co-promotor: Prof. M.T. Labuschagne

DECLARATION

I hereby declare that the dissertation submitted by me in the fulfilment of the requirement of a Masters degree in plant breeding at the University of the Free State, is my own independent work and has not previously been submitted by me at another university/ or faculty. I furthermore cede copyright of the dissertation in favor of the University of the Free State.

Tesfaye Abebe Desta

DEDICATION

I fully dedicate this thesis work to my late aunt Woizero Wogayehu Nesibu Taye and my cousin Ato Abiye Haile Dargie who played an incredibly significant role during my primary, secondary and tertiary education providing me with all the material need and spiritual encouragement for the betterment and success of my life as a whole. I thank my God for He gave and blessed me with such enthusiastic family.

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CONTENTS

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ARC	Agricultural Research Council
kb	Kilobases (1kb=10^3 base-pairs)
CIP	Centro Internacional de la Papa
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EARO	Ethiopian Agricultural Research Organization
EDTA	Ethylene Diamine Tetracetic Acid
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polmorphism
TAE	Tris-acetate-EDTA
Tris	Tris(hydroxymethyl)-aminoethane
Taq	*Thermus aquaticus*
UV	Ultraviolet
VOPI	Vegetable and Ornamental Plant Institute

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the world's most important food crops, being surpassed in total production only by wheat, maize and rice. According to the FAO statistics (FAO, 1999), world potato production amounts to 290 million tons (MT), covering nearly 18 million hectares. China leads in production with 47.8 MT, followed by the Russian Federation (31.3 MT), Poland (25.95 MT), U.S.A. (21.4 M T), India (19.2 MT) and Ukraine (17.5 M T) (FAO, 1999).

An almost untold number of varieties have been cultivated since the time the Indians of South America first used potatoes for food centuries ago (Caligari, 1992; Smith and Plaisted, 1968). The premodern development of new potato varieties was mainly based on selection from naturally set berries even after its introduction into Europe at the end of the sixteenth century. This approach was characterized by events such as the disastrous late blight epidemics in Ireland during 1845 and 1846 that resulted in practically complete loss of the crop including more than one million lives, as well as the subsequent blight epidemics in Britain and Europe (Hawkes, 1994). This epidemic demonstrated the lack of blight resistance in contemporary varieties, which was not surprising as previous selection had been done in the absence of late blight.

Late blight of potato that stimulated tremendous interest to develop resistant varieties ultimately made potato probably the first crop plant in which breeding for disease resistance was attempted (Hawkes, 1994). All previous attempts of transferring valuable economic characters from the *Solanum* species to the common potato have largely involved the use of tetraploid selections of *S. tuberosum* (Hougas and Peloquin, 1960) through induced polyploidy (Ross et al., 1967) although interspecific hybrids or multiple crosses (Howard and Swaminathan, 1952) have also been utilized to a smaller extent.

Efficient identification and selection of desirable genotypes largely depends on a comprehensive understanding of the genetic relatedness and variation existing within the crop

and its closely related wild species (Kearsey, 1993; Muench *et al.*, 1991).

Information concerning genetic relatedness is crucial, for it indicates the rate of adaptive evolution and the extent of response in crop improvement (Vega, 1993). Furthermore, it is essential as a guideline in the choice of parents for breeding hybrids (Loarce *et al.*, 1996), to detect genetic duplicates in germplasm collections and implement an effective genetic conservation program (Muench *et al.*, 1991).

Before the advent of DNA technology patterns of genetic diversity in crop species could only be studied using morphological and physiological descriptors (Liu and Furnier, 1993; Neinhuis *et al.*, 1995). This approach of characterization and evolutionary study involves the cultivation of sub samples and their subsequent morphological and agronomic description (Vega, 1993).

Morphological grouping of potatoes is based on characters such as tuber skin, tuber flesh type, tuber shape, sprout appearance, growth habit, leaf type, flower color and disease reaction (Douches and Ludlam, 1991). Unfortunately, these characteristics are a result of interactions of genes and their product, and environment in which they are grown (Russell, 1986; Tanksley *et al.*, 1989). Furthermore, traits of agronomic interest like vigor, disease resistance and cold tolerance usually involve high genotype-environment interactions. As a result there are limited numbers of stable traits that can be used to distinguish differences. Moreover, the requirement of several months to observe the distinguishing characteristics (Vega, 1993), subjectivity included in observation (Douches and Ludlam, 1991), difficulty of discriminating closely related genotypes and species due to low level of polymorphism (Demeke *et al.*, 1993; Smith and Smith, 1992) makes this method problematic. Hence, morphological and agronomic evaluation of population variability needs to be supplemented through direct study of the genome.

According to Arus and Moreno-Gonzalez (1993) the most important properties for good quality markers are: (1) easy recognition of all possible phenotypes (homo- and heterozygotes) from all different alleles; (2) early expression in the development of the plant; (3) no effect on the plant morphology of alternate alleles at the marker loci; (4) low or null interaction among marker

allowing the use of many at the same time in segregating populations. The recently established amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR also called microsatellite) fulfill most of the requirements of good quality markers. Their high informative value, identification of polymorphisms, reproducibility as well as independence to environment and interaction among other markers have been demonstrated and reported across a wide range of plant species including potatoes (Tautz and Renz, 1984; Kim *et al.*, 1998; Maheswaran et al., 1997; Milbourne *et al.*, 1997; Caicedo *et al.*, 1999; Hill *et al.*, 1996; Gupta and Varshney, 2000; McGregor *et al.*, 2000). Consequently, AFLP and SSR markers were employed in this study together with the traditional morphological marker to fulfill the following objectives.

OBJECTIVE OF THIS STUDY

To analyze the genetic distance among diploid, wild and cultivated, dihaploids and tetraploid potatoes

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

The success of any breeding or genetic conservation program is dependent on an understanding of the amount and distribution of the genetic variation present in the gene pool (Paul *et al.*, 1997). In plant breeding programs information concerning the genetic diversity within a crop species is essential for the efficient selection, grouping and utilization of parents that are apparently unrelated in a breeding program (Ford and Taylor, 1997; Frei *et al.*, 1986; Hussain *et al.*, 1989; Muench *et al.*, 1991; Autrique *et al.*, 1996; Yayeh and Zeven, 1997; Zeven, 1990). It is also essential in pure and applied plant research (Morell *et al.*, 1995). Cervera *et al.* (1998) further discussed the importance of characterization of genetic resources in resolving homonyms, genotypes maintained under the same name, and synonyms, the same genotypes maintained under different name, in germplasm collections. This will definitely help eliminate redundant germplasm in the collections. Hence, knowledge of the genetic relationships among cultivars and their wild relatives is useful to plant breeders to rationally stratify collections into smaller core groups (Ghislain *et al.* 1996), efficiently sample available genetic diversity (Nienhuis *et al.*, 1995) and enable curators to better organize and effectively manage their collections (Fanizza *et al.*, 1999; Hosaka *et al.*, 1994; Loarce *et al.*, 1996; Powell *et al.*, 1996). Its usefulness for the understanding the evolutionary relationships between accessions (Abebe *et al.*, 1997), commercial seed production, crop certification, registration and ultimately plant variety protection (Powell *et al.*, 1991; Staub *et al.*, 1996) was also reported. Characterization and identification of cultivated varieties is also a practical necessity for farmers to identify genotypes with proved performance (Smith and Smith, 1992).

Consequently, characterization and quantification of genetic diversity, both within and between populations, has long been a major goal in evolutionary biology (Loarce *et al.*, 1996).

2.2 Origin, domestication and diffusion of potato

2.2.1 Origin and domestication

Although knowledge of its early stages of domestication is not so precise as that of some other crops, such as wheat and barely, the potato is one of mankind's ancient cultivated plants. It is undoubtedly of New World origin, domesticated in South America (Hawkes, 1990; Hawkes, 1992).

The center of origin of potato cultivation may well have been the Andes of southern Peru and northern Bolivia, where likely wild prototypes still exist. Archaeological remains of potatoes and an unrelated tuber crop, *Ulluco*, from Chilca valley near Lima have been radiocarbon-dated to 7000 years before present from detailed studies of the starch and cell structure using light and scanning electron microscopy (Hawkes, 1990). Historical evidence of the chroniclers at the time of the Spanish conquest in the early sixteenth century accounts for the cultivation of potato in what are now Colombia, Ecuador, Peru, Bolivia and Chile (Hawkes, 1992). Furthermore, the reference to potato as iomza, iomy or iomuy in the Chibcha language of central Colombia, papa of Quechua (the language of the Inca empire by then), amka and choque, Bolivian Aymara Indians' language, and poni of the Chile Araucanians also indicates an ancient and widespread cultivation of potato (Hawkes, 1990). Hence, it can be said with some certainty that historical and linguistic evidence clearly corroborate archaeological evidence as the origin of the cultivated potato is in the Andes of South America (Hawkes, 1992).

2.2.2 Potato diffusion

The potato reached Europe in the late sixteenth century via Spain and England some years after the discovery and conquest of Peru by the Spaniards (Hawkes, 1990). They were spread throughout Europe first as a botanical curiosity and grown in physic gardens (Burton, 1989). They were rejected as being unclean, unhealthy, or even poisonous and were fed to livestock long before they become staple human diets (Horton and Anderson, 1992). Hence, it was not

generally grown as a field crop until about the mid-eighteen century during which it reached its zenith (Burton, 1989). Olivier de Sorres, a France landlord with big estate, was the first to have considered the potential of potatoes as an agricultural crop. But Ireland is considered to be the most probable country in the real establishment of the potato as a European food crop (Burton, 1989). Starvation due to the perpetual state of unrest and warfare in Europe in the seventeenth and eighteenth century accompanied by destruction of standing crops, livestock and appropriation of harvested crops by opposing armies contributed in the spread of potatoes in Europe as an agricultural crop (Burton, 1989).

Historical accounts on the further spread of potatoes note that potatoes reached most other parts of the world through European colonists, rather than directly from South America (Burton, 1989; Horton and Anderson, 1992). Although there is scanty information concerning potatoes introductions and diffusion in Africa, it is known that potatoes were grown in parts of Africa by the late seventeenth century (Burton, 1989; Howard, 1970). Traders and christian missionaries also contributed to the spread of potatoes in Africa (Horton and Aderson, 1992; Hawkes,1990). According to FAO statistics (FAO, 1999), Egypt, South Africa, Algeria, and Morocco produce more than 80% of all the potatoes in Africa. In the course of four hundred years a plant confined to the South America until the Sixteenth century has become now a crop of world importance ranking fourth in world production next to wheat, rice, and maize (Hawkes, 1992).

2.3 Utilization

Potatoes are consumed by more than three billion people, fed to animals and serve as raw material for starch and alcohol production. Potato provides more edible food in the world than the combined output of fish and meat. Nutritionally, the potato provides not only energy but also substantial amount of high quality protein and essential vitamins, minerals, and trace elements to the diet. The biological value of potato protein, as an index of the nitrogen absorbed from a food and retained by the body for growth and maintenance, is 73%, second to poultry eggs at 96%, just ahead of soybeans at 72%, and far superior to maize and wheat at 54 and 53 percent respectively. It is comparable to that of cows milk or about 70 percent that of whole egg.

Furthermore, as little as 200 grams of boiled potato provides an adult's recommended daily allowance of vitamin C. It is a moderately good source of iron, a good source of phosphorus and magnesium, and an excellent source of potassium (Horton, 1987; Horton and Anderson, 1992). Potatoes are increasingly consumed in the form of processed food such as French fries or potato chips. Hence, the potato is the best all-round source of nutrition.

2.4 Cultivation

Potatoes are grown as an economic crop under a wide range of day length latitude regimes. This ranges from 12 hours of sunlight in the Andes and equatorial zones of Africa and Asia, to over 16 hours of sunlight in Alaska at 60 degrees north latitude and in Puntana Arenas, Chile, at 53 degrees south latitudes (Horton,1987).

Potatoes grow well on a wide variety of soils. However, ideal soil for potato growing is deep, well drained, and friable with high water-holding capacity lacking a tendency to become puddled when wet and cloddy when dry, which limits the rooting of its relatively weak root system. A temperature range of 16-22^{0}C and rainfall of 550-750 mm is ideal for high tuber yield production. Potato is an exceptionally productive crop producing more energy per hectare per day than any other crop with the quickest maturity than any other staples in 90- 120 days.

2.5 Cytology

The basic chromosome number for the genus *Solanum* is x=12. But several authors have suggested that this is a derived number, the true basic number being x=6. The main evidence for suggesting this is obtained from secondary associations at meiosis in diploid species. Howard (1970) cited Gilles's (1955) findings of haploid plants (n=12) in the diploid species *Solanum polyadenium* and similar findings by Belling and Blakeslee's (1923) in *Datura stramonium*, Campos and Morgan's (1958) in *Capsicum frutescens*, and Rick and Butler's (1956) in *Lycopersicon esculentum*, all allied genera of the family Solanacaea which include the genus *Solanum*, indicate the lack of tangible evidence for assuming a lower basic

chromosome number than 12. Currently seven cultivated and some 228 wild potato species are recognized, which form a polyploid series ranging from diploid (2n=2x=24) to hexaploid (2n=6x=72) with 75% of them being diploid (Hawkes, 1990).

2.6 Structure, morphology and taxonomy

2.6.1 Taxonomy

The genus *Solanum*, to which the cultivated potato belongs, is extremely large. It contains about 1000 species, of which, however, only some 230 are tuber bearing and related to the cultivated potatoes (Hawkes,1990). Many of them are of considerable interest to potato breeders because of their resistance to pest and pathogens and their adaptation to climatic extremes.

The genus solanum is divided into two sub-genera: Pachystemonum (short, thick anthers; plant without thorns) and Leptostemonum (long, narrow anthers; stems and leaves with thorn) (Howard,1970). This genus extends all over the world except for the far north and south, with a strong concentration of species diversity in South America and Central America on the one hand and Australia on the other (Hawkes, 1992).

The potato of commercial importance belongs to a single species, *Solanum tuberosum* L., and is considered to be an autotetraploid with genomic formula of 2n= 4x= 48. This is the only species of tuber-bearing *Solanum* that has been cultivated outside its native area (Hawkes, 1992). Other well-known cultivated plants in the genus *Solanum* are the eggplant or *aubergine (S. melongena)*, the Pepino (*S. muricatum*) and the narajillo (*S. quitoense*). The chili (*Capsicum* spp.) and the tomato (*Lycopersicon esculentum*) are widely grown species in the genus (Hawkes, 1992).

2.6.2 Structure and morphology

The potato is a bushy, sprawling and dark green plant with compound leaves that resemble those of its relatives, the tomato. The above ground part consists of stems, leaves and floral parts.

2.6.2.1 Stem

The stem is herbaceous and erect in its early stages of development. Later it becomes erect (upright) forming an angle of more than 45 ^{0} with the ground, semi-erect (spreading) with an average of 30-45 ^{0}, decumbent in which the stem trail on the ground rising at the apex, and prostrate with trailing stem on the ground. It attains a height of 0.6 m to 1.5 m or more. And it may be simple or branched the primary one being single; few (2-3); medium (4-9) or many (10 or more) depending on the variety and storage of seed tubers. Besides, several axillary branches are usually produced. The stems are round to subtriangular or quadrangular in cross section with a hollow internode in most of fully-grown plants. It has a green, red brown, or purple colour with varying degrees of localized mottling pigment at nodes or certain internodes. The angles of the potato stem are extended to form structures called wings or ridges, which may be prominent or inconspicuous, narrow or broad, straight, or wavy.

2.6.2.2 Leaf

A potato leaf is compound and made up of a petiole, a terminal leaflet, and two to four pairs of large oval primary leaflets with entire or serrate margins interspersed with secondary or rudimentary leaflets along the mid rib (Fig.1). Leaves are alternate in a counter-clockwise spiral. Based on its varying angle of insertion on the stem, the potato leaf is arbitrarily divided as obtuse (upper angle between stem and leaf more than 45 ^{0} on the average) and acute (45 ^{0} or less on the average).

Petioles are semicircular in cross section, convex on the lower side, and slightly concave on

the upper side with flattened base with wing- like margins. The first leaves arising from the seed piece usually are simple while later formed ones are compound, irregularly odd-pinnate with petioled leaflet (Smith, 1968). The leaf may be described as open, intermediate or close depending on the proximity and number of interjected secondary leaflets.

2.6.2.3 Inflorescence

It may be simple flowered (very rarely) in which the peduncle divides usually into two each of which forms a monochasial cyme (Fig.1); or compound, in which the secondary axis divide again by successive divisions to give an inflorescence resembling a simple umbel (Burton, 1989). The corolla is five lobed with white, yellow, blue, purple which may be solid or a combination of colors and shape of stellate (star shape); semi-stellate; pentagonal; or rotate. It has five stamens born on the corolla tube and converges around the pistil.

2.6.2.4 Fruit and seed

The fruit or berry ball may be globose (globe like shaped or spherical), ovoid (egg-shaped) short conical, long conical or pyriform (pear shaped with smooth or verrucose veins) which is green or purplish green tinged with violet color. Seed set in the berry may be none to over three hundred. The seeds are small, flat, oval, or kideny-shaped that are yellow to yellowish brown in color. The underground part of potato consists of roots, stolons and tubers.

2.6.2.5 Roots

Potatoes have a fibrous root system. Plants grown from true seed develop a slender taproot from which lateral branches arise to form a fibrous system. Plants arising from tubers also have a fibrous system consisting of adventitious roots arising in groups of three just above the nodes of the underground stems. It may penetrate as deep as 1.5 meter in a soil without obstructive layers. Roots also extend laterally from plants for 0.6 m or more with extensive branching throughout the system for an efficient water and nutrient absorption.

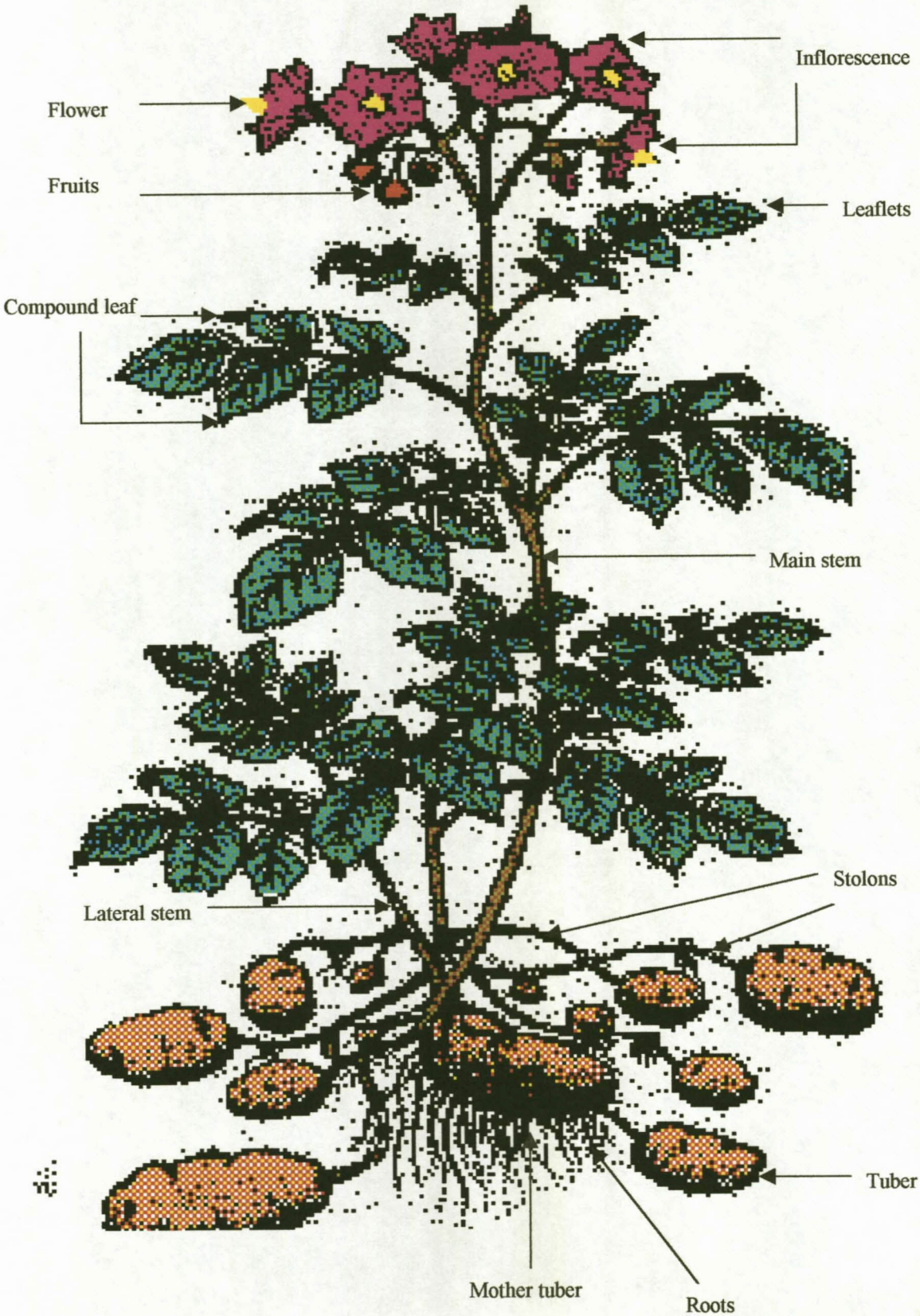

Figure 2.1. The potato plant: foliage, root system, and tubers.

2.6.2.6 Stolons

Stolon is an underground stem that appears comparatively early in the growth of the plant, normally within a week or 10 days after plants have appeared above ground. Under normal conditions of growth stolons are well started by the time plants are 10 centimeter tall. Length of stolons varies from less than 2.54 cm to 46 cm or more for cultivated varieties and with some wild species they may reach 457 cm 610 cm in length (Smith, 1968)

2.6.2.7 Tuber

Morphologically the tuber is an enlarged portion of an underground stem adapted to storage of photosynthates and reproduction of the plant (Fig.1). The tubers that originate from the tips of the stolons contain all the characteristics formed at the base of a leaf scar (the "eye brows"). In general, tuber formation begins approximately at the end of the period of flower bud development. The outer layer of the tuber's cell is known as epidermis. Immediately below the epidermis is the periderm consisting of several layers of corky cells. The epidermis and periderm together constitute the tuber's "skin". In early varieties tubers start to form when buds are fully developed whereas in late varieties tuber formation may not begin until buds begin to open. First indications of tuber formation are the swelling of ends of stolons. Stolons may be single or branched in arrangement (Smith, 1968).

2.7 Morphological characterization

2.7.1 Introduction

The assessment of genetic variation is a major concern of plant breeders and population geneticists due to its importance in controlling the material entering a breeding program, population genetic analyses and predicting potential genetic gain in a breeding program (Liu and Furnier, 1993). Welsh and McCelland (1990) also reported the significance of accurate identification in epidemology and ecology.

Historically, genetic analysis is primarily based on morphological characteristics. It involves cultivation/culturing of sub samples and agronomic and morphological description based on a good record keeping (Demeke *et al.*, 1993; Douches and Ludlam, 1991; Powell *et al.*, 1991; Sosinki and Douches, 1996; Welsh and McCelland, 1990; Vega, 1993). The traditional method of describing and naming cultivated plants by means of a study of their morphological characteristics is referred to as 'alpha taxonomy' (Hawkes, 1994).

2.7.2 The use of morphological characterization in diversity analysis

A combination of morphological and agronomic traits has been used and continue to be used to measure, describe and classify the genetic diversity of wild and cultivated plants and distinctly identify cultivars (Hawkes, 1994). The usefulness of morphological characterization has been clearly demonstrated in a wide variety of crop plants including pepper in which 67 hot pepper accessions were grouped into six cluster based on their variability across 35 morphological and physiological characters (Yayeh and Zeven, 1997). Fruit weight, 1000 seed weight, and fruit number per plant were, however, reported to contain the significant proportion of the variance in distinctly clustering the tested accessions. In a similarly study Zeven (1990) reported the classification of 51 land races and improved cultivars of wheat into four cluster groups mainly based on two characters, i.e., length and density of the ear. Very recently Amsalu and Endashaw (2000) have also reported the variability among 415 sorghum accessions mainly based on plant height and maturity date.

Tatineni *et al.* (1996) have also demonstrated the strong correlation (r=0.63) between the results of 19 easily distinguishable, highly heritable and stable morphological characters and RAPDs measure of distances in their genetic diversity study in elite cotton germplasm. This result clearly indicates the need to distinctly identify those morphological traits that are easily discernable and show high heritability and stability.

Similar results with a reasonable correlation (r=0.47) between average taxonomic distance and Nei's genetic distance have been reported by Autrique *et al.* (1996) from their diversity study in

durum wheat based on restriction fragment length polymorphism (RFLP), morphological trait, and coefficient of parentage distance measures. This clearly indicates the potential of numerical taxonomy to classify plants into distinct groups. Hosaka (1986) also reported on the strong correlation observed among the morphological, genetic, and biochemical markers in grouping some potatoes. The two most comprehensive taxonomic treatments of genus *Solanum* by Correll (1962) and Hawkes (1963) also employed morphological descriptors. The key morphological and agronomic markers used in the identification and grouping of potatoes is based on leaf type, growth habit, flower color, disease reactions, tuber shape, tuber skin and flesh colour, maturity, sprout appearance (Douches and Ludlam, 1991).

Contrary to these results, Johns *et al.* (1997) reported the discrepancy between morphological and RAPD distance measure in classifying common bean landraces. Similarly, an evident difference in the results of the two potato taxonomists have been reported at the number of series and species organization of the potato in the tuber bearing sub section. Such fallacy generally stems from the inherent general properties of morphological markers such as dominance and late expression of distinguishing characteristics (tuber vs leaf and flower), and pleiotropy (Arus and Moreno-Gonzalez, 1993)

The effect of environmental and management practices on morphology (Smith and Smith, 1992) and development of new cultivars resulting from hybridization between members of an elite group of genetically similar parents, which reduces the amount of genetic variability among them, will further complicates the task of unambiguous identification of plants by the use of the conventional characteristic alone (Rongwen *et al.*, 1995; Welsh, 1981).

Furthermore, the requirement of a large number of polymorphic markers to measure genetic relationships and diversity in a reliable manner aggravates the limitation of numerical taxonomy with a few or low level of polymorphism (Tatineni *et al.*, 1996). Thus the development of molecular markers as a powerful tool to analyze genetic relationships and diversity would provide solutions for unambiguous identification (Tatineni *et al.*, 1996)

This, however, does not rule out the use of alpha taxonomic systems' complementarity effect as DNA markers alone are not an omnipotent solution to the limitations of classical taxonomy (Paterson *et al.,* 1991). Li *et al.* (2000) also discussed the need for a synchronous utilization of the old and the newly established techniques.

With the advent of DNA-based genetic markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs), however, the fingerprinting of plant has become more efficient, reliable, and useful (Caetano-Anolles *et al.*, 1991; Nybom, 1994).

2.8 DNA profiling techniques

Identifying plant genotypes by molecular fingerprinting procedure is becoming a practical necessity (Smith and Smith, 1992). Of the molecular fingerprinting procedures, DNA-based markers offer a number of advantages over biochemical markers for demonstrating distinctness. Biochemical markers are often limited by their low frequency in many crop species for adequate discrimination purpose (Goodman and Stauber, 1980; Lu *et al.,* 1996).

However, DNA markers which often reveal neutral sites of variation are much more numerous than morphological and biochemical markers. Besides, unlike the morphological markers, these variations do not always express themselves in the phenotype or disturb the physiology of the organism as each might be nothing more than a single nucleotide difference in the gene or a piece of repetitive DNA (Jones *et al.*, 1997).

The DNA content of an organism is independent of environmental conditions, management practices and growth stage. Furthermore, certain DNA based techniques are simple and quick especially if polymerase chain reaction (PCR) based DNA profiling techniques are employed (Morell *et al.*, 1995). However, no matter what type of population or DNA marker one plans to use, DNA which will be used as a substrate for restriction enzyme digestion or as a template for

polymerase chain reactions must first be isolated from the plants.

2.8.1 DNA isolation

The application of molecular biology techniques to the analysis of complex genome depends on the ability to prepare pure, high molecular weight DNA. Fortunately, plants can be grown in a variety of environments and still provide starting material for DNA isolation. This is in contrast to phenotypic markers, such as morphological, physiological or disease resistance traits, whose expression tend to be highly dependent on growth conditions.

Several methods of DNA extraction suitable for DNA marker analysis have been described (Ausubel *et al.*, 1993; Edwards *et al.*, 1991; Pich and Schubert, 1993; John, 1992; Kamalay *et al.*, 1990; Rogers and Bendich, 1988; Stacey and Isaac, 1994). With each method, the goals have been simplicity, rapidity, low cost, and utilization of a small amount of starting material (Kamalay *et al.*, 1990). Simplicity and rapidity are absolutely essential for processing large numbers of individuals. Small amounts of starting material are advantageous if larger quantities are hard to obtain, such as with seeds, seedlings, or physically small plants like *Arabidopsis* (Young, 1994). Furthermore, the recovery of maximum yield of high molecular weight (>50 killo base (kb) DNA devoid of protein and enzyme inhibitors is the primary objective of the isolation process (Ausubel *et al.*, 1987; Rogers and Bendich, 1988; Sambrook *et al.*, 1989; Pich and Schubert, 1993).

Genomic DNA of a suitable grade for enzymatic manipulations, Southern, or PCR- analysis in crop genetics study can be isolated from tissues as small as individual ovules and embryos, or small pieces of tissues from various parts of the same plant, such as leaf, fiber, root, stem, flowers, seeds, (John, 1992; Kamalay *et al.*, 1990). In addition, DNA can also be isolated from milligram amount of herbarium and mummified tissues (Rogers and Bendich, 1988).

A problem encountered with the isolation of DNA from higher plants is often the presence of abundant polysaccharides, glycoprotiens, or secondary metabolites which tend to co-purify with

plant DNA and inhibit further reactions (Kochert, 1994; Rogers and Bendich, 1988). Ausubel *et al.* (1987) reported that placing plants in the dark for one to two days prior to DNA extraction could reduce the starch content in the tissues. Furthermore, extracting from younger plant tissue is preferable since the polysaccharide content is lower. The material used can be fresh, lyophilized or dried, in some cases even dried at room temperature.

The extraction procedure for plant DNA in general accomplishes breaking (or digesting away) of the cell walls by grinding plant tissues frozen in liquid nitrogen into a fine powder using a mortar and pestle followed by disruption of the cell membranes to release the DNA into the extraction buffer. Many types of extraction buffer can be used. Most contain a buffer that maintains a pH 8, salt such as NaCl to aid in dissociating proteins from the DNA, compounds such as Sodium Dodecyl Sulfate (SDS) and Cetyl Triethyle Ammonium Bromide (CTAB) is used to break open plant cells and solublize the cell membrane. Compounds such as Ethylene Diamine Tetraacetic Acid (EDTA) protect the DNA from endogenous nucleases (plants cells are rich in nucleases) by chalating magnesium ions, a necessary co-factor for most nucleases. Other added compounds can also aid in inactivation of Dnases as incubation of the extract at elevated temperatures. Emulsifiers such as chloroform or phenol are used to denature and remove the proteins from the DNA.

As the aim of any genomic DNA prep is to isolate DNA of high molecular weight with a length of 50-100 kb, which is quite acceptable for most applications and sufficient purity, and sufficient purity factors affecting the size of the DNA isolated, shear and nuclease activity, should be looked after carefully. Treating lysate gently and freezing the tissue quickly and thawing only in the presence of extraction buffer containing detergents and a high concentration of EDTA accomplish this.

Finally, the DNA extracted in such a way is reprecipitated with alcohol and washed in inorganic solvents before redisolve in a suitable buffer. Ribonuclease treatment can be used at this point to remove RNA, but RNA does not interfere with restriction enzyme action or electrophoresis. Total DNA extraction contains organelar DNA (mitochondrial and plastid DNA) in addition to

nuclear DNA and for most purposes it is not necessary to separate these unless the nuclear DNA is required for detailed analytical studies.

2.8.2 Restriction fragment length polymorphism (RFLP) technique

The first DNA profiling technique to be widely applied in the study of plant variation was restriction fragment length polymorphisms (RFLP) assay (Morell *et al.*, 1995). RFLPs are co-dominant markers that have proven to be abundant in all organisms, stable and virtually unlimited in number (Kochert, 1994). The RFLP technique has been successfully employed for identification of cultivars (Gebhardt *et al.*, 1989a; Gorg *et al.*, 1992), phylogentic studies (Debener *et. al.*, 1990), parental tracing (Hosaka, 1986), genetic map construction (Gebhardt *et al.*, 1989b; Gebhardt *et al.*, 1991), and genetic relationship and diversity studies (Miller and Tanksley, 1990).

RFLP analysis in its original form consisted of DNA isolation from a suitable set of plants followed by digestion of the DNA with a specific restriction endonuclease. The DNA fragments generated in such a way are then separated by agarose-gel electrophoresis and transferred to a nitrocellulose or nylon filter by Southern blotting. Subsequently, using nucleic acid hybridization with radioactively labeled cloned probes. RFLPs are then scored by direct comparison of banding patterns (Kochert, 1994; Morell *et al.*, 1995).

Conventional RFLP is limited by the relatively large amount of DNA required for restriction digestion, Southern blotting and hybridization plus the requirement of radioactive isotopes and autoradiography which makes this technique relatively slow, laborious and expensive (Kochert, 1994).

2.8.3 PCR based fingerprinting

The development of the polymerase chain reaction (PCR) technology, however, has promoted the development of a range of molecular assay systems, which has revolutionized the detection

of polymorphisms at the DNA level offering alternatives to the hybridization-based RFLP (Morell *et al.*, 1995). The PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA.

Random amplified polymorphic DNA (RAPD) was particularly one of the most widely applied PCR-based methods for DNA fingerprinting. RAPD analysis allows large numbers of markers to be assayed inexpensively using the PCR and single commercially available arbitrary sequence oligonucleotide primers with 10 base pairs (Welsh and McClelland, 1990; Williams *et al.*, 1990). RAPD analysis is technically straight forward compared to RFLP analysis and requires only nanograms of genomic DNA (Hill *et al.*, 1996). Furthermore, its rapidity, low cost and absence of radioactive/toxic reagents in its use gave popularity to RAPD as a molecular technique to evaluate the genotypic relationship (Demeke *et al.*, 1992; Hosaka *et al.*, 1994; Thormann *et al.* 1994), distance analyis (Fanizza *et al.*, 1999), diversity studies (Vierling and Nguyen, 1992) and cultivar identification (Demeke *et al.*, 1993; Koller *et al.*, 1993; Yang and Quiros, 1993) in a number of taxonomic groups including potatoes.

Sosinski and Douches (1996) reported their successful discrimination of 46 potato cultivars of both sexually derived and clonal variants using as few as 10 primers of 10 nucleotide length (10 mers). Similarly, Demeke *et al.* (1993) demonstrated the usefulness of RAPD markers by the identification of 36 commercial cultivars and clonal variants using only two primers. Hosaka *et al.* (1994) also reported similar result from their study of 73 Japanese breeding lines and commercial potato cultivars. Despite this merit of RAPD, its dominant quality, sensitivity to subtle changes in reaction conditions and PCR temperature profiles (Vos *et al.*, 1995) limited its application.

The recently developed potentially powerful, robust, reliable, highly reproducible and informative marker systems, the AFLP (Blears *et al.*, 1998; Crouch *et al.*, 1999; Vos *et al.*, 1995) and SSR also called microsatellite (Milbourne *et al.*, 1997; Morgante and Olivieri, 1993; McGregor *et al.*, 2000) have established as an alternative marker system of choice (Rhorer *et*

al., 1994; Sarikawa *et al.*, 1992; Simons *et al.*, 1997; Thomas *et al.*, 1995; Gupta and Varshney, 2000).

As a result these two PCR-based DNA fingerprinting techniques were chosen to study the genetic relationships and polymorphisms of the wild and cultivated potato species in this particular activity.

2.9 Amplified fragment length polymorphism (AFLP)

2.9.1 Introduction

A better understanding of the genetic structure and relationship between wild and cultivated plants populations helps to distinctly identify those which merit further study of their potential in the process of improvement of cultivated plants (Tohme *et al.*, 1996).

The recently developed AFLP marker technique (Vos *et al.*, 1995) is unprecedented with respect to the efficiency in the number of markers generated (Simons *et al.*, 1997; Thomas *et al.*, 1995). The AFLP procedure detects a large number of polymorphic DNA markers in a relatively short time. Hence, it is a useful technique when high throughput is desired (Vos *et al.*, 1995). Rouppe van der Voort *et al.* (1998) reported that this marker system allows the estimation of approximately up to 50 loci per assay. Meksem *et al.* (1995) also noted the greater number of polymorphisms detected per reaction in AFLP technique.

AFLP as compared to RFLP, which scans only the restriction sites for polymorphism in DNA, provides additional possibilities beyond the restriction site due to the selective nucleotides included (Blears *et al.*, 1998). AFLP also has a better reproducibility due to the stringent annealing conditions when compared to RAPD which is easier to perform (Becker *et al.*, 1995) but very sensitive to the reaction conditions, template DNA concentration and purity, and PCR temperature of low stringency annealing condition that limit its application (Blears *et al.*, 1998).

Hence, an AFLP marker exceeds RFLP and RAPD in reliability, reproducibility, and inspection of an entire genome for polymorphism (He and Prakash, 1997).

2.9.2 AFLP assay applications

The AFLP has practical application for DNA fingerprinting of prokaryotes and eukaryotes. It has proven extremely useful in generating high-resolution maps in plants. In a relatively well-characterized mapping population of potato, Van Eck *et al.* (1995) studied the localization of AFLP markers relative to the mapping population of 197 RFLP, nine isozyme, and 11 morphological markers which increased the total map length by 5% from 1120 to 1170 centimorgan (cM). In a similar study on barley Becker *et al.* (1995) reported the generation of additional markers that has bridged the gaps in the original RFLP maps resulting in an increase of the total map length by 58% from 1096 to 1873 cM.

This approach is also useful for specifying disease and agronomic markers more accurately. Meksem *et al.* (1995) and Bradshaw *et al.* (1998) reported the identification of markers at the vicinity of the R1 locus on chromosome V of potato, which confers race- specific resistance to *Phytophthera infestanse,* fungal pathogen as well as the quantitative resistance to potato root cyst nematodes, respectively. Ballvora *et al.* (1995) similarly reported the construction of a high-resolution map of the *Grol* locus on potato chromosome VII using AFLP marker together with RFLP and RAPD. Similar results demonstrated by Thomas *et al.* (1995) on tomato, Cnops *et al.* (1996) chromosome landing strategy on Arabidopsis, Cervera *et al.* (1996) on *Populus* species, a model tree, Brigneti *et al.* (1997) on potato, and Ellis *et al.* (1997) on barley. Cato *et al.* (1999) on Pinus radiata indicated that AFLP is very useful for marker enrichment and in some cases may extend the length of the existing linkage map.

Marker loci that are selectively neutral and easily identifiable offer the opportunity to track parental genetic contribution directly. Estimates of the proportion of parental genetic material in a cultivar, line, or breeding population is often useful to plant breeders in characterizing changes in the breadth of the germplasm base during selection (Gizlice *et al.*, 1994). Molecular

markers such as AFLP are best suited for such purpose due to their general availability in large numbers in most populations (VanToal *et al.*, 1997).

Maheswaran *et al.* (1997) noted the usefulness of AFLP markers for marker-assisted backcrossing. The importance of AFLPs marker in selecting better parental material in breeding programs and germplasm collections and conservation are reported by Caicedo *et al.* (1999). Van Toal *et al.* (1997) also demonstrated the power of AFLP markers in providing a consistent and satisfactorily precise estimate of parental contribution, which conformed to those, expected based on pedigrees. Maheswaran *et al.* (1997) further demonstrated the significance of AFLP in segregation analysis of rice population. AFLP is also an efficient tool in cultivar identification (Law *et al.*, 1998).

The efficiency of the AFLP approach above the other DNA based markers is clearly evident in the study of cultivated peanut (*Arachis hypogaea* L.). In this study, AFLPs detected 43% of polymorphic DNA markers in contrast to 3% using DNA amplification fingerprinting (DAF) approach and none using RAPDs and RFLPs (Kochert *et al.*, 1991; Halward *et al.*, 1992; Paik-Ro *et al.*, 1992). The failure of RAPD and RFLP in detecting DNA polymorphism is reported to be contrary to the substantial diversity existing among cultivated peanut genotypes for various morphological (plant habit, seed color, resistance to biotic and abiotic factors) and agronomic traits (Stalker, 1992).

Finally, the AFLPs technique has been successfully used in species diversity and relationship studies in soybean (Maughan *et al.*, 1996), wheat (Barrett and Kidwell, 1998), barely (Ellis *et al.*, 1997; Russell *et al.*, 1997; Pakniyat *et al.*, 1997), rice (Fuentes *et al.*, 1996; Mackill *et al.*, 1996), lettuce (Hill *et al.*, 1996), common beans (Caicedo *et al.*, 1999), tea and others (Paul *et al.*, 1997).

2.9.3. Use of AFLP in estimation of genetic variations and relationships in Potato

Kim *et al.* (1998) evaluated the AFLP technique for detecting DNA polymorphism and

relationship among 12 most important commercial potato cultivars that form the foundation of potato breeding in Korea. Consequently, using seven primer combinations, a total of 466 bands were scored. Of these 409 (88%) were polymorphic among the 12 cultivars while 106 were unique. The most attractive aspect of this result was that of the detection of up to 84 polymorphic bands with a single primer combination among the 12 cultivars. This clearly demonstrates the potential and power of AFLP to readily distinguish and identify all the potato cultivars by any one-primer combination. Hosaka *et al.* (1994) differentiated 67 potato cultivars by 84 RAPDs using 31 primers while Mori *et al.* (1993) distinguished 39 Japanese potato cultivars by 13 RAPDs using 5 primers. Demeke *et al.* (1993) also differentiated 36 North American potato cultivars using two RAPDs primers. In comparison to these RAPD studies on the same crop, AFLP assay is proved to be a powerful tool for cultivar identification in potato.

The results of McGregor *et al.* (2000) technique comparison study on 39 commercial cultivars of potatoes in South Africa agrees with the report of Kim *et al.* (1998). McGregor *et al.* (2000) also demonstrated that AFLP was the only marker type that could distinguished all the 39 cultivars using either one of the two-primer combinations used in the study. Furthermore, these results indicated that AFLP with a genotype index (GI) value of 1, a criterium used to delimit the order of merit of technique, comes first in generating large mean number of profiles per primer pairs.

Milbourne *et al.* (1997) also reported the results of genotyping of 16 potato cultivars using five AFLP primer combinations, 14 RAPD primers, and 17 database-derived SSR primer pairs. As a result, for RAPD analysis, the 14 chosen primers produced a total of 117 major scorable products, whilst using five 3-base extended primer combinations of AFLP analysis, a total of 273 different products were scored. Consequently, based on a single parameter, i.e., marker index (M.I.), a product of effective multiplex ratio (EMR= the number of loci revealed) and diversity index (DI= amount of polymorphism detected) which was used to evaluate the over all utility of each marker system, AFLP was found to be the best suited marker for the generation of the volume of information required to perform such a task. All the results obtained in potato employing AFLP markers is in agreement with the results reported in soybean germplasm

analysis studies by Powell *et al.* (1996) and in the analysis of breeding populations of banana (Crouch *et al.*, 1999).

Hence, the AFLP marker system was reported to be an efficient tool for assessing genetic diversity (Stappen *et al.*, 2000), and reliable PCR-based marker for studies of genetic relationships at a variety of taxonomic levels (Hill *et al.*, 1996). Reproducibility, heritability, a very important criteria for a marker to be useful, and intraspecific homology of AFLPs have been demonstrated in different studies (Mackill *et al.*, 1996; Tohme *et al.*, 1996; Schondelmaier *et al.*, 1996; Powell *et al.*, 1996; Rouppe van der Voort *et al.*, 1997; Milbourne *et al.*, 1997; Crouch *et al.*, 1999; McGregor *et al.*, 2000) indicated that AFLP offer a high level of utility when compared with other marker systems.

2.10 Simple sequence repeats (SSRs)

2.10.1 Introduction

Prior to the advent of DNA sequencing, the higher eukaryotic genomes were known to contain more DNA than necessary to encode required gene products (Shmid, 1996). Vogel (1964) and Ohta *et al.* (1971) as cited by Schmid (1996) claimed that the 3 billion base pairs in the haploid genome could potentially encode 6.7 million genes that would have amount to at least 17 times the number of human genes estimated from genetic load considerations. And the repeated copies of similar DNA sequences that are ubiquitously interspersed both within and between genes form part of this superfluous eukaryotic genome (Schmid, 1996).

The genomes of almost all eukaryotes contain a substantial amount of repeated DNA sequences. More than 20% of human DNA consists of repetitive sequences that have been largely identified and cataloged (Schmid, 1996). These repeated DNA sequences are organized in long arrays of tandem repeats or dispersed throughout the genome. Commonly, they are often localized around centromeres and at the telomers of chromosomes (Russell, 1986; Wintro *et al.*, 1992) and might play a role in chromosome pairing, meiotic recombination,

or speciation (Rose and Dolittle, 1983). Schmid (1996) further noted that these ubiquitously distributed repetitive sequences cause various effects through unequal cross-over between interspersed repeats which either duplicate or delete sequences and thereby mutate genes.

A class of repeated sequences is called microsatellites are comprised of highly variable arrays of tandomly repeated two to six base pairs (bp) long DNA (Senior *et al.*, 1998; Taramino and Tingey, 1996) have been reported in plants (Kochert, 1994). Condit and Hubbell (1991) first reported the presence of 10^4 to 10^5 copies of (GT) n and (AG) n microsatellites in genomes of tropicaltree and maize, respectively.

2.10.2 The use of microsatellites in determining genetic distance

DNA polymorphism assay for genetic mapping, marker assisted plant breeding, germplasm management, and investigation of genetic relatedness has become an important tool in recent years. Various systems and their related techniques are also available. PCR based ones are the most commonly used. The kind of variation that each method detects, however, is one of the yard sticks which will help decide the marker system and technique to be used in genetic analysis (Rus-Kortekaas *et al.*, 1994).

Microsatellite-containing DNA has been found to be polymorphic and co-dominantly inherited (Rus-Kortekaas *et al.*, 1994). These simple DNA motifs can be found in transcribed as well as non-transcribed sequences of eukaryotic genome (Stallings *et al.*, 1991). And the frequency of each class of SSR is also different between species (Wang *et al.*, 1994). Consequently, (GT) n repeats were reported to be the most abundant microsatellite in higher vertebrates and occur every 30 kilo bases (kb), 21 kb and 18 kb, on average, in human, rat and mouse genomes, respectively (Beckmann and Weber, 1992). (CT) n repeats form another abundant class of microsatellites in mammalian species (Wintro *et al.*, 1992). Contrary to this, (AT) n dinucleotide repeat containing microsatellite were reported to be far more abundant in plant species (Akkaya *et al.*, 1992; Morgante and Olivieri, 1993; Lagercrantz *et al.*, 1993). Kawchuk *et al.* (1996) also reported the presence of more dinucleotide (AT) n repeat than other classes of

repeats within *Solanum tuberosum.*

Specific loci amplification and variability analysis performed on soybean (Akkaya *et al.*, 1992), rice (Wu and Tanksley, 1993), and lettuce (Bell and Ecker, 1994) indicates that microsatellites in plants can be up to tenfold more variable than other marker systems such as RFLPs. SSRs efficiency in detecting high level of polymorphism as compared to RFLPs, RAPDs and AFLP was also reported by Powell *et al.* (1996) and Milbourne *et al.* (1997). Similarly, Vosman *et al.* (1992) reported that sufficient levels of polymorphism could be detected with $(GATA)_4$ to distinguish among 15 tomato cultivars studied.

While Yang *et al.* (1994) demonstrated the usefulness of microsatellites for genotype differentiation in rice, the presence, frequency, number and informativeness of SSRs have been estimated for tropical crops (Condit and Hubbell, 1991), Brassica (Lagercrantz *et al.*, 1993; Szewc-McFadden *et al.*, 1996), soybean (Akkaya *et al.*, 1992) rice (Wu and Tanksley, 1993; Yang *et al.*, 1994), chickpea (Sharma *et al.*, 1995), maize (Senior and Heun, 1993; Taramino and Tingey, 1996), lettuce (Bell and Ecker, 1994), barely (Liu *et al.*, 1996), beet root (Morchene *et al.*, 1996), tomato (Smulders *et al.*, 1997), potato (Provan *et al.*, 1996b) and other plant species. Roder *et al.* (1995) reported that (GT) n microsatellite types are the most abundant in plants.

Westman and Kresovich (1999) reported that SSR polymorphism is consistent with the variation patterns revealed by the species breeding history. Senior *et al.* (1998) also found SSRs consistent with the variation among pedigree and discussed the usefulness of SSR as a crucial marker type for genetic analysis of organisms with a narrow genetic base and are genetically very close to each other (Akkaya *et al.,* 1995; Roder *et al.,* 1998; Smulders *et al.*, 1997; Westman and Kresovich, 1999).

Sequence tagged site (STS) microsatellites appear to be the markers of choice for creating high density maps even for species with little intraspecific variation in inbreeding crops (Kwiatkowski *et al.*, 1992; Lagercrantz *et al.*, 1993; Roder *et al.*, 1995; Tautz, 1989). The

codominant mode of inheritance of such STS type markers (Garland *et al.*, 1999) permits their easy transfer between genetic maps of different crosses in contrast to the dominant PCR marker types based on arbitrary primers requiring the generation of a new map for each cross (Thomas and Scott, 1993). SSR markers have also proven useful for studying genetic variation (Plaschke *et al.*, 1995).

Microsatellites offer their greatest potential in parentage studies, studying the gene flow within and between populations, and the extent and maintenance of genetic diversity studies (Condit and Hubbell, 1991). Microsatellites are extremely informative in pedigree tracing studies and analysis of progeny from multiparent matings (Akkaya *et al.*, 1995; Smulders *et al.*, 1997). Hartl and Clark (1997) also discussed the practical applications of this short sequence of bases repeated in tandem for DNA typing.

Buchanan *et al.* (1994) also discussed the potential of multiallelic microsatellites for evolutionary studies. This fact is clearly revealed by Plaschke *et al.* (1995) in which they detect 142 alleles with an average of 6.2 alleles per microsatellite using 23 wheat microsatellite markers located on 15 different chromosomes and 19 different chromosome arms. Similarly, a higher gene diversity values, 0.87, for 96 soybean genotypes was reported for SSR alleles present at seven loci which much higher than the diversity value obtained using RFLP markers. Smulders *et al.* (1997) also reported the detection of two to four alleles for each locus among seven *Lycopersicon esculentum* and accessions of three wild *Lycopersicon* species. Still a good result given the relatively low amount of genetic variation detected with RFLP and RAPD markers among *L. esculentum* cultivars (Miller and Tanksley, 1990; Rus-Kortekaas *et al.*, 1994).

2.10.3 The use of microsatellites in genetic distance analysis of potato

Kawchuk *et al.* (1996) reported the occurrence of one SSRs every 8.1 kb within *Solanum tuberosum.* And 42% of these repeat were dinucleotide repeats of which (AT) n amounts 82% of them. Their results are in agreement with the previous report of Akkaya *et al.* (1992) and

Lagercrantz *et al.* (1993).

Furthermore, these workers reported the generation of a unique DNA profile that distinctly differentiated 73 of 95 tetraploid potato cultivars using site specific amplified SSRs at the starch synthase gene and the sequence encoding mature protienase inhibitor I. Milbourne *et al.* (1997) reported the successful discrimination of 16 potato cultivars using five AFLP primer combinations, 14 RAPD primers, and 17 data based-derived SSR primer pairs which generated a total of 117, 273, and 98 major scorable different products, respectively. The exciting thing in this result is the fact that 89 (90.8%) of the SSR primer pairs generated products were polymorphic as compared to 77 (65.85%) for RAPD and 114 (41.75%) for AFLP.

Provan *et al.* (1996b) have also demonstrated the possibility of discriminating 18 cultivars using a single site specific amplified SSRs. This is in agreement to reports of Milbourne *et al.* (1997) and Powell *et al.* (1996) in which the discriminating power of SSRs have been shown. McGregor *et al.* (2000) also reported that SSR method has a higher resolution power in genetic relationship studies of potatoes with 100% reproducibility. This high level of polymorphism that is displayed due to variation in tandem repeat length renders them very useful as genetic markers to distinguish DNA profile for plant variety protection (Rongwen *et al.*, 1995; Smulders *et al.*, 1997). As a result it was concluded that SSRs are ideal for quick and accurate determination of cultivar identity of *Solanum tuberosum ssp. tuberosum.*

In spite of SSRs easy applicability, highly informativeness and reliability, this marker system, is not yet extensively used in plants because of the laborious, time-consuming and costly exercise requiring the characterization of the sequences flanking the repeat region that allows the development of suitable primer for PCR amplification (Plaschke *et al.*, 1995; Provan *et al.*, 1996b). Thomas and Scott (1993) demonstrated one possible way, which would overcome the relative lack of available sequence data without embarking on a microsatellite isolation program. They successfully used grape vine (*Vitis vinifera*)- derived primers to amplify polymorphic microsatellites from other vitis species. Similarly Provan *et al.* (1996b) proved the possibility of cross-species amplification of microsatellites and arrived at a conclusion that a

carefully designed primer with respect to maximal homology from related species may provide a rich source of polymorphic microsatellite markers for application in genomic analysis within a genus.

This technique involves PCR amplification of SSR loci using oligonucleotide primers that are complementary to the regions flanking the repeat sequences. The resulting variable numbers of products are separated and resolved electrophoretically using denaturing polyacrylamide gel electrophoresis (Westman and Kresovich, 1999; Wu and Tanksley, 1993).

Hence, SSR markers provide an excellent complement to the conventional markers that are currently used to characterize crop species including potato. And it is due to their specific merit and complementarity effect that the alpha taxonomic marker and the most recently developed DNA marker systems (AFLP and SSR) were chosen for this particular study.

CHAPTER III

GENETIC DISTANCE ANALYSIS OF POTATO (*Solanum tuberosum* L.) GENOTYPES USING MORPHOLOGICAL DESCRIPTORS

Abstract

Advanced breeding materials and commercial cultivars, which are known to have economically important attributes, are the building blocks of a successful breeding program. However, their utilization depends on the knowledge of the genetic distance between them. A total of 15 potato genotypes consisting of 11 advanced breeding lines at multi-location performance trial stage and four Ethiopian converted commercial cultivars obtained from the Ethiopian National Potato Research Program were evaluated based on 39 morphological and physiological characters to determine the genetic distance between them. Consequently, the 15 genotypes were grouped into two main clusters and three singletons based on leaflet number, leaf insertion, leaf length, growth habit, stem number and height, stem cross-section, stem wing, tuber number per plant, tuber flesh colour and length of tuber dormancy. Hence, it could be concluded that studying the morphological characters of breeding material is crucial to identifying potential parental material.

3.1 Introduction

In plant breeding, variation and its identification is the key to the successful development of new varieties (Welsh, 1981). Evaluation of the extent of genetic diversity among adapted and elite germplasm can provide predictive estimates of potential genetic gain in a breeding program (Barbosa-Neto *et al.*, 1996; Liu and Furnier, 1993; Cox and Murphy, 1990). This is also fundamental for the effective conservation of germplasm resources (Lubberstedt *et al.*, 2000; Caicedo *et al.*, 1999; Ellis *et al.*, 1997; Smith and Smith, 1992) and their efficient utilization through avoiding the risk of increased uniformity in collections (Barrett and Kidwell, 1998; Ford and Taylor, 1997; Hayward and Breese, 1993; Moreno-Gonzalez and Cubero, 1993; Lubberstedt *et al.*, 2000; Messmer *et al.*, 1993). Hence, the assessment of genetic diversity is of major importance to plant breeders (Hayes *et al.*, 1997; Liu and Furnier, 1993).

It is important to determine the genetic diversity in crop plants that are phenotypically plastic, of which potato is an example, in order to identify potential breeding material of interest (Hawkes, 1990). The genetic similarity between two genotypes can be estimated at phenotypic and genotypic levels. Morphological markers are the oldest marker system used in the identification of varieties (Smith and Smith, 1992), genetic diversity analysis and the grouping of species (Amsalu and Endashaw, 2000; Yayeh and Zeven, 1997; Hawkes, 1990) and the development of linkage maps (Weber and Wricke, 1994). This method generally relies upon the overall phenotypic resemblance judged from the phenotype of the organism without any implication as to their relationship by ancestry (Sneath and Sokal, 1973).

Consequently, 39 morphological markers were used in this study to assess the genetic distance and relationships among four Ethiopian converted commercial cultivars introduced from Europe in around 1985 and 11 advanced genotypes of CIP germplasm.

3.2 Materails and Methods

3.2.1 Genetic materials

Fifteen potato (*Solanum tuberosum* L.) genotypes, consisting of four Ethiopian converted commercial cultivars from Europe and 11 advanced clones of CIP (Table 3.1) at multi-location performance trial stage, were used in this study. Six seed tubers of each of these genotypes were obtained from the Ethiopian National Potato Research-Coordinating Centre based at the Holetta Agricultural Research Centre of the Ethiopian Agricultural Research Organization (EARO).

The experiment was laid out in a Randomised Complete Block Design (RCBD) with two replications, and was carried out at the Free State University, Bloemfontein, in 1999 under glasshouse conditions. Six tubers of each of the 15 clones were planted separately per pot (23 cm x 21.5 cm), with three pots of each genotype considered as one plot of the two replications.

They were planted on 20th of July 1999 and all the necessary cultural practices were undertaken as required. The plants generally took 97 to 109 days to mature depending on the type of clone.

3.2.2 Data collection

Thirty-nine morphological and physiological characters (Table 3.2) were measured on all the 15 clones. The UPOV descriptor list produced by the International Board for Plant Genetic Resources (IBPGR/77) was used in describing genotypes according to Stevens's (1951) classification of variables, which were recorded on quantitative (interval and ratio variables) and qualitative scales (ordinal and nominal variables). These included 10 quantitative and 29 qualitative characters covering vegetative, flowering and tuber characteristics. Three observations were averaged for each plot of two replications used for each of the quantitative characters.

3.2.3 Data analysis

A simple statistical analysis of variance was computed for the 10 quantitative morphological characters using the MSTAT C program (Table 3.4). This was done with the main objective of quantifying the relative importance of each character in the formation of distinct clusters. In addition, bivariate statistics of phenotypic simple correlation coefficient was also computed for these variables to examine the degree of association among these same quantitative characters (Table 3.3). For multivariate analysis, the Numbers Cruncher Statistical System for windows (NCSS, 2000) was employed. Mean values of the two replications were used for quantitative and ordinal variables. Nominal variables were used as recorded for they were identical across the six observations of the two replications. Hierarchical agglomerative clustering method using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) was carried-out to examine the resemblance and grouping of genotypes. The following step by step procedures were followed:

a) Univariate transformation was carried for each nominal variable through creating a set of dummy (0/1) variables. The quantitative and ordinal variables were not transformed.

b) The means were normalized prior to clustering by subtracting the mean and dividing by the standard deviation for each trait to alleviate a scale change caused by a simple changing of the units of measurements.

c) Then principal components (PCs) were extracted from the standardized variables and the extracted variances. Decaying of eigenvalues (variances) determined the number of principal components (PCs). This step was used to reduce the number of variables without losing much of the information through transforming the original variables into new, uncorrelated variables. This reduces the influence of noise and outliers in the data on the clusters. The variance of each PCs was used as a measure of the amount of information conveyed to select the most informative PCs. Finally the matrix of "average" taxonomic distances (Eij) for individuals I and j and morphological traits k was computed using Euclidian distance function, where,

$$E_{ij} = [\Sigma_k (n^{-1})(X_{ki} - X_{kj})^2]^{1/2}$$

For p variables the Euclidian distance is then computed as the square root of the sum of squared differences between the coordinates of each variables for the two observations. The "average" taxonomic distance matrix produced using the selected standardized PCs and transformed data was then used to perform cluster analysis using NCSS (2000) analysis software package.

Principal component analysis (PCA) was performed in order to simplify the description of interrelated variables. In other words PCA is used when simpler representation is desired for a set of interrelated variables. The technique can be briefly summarized as a method of transforming the original large number of variables into new, uncorrelated variables called principal components, which is linear combination of the original variables. Based on the amount of information conveyed by each PCs, which is discerned from its variance, components of most informative are selected. This allows the analysis of a small number of

uncorrelated variables without losing much of the information contained by the original total number of variables (Afifi and Clark, 1996; Tabachinick and Fidell, 1989). Hence, PCA helps to reduce variables with little information content.

Table 3.1 List of potato genotypes studied.

Treatment number	Plant identity
1	CIP-387675.8
2	MENAGESHA
3	CIP-389701.3
4	KP-90108.5
5	GENET
6	KP-90143.5
7	CIP-386423.13
8	WOCHCHA
9	CIP-387675.20
10	KP-90134.5
11	KP-90134.2
12	KP-90138.12
13	TOLCHA
14	CIP-387676.24
15	AWASH

Note: The country of origin for all genotypes studied is Ethiopia, their ploidy level is 2n=4x=48, all are *Solanum tuberosum*, and the source of the materials is CZP. The commercial cultivars are Ethiopian converted varieties introduced from Europe in 1987.

Table 3.2 Descriptor list and values used in morphological characterisation of 15 potato genotypes.

Character number	Character abbreviation	Description and values
1	LD	Leaf dissection, 1=undissected; 2=pinnatilobed; 3=scarcely dissected; 4=weakly dissected; 5=medium dissected; 6=strongly dissected; 7=very strongly dissected; 8=others
2	NPLL	Number of primary leaflets, 1=few (1-3); 2=medium (4-6); 3=many (≥7)
3	LI	Leaf insertion, 1=acute (upper angle between stem and leaf ≤ 45^0; 2=obtuse (upper angel between stem and leaf ≥ 45^0
4	LMRC	Leaf midrib colour, 0=unpigmented; 1=axillary pigmentation; 2=whole midrib pigmented
5	AbLP	Abaxial leaf pubescence, 0=gabrous; 1=glabrescent; 2=pubscent; 3=strongly pubescent
6	AdLP	Adaxial leaf pubescence; same description and values as above
7	LLL	Leaflets length in cm
8	LLW	Leaflet width; 1= narrow (<0.60); 2= Average width (0.60-0.67); 3=broad (.0.67)
9	LL	Leaf length in cm
10	GH	Growth habit, 1= erect; 2=semi-erect; 3=decumbent; 4=prostrate; 5=semi-rosette; 6=rosette
11	BH	Branching habit, 1=single; 2=branched
12	NPS	Number of primary stems, 1=single; 2=few (1-3); 5=medium (4-8); 7=many
13	SCSec	Stem cross section, 1= round; 2= angular
14	SW	Stem wing, 0= absent; 1= straight; 2= undulate; 3=dentate
15	S C	Stem colour, 1=green only; 2=red brown only; 3=purple only; 4=cream with some red brown; 5=cream with purple; 6=red brown with some green; 8=other
16	STNO	Stem number per plant, recorded in count
17	STHT	Stem height at flowering in cm
18	DFLR	Days to flowering= counted as the number of days from emergence to flowering of 50% of the plants of an Accession
19	CSH	Corolla shape, very rotate =1; rotate =3; pentagonal =5; semi-stellate =7; stallate =9
20	PFLRC	Predominant flower colour, white =1; light red =2; intense red =3; light blue =4; intense blue =5; light purple =6; intense purple =7; yellow =8
21	DHARV	Days to harvest recorded when 50% of the plants of an accession are ready for harvest as indicated by the senescence of vines
22	NTPP	Number of tubers per plant in number
23	WTUB	Average weight of a tuber in gm
24	TYPP	Tuber yield per plant in gm.
25	PTSC	Predominant tuber skin colour, 1=white-cream; 2=yellow; 3=orange; 4=brownish; 5=pink; 6=red; 7=purplish-red; 8=purple; 9=dark purple-black
26	STSC	Secondary tuber skin colour; 0= absent and all stated for PTSC
27	PTFC	Predominant tuber flesh colour; 1=white; 2=cream; 3=yellow-cream; 4=yellow; 5=red; 6=violet; 7=purple; 8=other
28	TST	Tuber skin type; 1=smooth; 2=rough (flaky); 3=partially netted; 4=totally netted; 5=very heavily netted; 6=other
29	GTS	General tuber shape, 1=compressed; 2=round; 3=ovate; 4=obovate; 5=elliptic; 6=oblong; 7=long-oblong; 8=elongate
30	TED	Tuber eye depth, 1=protruding; 2=shallow; 3=medium; 4= deep; 5=very deep
31	UTSH	Uniformity of tuber size, 3=low; 5=medium; 7=high
32	DORM	Dormancy after two weeks of storage; 1= dormant; 2= initiated sprouting
33	SPTS	Sprout size, small=3; medium=5; large=7
34	PSC	Predominant sprout colour, 1=white green; 2=pink; 3=red; 4=violet; 5=purple; 6=other
35	SSC	Secondary sprout colour, 0=absent; and all others stated for PSC
36	PUBST	Pubescence of sprout tip, absent or very weak=1, weak=3; medium=5; strong=7; very strong=9
37	PUBSB	Pubescence of tip, same scale as above
38	ST	Size of sprout tip, very small=1; small=3; medium=5; large=7; very large=9
39	HST	Habit of sprout tip, closed=3; medium=5; open=7

3.3 Results

Significant variability in morphological characters was observed among the genotypes studied (Table 3.4). A highly significant positive (P<0.01) association was observed between stem height and tuber weight. A positive and highly significant (P<0.01) correlation also existed between stem height and average weight of tubers. Also significant positive association (P<0.05) was seen between days to flowering and days to harvest. A significantly negative (P<0.01) association was also observed between number of tubers and average weight of a tuber as well as between leaflet width and days to flowering and stem height and days to maturity or harvesting (Table 3.3).

Table 3.3 Correlation coefficient matrix for 10 quantitative characters.

VARIABLES	LLL	LLW	LL	STNO	STHT	DFLR	DHARV	NTPP	WTUB
LLL									
LLW	0.01								
LL	-0.36	0.26							
STNO	-0.25	0.13	-0.18						
STHT	0.17	-0.02	0.02	-0.31					
DFLR	-0.35	-0.66**	-0.27	0.19	-0.02				
DHARV	-0.13	-0.36	-0.18	0.25	-0.47	0.55*			
NTPP	0.28	0.17	-0.18	0.38	-0.44	-0.15	0.19		
WTUB	-0.08	-0.33	0.04	-0.36	0.63**	0.27	0.10	-0.73**	
TYPP	0.3	-0.12	-0.29	0.19	0.34	0.16	0.32	0.40	0.29

LLL=Leaflet length; LLW= Leaflet width; LL= Leaf length; STNO= Stem number per plant; STHT= stem height; DFLR= Days to flowering; DHARV= Days to harvesting; NTPP= Number of tubers per plant; WTUB= Weight of a tuber; TYPP= Tuber yield per plant.

** Indicates those variables with very highly significant (P<0.01) correlation values
* Indicates those variables that are highly (P<0.05) correlated to each other
The remaining variables had no significant correlation to each other

Separate analysis of variance on the 10 quantitative characters identified that leaflet width and leaflet length is insignificant in defining the clusters for the genotypes tested in this particular study (Table 3.4). In other words the clusters are not different from each other in terms of these

two characters.

However, it was clearly observed that the tested genotypes substantially vary with regard to the remaining eight variables (Table 3.4). This information is used for comparing variables only and does not serve for the purpose of hypothesis testing.

Table 3.4 Mean, standard deviation values and Duncans Multiple Range Test for the 10 quantitative characters.

	VARIABLES									
GENOTYPES	LLL	LLW	LL	STNO	STHT	DFLR	DHARV	NTPP	WTUB	TYPP
CIP-387675.8	7.72^{a}	6.643^{a}	30.25^{ab}	4^{ab}	43.4^{bc}	31^{abc}	97^{b}	13^{abc}	20.047^{c}	256.6^{cd}
MENAGESHA	5.938^{a}	7.332^{a}	29.125^{ab}	8^{a}	56^{abc}	37^{a}	100^{ab}	14^{abc}	27.329^{abc}	399^{abc}
CIP-389701.3	6.589^{a}	7.055^{a}	26.875	8^{a}	44^{bc}	36^{a}	109^{a}	13^{abc}	25.942^{abc}	337.25^{abcd}
KP-90108.5	7.052^{a}	6.982^{a}	28	3^{b}	77.35^{a}	36^{a}	100^{ab}	11^{abc}	39.241^{abc}	423.8^{a}
GENET	7.721^{a}	6.686^{a}	30.5^{ab}	3^{b}	80.5^{a}	34^{abc}	97^{b}	8^{bc}	41.439^{ab}	339.8^{abcd}
KP-90143.5	7.838^{a}	6.204^{a}	29.375^{ab}	3^{b}	53.65^{abc}	35^{ab}	109^{a}	10^{abc}	42.938^{a}	412.2^{ab}
CIP386423.13	6.434^{a}	6.355^{a}	30.125^{ab}	5^{ab}	33.75	39^{a}	109^{a}	17^{a}	20.44^{c}	340.67^{abcd}
WOCHECHA	3.41^{b}	6.922^{a}	34^{a}	3^{b}	50.9^{abc}	36^{a}	103^{ab}	7^{c}	36.857^{abc}	258^{cd}
CIP387675.20	8.118^{a}	7.438^{a}	31.25	3^{b}	55.75^{abc}	33^{abc}	103^{ab}	17^{a}	21.5^{c}	378.4^{abc}
KP-90134.5	7.126^{a}	7.724^{a}	30.7^{ab}	3^{b}	58.75^{abc}	32^{abc}	103^{ab}	9^{abc}	33.044^{abc}	304^{abcd}
KP-90134.2	6.204^{a}	5.938^{a}	28.875	4^{ab}	69.5^{ab}	38^{a}	100^{ab}	11^{abc}	29.844^{abc}	335.75^{abcd}
KP-90138.12	7.479^{a}	7.811^{a}	28.125	3^{b}	51^{abc}	23^{c}	97^{b}	16^{ab}	21.092	342.75^{abcd}
TOLCHA	7.736^{a}	6.397^{a}	27.667	2^{b}	54.75^{abc}	35^{ab}	100^{ab}	8^{bc}	33.455^{abc}	276^{abcd}
CIP387676.24	6.355^{a}	7.76^{a}	30.375^{ab}	4^{ab}	52^{abc}	32^{abc}	97^{b}	9^{abc}	23.255^{bc}	218.6^{d}
AWASH	7.199^{a}	7.721^{a}	34^{a}	5^{ab}	62.5^{abc}	24^{bc}	97^{b}	12^{abc}	28.66^{abc}	336.75^{abcd}
MEAN	6.86	7	29.95	4.07	56.25	33.4	101.4	11.67	29.67	330.64
SD	1.17	0.62	2.06	1.79	12.42	4.61	4.52	3.29	7.85	59.6

Means that are followed by the same letter/s are not significantly different from each other.

Pair wise taxonomic distance estimates for the 15 genotypes were produced based on the morphological data using Euclidean distance measure. Genetic distances ranged from 0.46 to 1.68 (Table 3.5). A maximal difference of 1.68 was obtained between Genet and KP-902134.5. Conversely a minimal genetic distance of 0.46 was observed between Wichita and CIP-387676.24 (Table 3.5). The genetic distance matrix was further used to construct a dendrogram based on the UPGMA clustering method (Fig. 3.1). A goodness of fit for the original distances and those that result from the cluster configuration was confirmed by the cophenetic coefficient of r=0.80. The dendrogram constructed based on this distance matrix divided the 15 genotypes into two main clusters and three singletons (Fig. 3.1).

The first cluster contained two sub-clusters. The first sub-cluster consisted of three genotypes namely, Tolcha, KP-90138.12 and KP-90134.2. The second sub-cluster consisted of one genotype, KP-90143.5. This first cluster contained genotypes that are generally characterized by leaves with medium leaflet number, semi-erect growth habit, medium number of stems, tall stem height, angular stem cross-section, undulate stem wing and white tuber flesh colour.

Table 3.5 Distance matrixes between the 15 potato genotypes based on morphological characters produced by Euclidean distance

No.	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	CIP-387675.8														
2	MENAGESHA	0.78													
3	CIP-389701.3	0.73	0.87												
4	KP-90108.5	0.84	0.86	0.70											
5	GENET	0.89	0.96	1.01	0.97										
6	KP-90143.5	0.78	1.07	1.07	1.04	1.13									
7	CIP-386423.13	0.74	0.68	0.77	0.79	0.93	1.00								
8	WOCHECHA	0.61	0.71	0.66	0.78	0.98	0.84	0.68							
9	CIP-387675.20	1.27	1.14	1.36	1.38	1.62	1.16	1.16	1.12						
10	KP-90134.5	0.99	1.25	1.00	1.13	1.68	0.85	1.27	1.08	1.17					
11	KP-90134.2	0.84	0.93	0.89	1.07	1.18	0.71	0.90	0.70	0.96	0.98				
12	KP-90138.12	0.95	1.00	0.84	0.90	1.31	0.91	1.09	0.73	1.24	0.90	0.76			
13	TOLCHA	0.76	0.89	0.81	0.84	1.04	0.84	0.89	0.66	1.12	0.95	0.71	0.73		
14	CIP-387676.24	0.63	0.73	0.75	0.76	0.83	0.71	0.59	0.46	1.05	1.19	0.78	0.94	0.75	
15	AWASH	0.74	0.91	0.70	0.92	1.01	0.79	0.83	0.74	1.20	0.86	0.73	0.75	0.72	0.75

The second main cluster consisted of three sub-clusters. The first sub-cluster consisted of the largest number of genotypes, namely, CIP-387675.8, CIP-387676.24, CIP-386423.13, Menagesha and Wochecha. This sub-cluster is characterized by semi-erect growth habit, short stem height, straight stem wing, white flower colour, medium maturity date, oblong tuber shape, average number and weight of tuber, relatively lower yield and blue-violet anthocyanin colour sprout base (Table 3.6). The second sub-cluster contained two genotypes, CIP-389701.3 and Awash. These genotypes are characterized by an erect growth habit, large number of stems per plant, light purple flower colour, red-violet anthocyanin coloured sprout bases, closed sprout tip, high tuber numbers per plant average tuber weight and high tuber yield (Table 3.6). The third sub-cluster contained a single genotype, KP-90108.5, which is distantly linked with the rest of the genotypes in this second main cluster. The second main cluster was characterized by large leaf length, acute leaf insertion, and straight stem wing, large number of tubers per plant and long period dormancy of tubers (Table 3.6). Morphologically, the remaining three singleton genotypes were observed to be distant from other genotypes and distinct from

each other as explicitly reflected in distance values (Table 3.5).

Table 3.6 Mean value of the 10 quantitative characters for each cluster and singletons.

Characters										
Cluster/Singleton	LLL	LLW	LL	STNO	STHT	DFLR	DHARV	NTPP	WTUB	TYPP
Cluster I	7.31	6.59	28.51	3	57.23	33	102	11	31.83	341.68
Cluster II	6.34	7.06	30.34	5	52.49	34	102	12	27.72	321.13
Genet	7.21	6.69	30.50	3	80.50	34	109	8	41.44	339.80
CIP-387675.20	8.12	7.44	31.25	3	55.75	33	103	9	21.5	378.40
KP-90134.5	7.13	7.72	30.70	3	58.75	32	100	11	33.04	304

Dendrogram

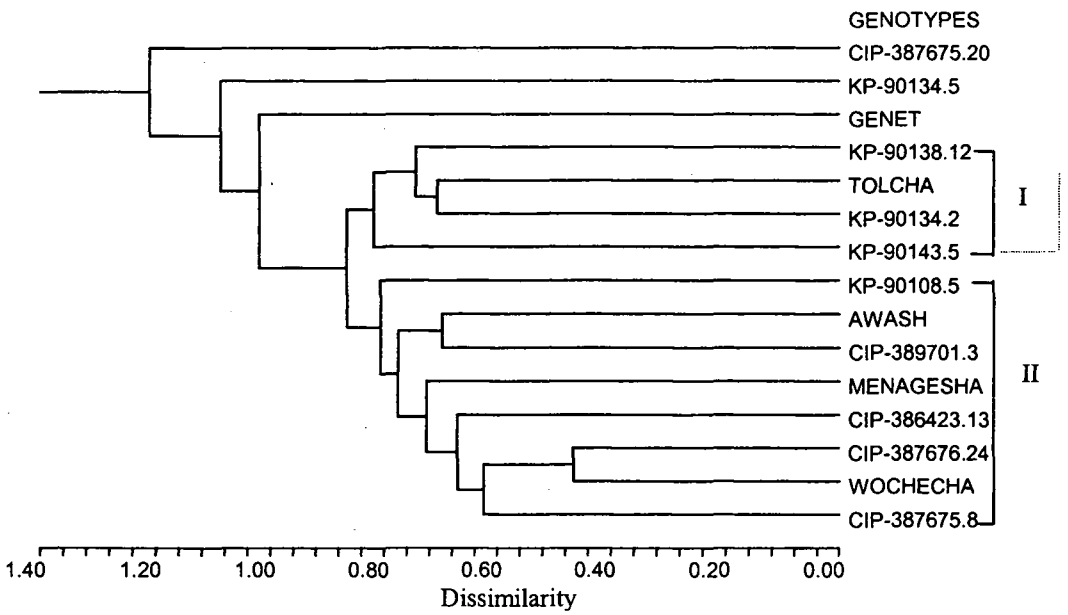

Figure 3.1 Dendrogram depicting the interrelationship between 15 potato genotypes constructed based on 40 morphological characters using UPGMA using a 0.45 genetic distance as a cut-off point.

3.4 DISCUSSION

The simple analysis of variance identified the relative importance of each of the quantitative characters considered in this study. Eight of the total 10 characters were observed to have substantial contribution in differentiating the 15 genotypes studied. These characters are leaf length, stem number per plant, stem height, days to 50% flowering, days to harvesting, number

of tubers per plant, average weight of tuber and tuber yield per plant. Growth habit, flower colour, corolla shape, anthocyanin coloration of sprout and general tuber shape have already been reported to play a significant role in the differentiation of genotypes. It was found that qualitative characters alone were not suitable for cluster analysis due to their lack of genotypic discrimination. This result compares to the findings of Hawkes (1990) that is used as a basis in the classification and biosystematics organization of potato species. Sosinski and Douches (1996) reported the clustering of 46 North American potato cultivars into four groups based on easily identifiable tuber traits, tuber shape and flesh and skin colour.

The association of several characters in potato is quite common. Indeed, it is difficult to find varieties in which correlated features do not appear. Accordingly, the simple correlations between each pair of quantitative characters recorded in this study (Table 3.3) clearly depicts how closely interrelated or associated the variables are. The significant positive ($P<0.01$) association between stem height and average tuber weight is a result of the provision of large amounts of photosynthetic surface by haulm of the potato plant containing more number of leaves as reported by Milthorpe (1963) and Whitehead *et al.* (1953).

Furthermore, while the positive relationship between stem number and number of tubers is a result of more number of stems that act as an independent plant to produce its own roots, stolons and ultimately tuber yields as previously reported (Beukema and Van der Zaag, 1979; Tesfaye *et al.*, 1997; Wiersema, 1987), the significantly negative ($P<0.01$) association between number of tubers and average weight of a tuber is a result of a strong within plant stems competition for growth factors especially of mineral nutrients and moisture. The significant negative association ($P<0.01$) between leaflet width and days to flowering and stem height and days to maturity or harvesting (Table 3.3) may be due to the high leaf area index earlier in the season for more light interception and assimilation which will enhance the growth and development of the plant of genotypes with larger leaflet width. This correlates with the findings of Whitehead *et al.* (1953) on the same crop. A significant positive association ($P<0.05$) was seen between days to flowering and days to harvest and the obvious explanation for this lies in the fact that these two sequential phonological developmental stages are interdependent, as

one should naturally happen before the other. Beukeman and Van der Zaag (1979) and Wiersema (1987) further reported the presence of a positive correlation between stem number and tuber yield. This is due to the in increased tuber number from increased stem number, which ultimately increases tuber yield.

In contrast to this, a very clear relationship between the interrelated quantitative characters and the distinct clusters is formed (Table 3.6; Fig. 3.1). Accordingly, the positive association between stem number and tuber number in one hand and the negative association between number of tubers and average weight of a tuber are quite obvious in the genotypes of cluster II. Similarly, the positive association between stem height and average weight of a tuber and negative association between leaflet width and days to flowering is clearly seen in the singleton commercial variety Genet and advanced clone KP-90134.5. Hence, an indirect selection can be performed for the genotypes of interest from the different cluster group based on the knowledge of these character associations.

Furthermore, the distance matrix and the dendrogram clearly identify different grouping of genotypes. When the overall morphological characters are considered, the advanced clone CIP-387675.20 has an average maximal genetic distance value of 1.20 from all other genotypes. The commercial cultivar Genet, on the other hand, is relatively distant from the advanced clones when compared to other commercial varities. The least genetic distance value exists between the commercial variety Wochecha and the advanced clone CIP-387676.24. It is also interesting to note that although CIP-387675.20 and CIP-387675.8 were developed from seedlings of a single cross between a female and male parent they are genetically very distinct from each other. Caligari (1992) and Poehlman and Steper (1995) qualify this phenomenon of genetic uniqueness of seedlings arising from a given cross as the effects of genetic segregation of the heterozygous parental genetic material. This underscores the merits of clonal reproduction, which maintains desirable genetic combinations in individuals, with low fertility, arising from wide or interspecific crossing blocks and the fixation of combinations of desirable genes in contrast to sexual reproduction in which segregation is a common phenomenon. Hence, selections in clonal progenies does not suffer the complications

that can arise, particularly in inbreeding crops where selection in early generations can be distorted by dominance that is not present in the final inbreeding lines produced.

Finally, it is reasonable to conclude that genetic distances can be successfully determined in potato genotypes through the use of morphological characters that are observed to play an important role in the separation of genotypes into distinct groups. It can also be concluded that those genotypes that clustered distantly as could serve as a germplasm source for unique alleles. This, however, does not rule out the major drawbacks of morphological characterization caused by low levels of environmentally stable polymorphic characters. The identification and use of few heritable, stable and easily identifiable characters will, however, alleviate the hit and miss system of genotype identification through using bulk characters.

CHAPTER IV

GENETIC DISTANCE ANALYSIS OF DIFFERENT SPECIES OF POTATO GENENOTYPES USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) MARKERS

Abstract

Knowledge of the relative genetic distances between a cultivated variety and its closely related wild species is useful in a breeding program to increase the efficiency of breeding efforts of improving crop species. A total of seven species represented by 53 different genotypes were evaluated using AFLPs to examine the genetic distances and evolutionary relationships among them. The pairwise genetic distances computed for these genotypes indicate a high degree of diversity. The genetic distance among them ranged between 0.17 and 0.74. A dendrogram constructed, based on the distance matrix, confirmed the hypotheses reports made in earlier reports on the cultivated *Solanum tuberosum subsp. tuberosum*. Furthermore, the hypotheses on the species groups frequently used to contribute new traits to modern potato varieties was also confirmed by the associations revealed in the dendrogram. Consequently, AFLPs are shown to be suitable for assessing the genetic variability in potato germplasm and are useful in describing the evolutionary relationships among genotypes, which allows questions on breeding ancestry to be addressed.

4.1 Introduction

Potato (*Solanum tuberosum* L.) is a tuberous-rooted tropical and subtropical crop plant grown as an annual under a wider range of environmental conditions than most crops (Horton, 1987). It is currently the fourth major food crop in the world after rice, wheat, and maize with nearly 290 million tons of production and global coverage of about 18 million hectares (FAO, 1999). With eight cultivated and 230 wild ancestors, the potato is genetically one of the most complex and diverse of all food crops (Hawkes, 1990).

Nevertheless, efficient utilisation of available genetic resources such as this demands a comprehensive knowledge of the variation and relationships between cultivated and its related wild species (Ford and Taylor, 1997). Such information is particularly useful to plant breeders

as a general guide in the choice of parents for breeding hybrids (Loarce *et al.*, 1996).

Patterns of genetic diversity have been studied in crop species using a variety of molecular, chemical and morphological descriptors (Nienhuis *et al.*, 1995). Isozymes and seed proteins are among the most commonly used molecular markers for measuring genetic relationships. Although informative and practical, the use of these markers is limited by their low frequency in many crops and expresson under different environmental conditions (Goodman and Stuber, 1980; Liu and Furnier, 1993). Molecular markers that are selectively neutral, however, provide an opportunity to measure genetic relationships more precisely than morphological markers, which are highly selective, due to their availability in large numbers in most crop plants and environmentally stable quality (Soller and Beckmann, 1983; Helentjaris *et al.*, 1986). These markers allow a direct comparison of similarity of genotypes at the DNA level (Lubberstedt *et al.*, 2000). The recently developed AFLP marker technique (Vos *et al.*, 1995) is unprecedented with respect to the efficiency in the number of markers generated (Thomas *et al.*, 1995; Pillay and Myers, 1999; Van Toal *et al.*, 1997). The usefulness of AFLP markers for genetic diversity analysis has been demonstrated for a number of crop species including potatoes (Sharma *et al.*, 1996; Mackill *et al.*, 1996; Powell *et al.*, 1996; Russell *et al.*, 1997; Seefelder *et al.*, 2000; Kim *et al.*, 1998). Besides, it has been successfully used to identify markers tightly linked to disease resistance loci (Meksem *et al.*, 1995; Thomas *et al.*, 1995) and construction of genetic linkage maps (Becker *et al.*, 1995; van der Voort *et al.*, 1998). To date, all comparative studies concur in identifying AFLP as a unique technology with high marker utility (Powell *et al.*, 1996; Milbourne *et al.*, 1997; Crouch *et al.*, 1999). Reproducibility and heritability of AFLPs have been indicated (Jones *et al.*, 1997; Mackill *et al.*, 1996). In the present study AFLP technique was used to examine the relationships among 53 wild, cultivated and advanced potato genotypes.

4.2 Materials and Methods

4.2.1 Plant materials

A total of 53 genotypes belonging to the most commonly used seven species in potato

improvement program as important sources of economically important traits were evaluated in this study. The species name, place of origin, ploidy level, and plant identity of these genotypes is listed in Table 4.1 shown below.

Table 4.1 List of potato genotypes used for genetic analysis.

Treatment Number	Species name	Plant identity Number	Country of origin	Ploidy level	Source	Status
1	Solanum stenotomum	CPC-1839	Peru	2n=24	VOPI	Cultivated
2	*S. stenotomum*	GAN-56	Bolivia	2n=24	VOPI	Cultivated
3	*S. stenotomum*	GAN-30	Bolivia	2n=24	VOPI	Cultivated
4	*S. stenotomum*	GAN-29	Bolivia	2n=24	VOPI	Cultivated
5	*S. stenotomum*	GAN-19	Bolivia	2n=24	VOPI	Cultivated
6	*S.stenotomum*	CPC-1514	Peru	2n=24	VOPI	Cultivated
7	*S.stenotomum*	OCH-1922	Peru	2n=24	VOPI	Cultivated
8	*S. stenotomum*	OCH-1933	Peru	2n=24	VOPI	Cultivated
9	*S. stenotomum*	CPC-1793	Peru	2n=24	VOPI	Cultivated
10	*S. sparsipilum*	EBS-1890	Peru	2n=24	VOPI	Cultivated
11	*S. sparsipilum*	UGT-905	Peru	2n=24	VOPI	Wild
12	*S. sparsipilum*	OCH-2064	Peru	2n=24	VOPI	Wild
13	*S. sparsipilum*	EBS-1801	Bolivia	2n=24	VOPI	Wild
14	*S. canasense*	189219x189218	Hybrid	2n=24	VOPI	Wild
15	*S. stoloniferum*	CPC-2093	Mexico	2n=48	VOPI	Cultivated
16	*S.stoloniferum*	CPC-2092	Mexico	2n=48	VOPI	Cultivated
17	*S. stoloniferum*	CPC-28.4	Mexico	2n=48	VOPI	Cultivated
18	*S.stoloniferum*	COR-14247	Mexico	2n=48	VOPI	Cultivated
19	*S. stoloniferum*	HAW-1293	Mexico	2n=48	VOPI	Cultivated
20	*S. stoloniferum*	COR-14213	Mexico	2n=48	VOPI	Cultivated
21	*S.tuberosum ssp..andigena*	COR-14255	Mexico	2n=48	VOPI	Cultivated
22	*S. acaule*	EBS-9	Argentina	2n=48	VOPI	Cultivated
23	*S. tuberosum*	CIP-387675.8	CIP Peru	2n=48	Ethiopia	Cultivated
24	*S. tuberosum*	CIP-389701.3	CIP Peru	2n=48	Ethiopia	Cultivated
25	*S.tuberosum*	CIP-387675.20	CIP Peru	2n=48	Ethiopia	Cultivated
26	*S.tuberosum*	CIP-387676.24	CIP Peru	2n=48	Ethiopia	Cultivated
27	*S.tuberosum*	CIP-386423.13	CIP Peru	2n=48	Ethiopia	Cultivated
28	*S.tuberosum*	CIP-378501.3	CIP Peru	2n=48	Ethiopia	Cultivated
29	*S.tuberosum*	MENAGESHA	CIP Peru	2n=48	Ethiopia	Cultivated
30	*S.tuberosum*	TOLCHA	CIP Peru	2n=48	Ethiopia	Cultivated
31	*S.tuberosum*	WOCHECHA	CIP Peru	2n=48	Ethiopia	Cultivated
32	*S.tuberosum*	GENET	CIP Peru	2n=48	Ethiopia	Cultivated
33	*S.tuberosum*	KP-90108.5	CIP Peru	2n=48	Ethiopia	Cultivated
34	*S.tuberosum*	KP-90143.5	CIP Peru	2n=48	Ethiopia	Cultivated
35	*S.tuberosum*	KP-90134.5	CIP Peru	2n=48	Ethiopia	Cultivated
36	*S.tuberosum*	KP-90134.2	CIP Peru	2n=48	Ethiopia	Cultivated
37	*S.tuberosum*	KP-90138.12	CIP Peru	2n=48	Ethiopia	Cultivated
38	*S.tuberosum*	DG-18.2	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
39	*S.tubrosum*	DG-18.32	ARC_Roodplaat SA	Dihaploids,	VOPI	Reduced genome
40	*S.tuberosum*	DG-18.9	ARC_Roodplaat SA	Dihaploids,	VOPI	Reduced genome
41	*S.tuberosum*	DG-19.18	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
42	*S.tuberosum*	DG-19.38	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
43	*S.tuberosum*	DG-19.43	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
44	*S.tuberosum*	DG-20.32	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
45	*S.tuberosum*	DG-20.34	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
46	*S.tuberosum*	DG-20.6	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
47	*S.tuberosum*	DG-32.10	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
48	*S.tuberosum*	DG-32.2	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
49	*S.tuberosum*	DG-32.8	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
50	*S.tuberosum*	DTO-33	USA	2n=4x	VOPI	Cultivated
51	*S.tuberosum*	LT-2	Peru	2n=4x	VOPI	Cultivated
52	*S.tuberosum*	LT-7	Peru	2n=4x	VOPI	Cultivated
53	*S.tuberosum*	Buffelspoort	ARC_Roodplaat SA	2n=4x	VOPI	Cultivated

4.2.2 DNA Extraction

DNA was extracted from fresh young tissues based on the modified monocot protocol of Edwards *et al.* (1991). Young plant leaf tissue harvested from one plant of each clone was used to isolate DNA for fingerprinting analysis. Tissue was frozen with liquid nitrogen and ground into a fine powder using a mortar and pestle. Ten ml of extraction buffer (1 M Tris-HCl pH 8; 0.25 M EDTA and 20% SDS) was added to each sample and then incubated at 65°C for half an hour with inversion every ten minutes, thereafter, 1 ml Cetyl triethyl ammonium bromide (CTAB) (10% w/v) and 2 ml 5 M NaCl were added to the homogenate and incubated for a further hour with gentle agitation every 10 minutes. Chloroform-isoamyl alcohol (24:1) extractions were performed at room temperature with centrifugation at 10 000 rpm for 15 minutes to remove protein. After centrifugation the DNA was precipitated by the addition of two volumes (v/v) ethanol and stored at 4°C. The precipitated DNA was spooled and washed with 70% ethanol to remove residual salt before redissolving in sterile double distilled water and stored at 4°C. DNA concentration was estimated using UV spectrophotometery. Then the DNA solution was diluted to a working concentration of 100 ng μL^{-1} and stored at 4°C. Finally the integrity and concentration of the DNA was confirmed by agarose electrophoresis and visualized with ethidium bromide staining under UV light.

4.2.3 AFLP analysis

The AFLP assays were performed as described according to the manufacturer instructions (Gibco BRL) with minor modification.

4.2.4 Restriction Endonuclease digestion and ligation of adapters

Genomic DNA (250 ng) was subjected to digestion at 37°C for 2 h with EcoR I and Mse I. The digested fragments were then ligated with EcoR I and Mse I adapters (Table 4.2).

4.2.5 Polymerase Chain Reaction

A 51 µL pre-selective reaction was performed with 5 µL (1:10diluted) ligation product, pre-amp primer mix 10x PCR buffer and 1U of Ampli Taq DNA polymerase (Gibco BRL). A touch down Hybaid thermal cycler was used to perform the reaction for 20 cycles with the following profiles: 30 s at 94 ^{0}c, 60 s at 56 ^{0}c and 60 s at 72 ^{0}c. Pre-selective PCR products were diluted 50 fold in TE buffer.

Selective PCR-reactions were performed in a 20 µL PCR reactions containing 5 µL diluted pre-selective amplicon, 4.5 µL of MSE+3 primer (Table 4.2), 1 µL ECO+3 (fluorescently labelled), 2 µL 10x PCR buffer and 5 U of Ampli Taq DNA polymerase. Reactions were performed for 30 cycles with the following cycle profile: 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temprature is reduced by 0.7°C for 12 consecutive cycles and then maintained at 56°C for the remaining 18 cycles. A total of 2 primer combinations were tested. EcoR I primers (PE Biosystems) were labelled with NED and FAM.

Table 4.2 List of adapters, primer pairs and their sequences used in this study for selective reaction in AFLP amplification.

Mse I – adapter 5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'	EcoR I – adapter 5'-CTCGTAGACTGCGTACC-3' 3'-CATCTGACGCATGGTTAA-5'
MseI – primer 5'- GATGAGTCCTGAGTAA-3'	EcoR I – primers 5'-GATGCGTACCAATTC-3'
MseI + CAG	EcoR I + ACA (FAM)
	EcoR I + AAC (NED)

After amplification 5 µL of each of the selective reactions was added to 24 µL of formamide and 1 µL of Rox standard size marker, denatured at 94°C for 5 min and resolved on Perkin Elmer Prism 310 Automated capillary sequencer (PE Biosystems).

4.3 Results

An average of 20 to 31 fragments were amplified by individual primer combinations for any one genotype examined in this study. Scorable fragments ranged in size from 55 bp to 395 bp.
The pairwise genetic distance estimates computed for the 53 different potato genotypes is indicated on Table 4.3. The genetic distance overall genotypes ranged between 0.17 to 0.74. The smallest genetic distance of 0.17 was measured between LT-7 and DTO-33. The greatest genetic distance of 0.74 was found between CIP-387676.20 (*S.tuberosum subsp. tuberosum*) and DG-19.43 (dihaploid derivatives of *S. tuberosum subsp. tuberosum*) and KP-90134.5 (*S. tuberosum subsp. tuberosum*) and DG-19.43 (dihaploid), respectively (Table 4.3).

A dendrogram, summarizing the genetic distance between the 53 genotypes was constructed from the genetic distance matrix using the unweighted pair-group method with arithmetic mean (UPGMA) clustering method. The Numbers Cruncher Statistical System (NCSS, 2000) computer program was used to perform the cluster analysis. Goodness of fit of the dendrogram constructed was also confirmed by the co-phenetic correlation of r= 0.89.

The dendrogram constructed on the basis of shared AFLP fragments divides the 53 different genotypes into three main clusters at a genetic distance of 0.55. Cluster I consisted of 13 genotypes. This cluster is sub divided into three sub clusters. This cluster contained only the dihaploid genotypes of the tetraploid *Solanum tuberosum subsp. tuberosum.* The first sub cluster consisted of five genotypes, DTO-33, LT-7, LT-2, DG-32.8 and DG-32.10. In second sub cluster DG-32.2, DG-20.34, DG-20.6, DG-20.32, DG-19.43 and DG-19.38 grouped together. The third sub cluster consisted the genotypes DG-19.18 and DG-18.36. The genetic distance amongst the dihaploid genotypes ranged between 0.17 to 0.71.

The second main cluster consisted three genotypes KP-90134.2, KP-90138.12 and CIP-387675.20. All the genotypes in this cluster belong to the *S.tuberosum subsp. tuberosum.*
The third main cluster contained five sub clusters. The first sub cluster consisted of six genotypes, EBS-9 (*S.acaule*), Awash, CIP-386423.13, Wochecha and KP-90143.5, all with the

exception of *S. acaule* belonging to *S. tuberosum subsp. tuberosum.* The *S. tuberosum subsp. tuberosum* genotypes in this sub group are more closely related to each other than to EBS-9 (*S.acaule*). Sub-cluster number two contained four genotypes namely, CPC-28.4 (*S. stoloniferum*), GAN-19 (*S. stenotomum*), GAN-29 (*S. stenotomum*) and CPC-2093 (*S. stoloniferum*). The third sub-cluster consisted of CPC-2092 (*S. stoloniferum*), 189218x189219 (artificial hybrid species) and GAN-56 (*S. stenotomum*). The fourth sub-cluster is comprised of Menagesha (*S. tuberosum subsp. tuberosum*), KP-90108.5 (*S. tuberosum subsp. tuberosum*), CIP-387675.8 (*S. tuberosum subsp. tuberosum*) and CPC-1514 (*S. stenotomum*). The last sub-cluster contained the largest number of genotypes. These are DG-18.2, DG-18.9 and Bufflespoort (dihaploids of the *S. tubersum subsp. tuberosum*), EBS-1801 (*S. sparsipilum*), Tolcha (*S. tuberosum subsp. tuberosum*), KP-90134.5 (*S. tuberosum subsp. tuberosum*), OCH-2064 (*S. sparsipilum*), OCH-1933 (*S. stenotomum*), Genet (*S. tuberosum subsp. tuberosum*), COR-14255 (*S. tuberosum subsp. andigena*), COR-14247 (*S. stoloniferum*), COR-14213 (*S. stoloniferum*), OCH-1922 (*S. stenotomum*), GAN-30 (*S. stenotomum*), UGT-905 (*S. sparsipilum*), EBS-1890 (*S. sparsipilum*), HAW-1293 (*S. stoloniferum*), CPC-1793 (*S. stenotomum*) and CPC-1839 (*S. stenotomum*).

4.4 Discussion

With the use of two AFLP primer combinations, all the 53 genotypes studied could be distinctly identified. Similar findings have been reported previously by other workers (Milbourne *et al.*, 1997; Kim *et al.*, 1998 and McGregor *et al.*, 2000).

The genetic distances computed for the 53 genotypes based on the formula of Excoffier *et al.* (1992), ranged between 0.17 to 0.74. This wide range of genetic distance suggests the broad genetic base among the genotypes studied. The lowest genetic distance was observed between LT-2 and DTO-33, tetraploids of S. *tuberosum subsp. tuberosum.* Within species genetic distance values ranged between 0.17 to 0.71 for *S. tuberosum subsp. tuberosum*, 0.28 to 0.68 for genotypes of *S. stoloniferum*, 0.54 to 0.62 for *S. sparsipilum* and 0.28 to 0.70 for *S. stenotomum.*

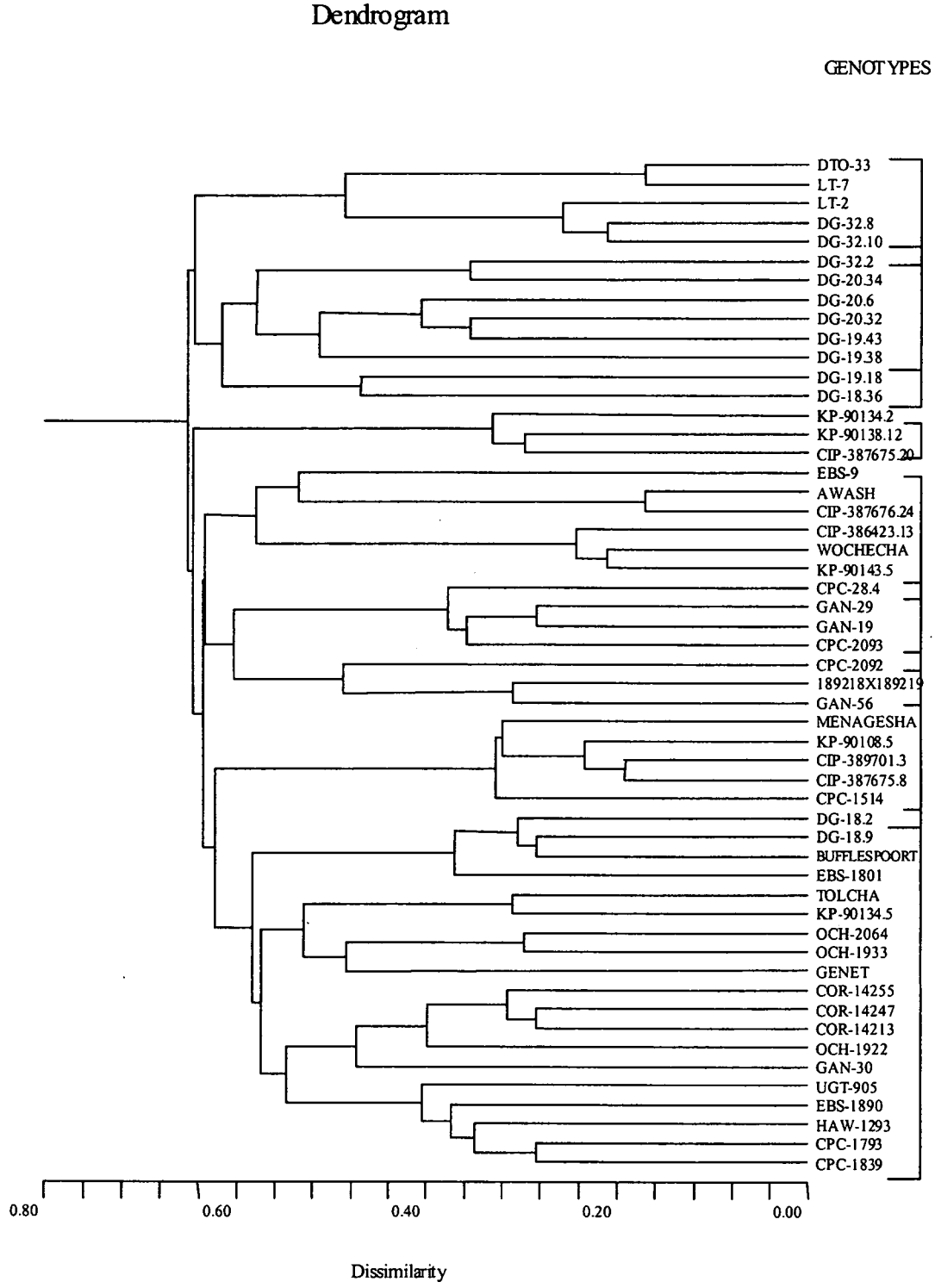

Figure 4.1 Dendrogram indicating the genetic distances between the 53 potato genotypes constructed based on AFLP data using UPGMA clustering method.

The wide range of genetic distance values observed among the advanced tetraploid *S. tuberosum subsp. tuberosum* breeding clones clearly indicate the development of these clones from parental genotypes with a wide genetic background. Similarly, the genetic distance values observed among other species follows the species structure and organization documented by Hawkes (1990) based on morphological characters.

It is interesting to note the grouping of the *S. stenotomum* species, *S. tuberosum subsp. tuberosum*, *S. stoloniferum*, *S. sparsipilum* and *S. tuberosum subsp. tuberosum* in the same cluster (cluster III). This explicitly correlates with the Hawkes (1990) hypothesis on the frequent utilization of these species in potato improvement and is a result of the which this in itself result in the presence of shared, fragments because of transferred genes. Furthermore, the grouping of *S.stenotomum* and *S. sparsipilum* together with the *S. tuberosum subsp. tuberosum* and *S. tuberosum subsp. andigena* supports Hawkes (1990) the suggested hypothetical putative ancestor of potato. It is also interesting to note that grouping of clonal genotypes reflects parental groupings in both morphological and the molecular markers.

In conclusion, using the AFLP method it was found that the seven species studied in this project do actually contribute some genes to the modern potato varieties thus far developed as reported by Hawkes (1990). This was observed from the clustering of the different species under same group. Futrhermore, our result correlates with the previous hypothesis concerning the evolutionary relationships between the *S. stenotomum* and *S. sparsipilum*, the two putative ancesters of the cultivated potato, *S. tuberosum subsp. tuberosum.* Hence, AFLP marker could be successfully used to study genetic distances and evolutionary relationships in potato.

Table 4.3 Genetic Distance matrix of the 53 different potato genotypes using Euclidean distance analysis of AFLP DNA data.

Number	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	CPC-1839																																																			
2	UQT-605	0,42																																																				
3	CPC-1793	0,28	0,39																																																		
4	HAW-1293	0,35	0,43	0,34																																																	
5	EBS-1890	0,37	0,37	0,36	0,38																																																
6	GAN-56	0,61	0,58	0,59	0,59	0,62																																															
7	189218x189219	0,69	0,61	0,63	0,62	0,64	0,31																																														
8	CPC-2092	0,64	0,62	0,62	0,62	0,62	0,49	0,49																																													
9	GAN-30	0,54	0,57	0,54	0,54	0,56	0,49	0,52	0,49																																												
10	OCH-1922	0,5	0,53	0,52	0,52	0,49	0,59	0,62	0,59	0,53																																										
11	CPC-2093	0,64	0,61	0,61	0,62	0,65	0,58	0,59	0,56	0,61	0,59																																									
12	CPC-28,4	0,64	0,62	0,61	0,62	0,64	0,58	0,59	0,59	0,63	0,61	0,37																																								
13	COR-14213	0,54	0,58	0,58	0,58	0,58	0,6	0,62	0,59	0,45	0,39	0,63	0,66																																								
14	GAN-19	0,55	0,66	0,56	0,66	0,68	0,61	0,61	0,62	0,63	0,65	0,39	0,36	0,67																																							
15	GAN-29	0,67	0,66	0,68	0,65	0,66	0,62	0,64	0,62	0,65	0,62	0,32	0,37	0,66	0,28																																							
16	COR-14255	0,56	0,58	0,56	0,55	0,55	0,61	0,6	0,59	0,47	0,36	0,66	0,68	0,33	0,71	0,7																																						
17	COR-14247	0,54	0,57	0,54	0,55	0,55	0,62	0,64	0,61	0,45	0,42	0,66	0,68	0,28	0,7	0,68	0,3																																					
18	CPC-1514	0,65	0,64	0,62	0,69	0,65	0,6	0,61	0,61	0,59	0,56	0,66	0,66	0,58	0,67	0,7	0,65	0,59																																					
19	CIP-387675.8	0,65	0,64	0,62	0,68	0,65	0,59	0,62	0,62	0,61	0,56	0,66	0,65	0,56	0,68	0,67	0,56	0,61	0,33																																					
20	MENAGESHA	0,64	0,64	0,62	0,68	0,64	0,59	0,61	0,6	0,56	0,59	0,65	0,64	0,56	0,67	0,66	0,59	0,6	0,32	0,31																																					
21	CIP-389701.3	0,64	0,64	0,61	0,66	0,64	0,59	0,62	0,61	0,6	0,55	0,64	0,65	0,54	0,66	0,66	0,56	0,56	0,32	0,19	0,32																																					
22	KP-90108.5	0,64	0,63	0,61	0,67	0,64	0,59	0,61	0,62	0,61	0,56	0,64	65	0,56	0,68	0,66	0,67	0,56	0,33	0,24	0,33	0,23																																					
23	GENET	0,59	0,6	0,57	0,58	0,56	0,58	0,56	0,61	0,51	0,52	0,68	0,66	0,61	0,65	0,66	0,49	0,49	0,63	0,62	0,61	0,61	0,61																																					
24	KP-90143.5	0,66	0,65	0,64	0,65	0,65	0,6	0,63	0,61	0,64	0,62	0,62	0,59	0,63	0,6	0,61	0,66	0,64	0,66	0,66	0,66	0,64	0,66	0,61																																					
25	CIP-388423.13	0,66	0,66	0,65	0,66	0,66	0,61	0,63	0,6	0,62	0,62	0,64	0,61	0,62	0,61	0,62	0,67	0,62	0,59	0,66	0,69	0,66	0,66	0,61	0,24																																					
26	WOCHECHA	0,66	0,64	0,64	0,66	0,66	0,6	0,63	0,59	0,62	0,6	0,61	0,59	0,61	0,6	0,59	0,65	0,62	0,66	0,65	0,66	0,63	0,65	0,62	0,21	0,24																																					
27	CIP-387675.20	0,62	0,63	0,64	0,64	0,65	0,64	0,65	0,66	0,64	0,59	0,64	0,69	0,62	0,71	0,66	0,62	0,62	0,66	0,69	0,7	0,67	0,69	0,66	0,62	0,62	0,61																																					
28	KP-90134.5	0,56	0,61	0,58	0,61	0,61	0,6	0,61	0,62	0,59	0,59	0,65	0,67	0,58	0,66	0,66	0,58	0,58	0,61	0,65	0,64	0,63	0,65	0,61	0,59	0,62	0,61	0,54																																					
29	KP-90134.2	0,62	0,64	0,63	0,65	0,66	0,64	0,62	0,66	0,65	0,62	0,68	0,7	0,64	0,72	0,72	0,62	0,64	0,65	0,69	0,69	0,65	0,69	0,65	0,65	0,64	0,63	0,35	0,56																																					
30	KP-90136.12	0,62	0,63	0,64	0,65	0,65	0,65	0,65	0,57	0,65	0,61	0,65	0,66	0,64	0,7	0,68	0,63	0,63	0,66	0,69	0,69	0,67	0,69	0,67	0,61	0,61	0,59	0,3	0,65	0,31																																					
31	TOLCHA	0,56	0,61	0,59	0,61	0,6	0,63	0,62	0,65	0,57	0,58	0,67	0,69	0,58	0,68	0,69	0,56	0,64	0,66	0,7	0,69	0,68	0,7	0,69	0,61	0,62	0,61	0,56	0,31	0,56	0,66																																					
32	CIP-387675.24	0,66	0,65	0,66	0,68	0,67	0,64	0,64	0,64	0,64	0,65	0,68	0,68	0,6	0,68	0,69	0,61	0,61	0,65	0,64	0,66	0,64	0,64	0,62	0,59	0,56	0,6	0,63	0,65	0,65	0,68	0,64																																					
33	AWASH	0,69	0,69	0,65	0,7	0,68	0,66	0,66	0,65	0,66	0,66	0,67	0,68	0,61	0,66	0,66	0,62	0,62	0,66	0,64	0,67	0,64	0,64	0,65	0,59	0,58	0,59	0,66	0,66	0,66	0,66	0,65	0,17																																					
34	OCH-1933	0,6	0,61	0,59	0,61	0,59	0,62	0,63	0,64	0,55	0,52	0,62	0,61	0,63	0,59	0,62	0,55	0,51	0,62	0,61	0,61	0,59	0,6	0,47	0,57	0,56	0,56	0,67	0,66	0,69	0,66	0,51	0,61	0,62																																					
35	OCH-2064	0,61	0,62	0,62	0,62	0,61	0,64	0,62	0,65	0,56	0,56	0,62	0,62	0,56	0,6	0,62	0,58	0,55	0,66	0,66	0,65	0,64	0,65	0,5	0,56	0,56	0,58	0,67	0,58	0,69	0,66	0,62	0,61	0,61	0,3																																					
36	EBS-6	0,65	0,62	0,64	0,64	0,64	0,66	0,66	0,66	0,65	0,59	0,63	0,64	0,59	0,64	0,65	0,65	0,61	0,69	0,7	0,71	0,69	0,7	0,66	0,59	0,58	0,57	0,66	0,7	0,65	0,65	0,69	0,64	0,64	0,66	0,64																																					
37	EBS-1801	0,63	0,61	0,59	0,61	0,6	0,64	0,61	0,56	0,56	0,61	0,64	0,64	0,65	0,62	0,62	0,67	0,56	0,6	0,62	0,6	0,61	0,62	0,64	0,62	0,6	0,6	0,63	0,6	0,62	0,63	0,63	0,58	0,58	0,52	0,61	0,59																																					
38	BUFFLESPOORT	0,62	0,64	0,61	0,6	0,61	0,66	0,66	0,65	0,56	0,59	0,66	0,69	0,64	0,68	0,68	0,56	0,53	0,64	0,63	0,64	0,62	0,63	0,56	0,65	0,62	0,61	0,65	0,61	0,68	0,69	0,62	0,61	0,59	0,52	0,52	0,64	0,34																																					
39	DG-19,2	0,61	0,64	0,61	0,62	0,63	0,65	0,69	0,65	0,56	0,59	0,66	0,69	0,53	0,69	0,68	0,56	0,55	0,64	0,64	0,63	0,63	0,63	0,62	0,65	0,64	0,62	0,62	0,61	0,64	0,65	0,6	0,62	0,61	0,56	0,56	0,62	0,37	0,31																																					
40	DG-19,36	0,71	0,72	0,72	0,7	0,71	0,66	0,65	0,67	0,64	0,66	0,69	0,69	0,62	0,66	0,69	0,65	0,64	0,65	0,68	0,65	0,66	0,66	0,65	0,63	0,64	0,65	0,69	0,66	0,7	0,66	0,65	0,64	0,61	0,57	0,61	0,54	0,48	0,51																																					
41	DG-19,9	0,61	0,65	0,62	0,61	0,63	0,69	0,69	0,66	0,56	0,59	0,66	0,69	0,53	0,69	0,69	0,55	0,54	0,64	0,64	0,65	0,65	0,67	0,61	0,69	0,65	0,65	0,64	0,62	0,65	0,66	0,6	0,6	0,6	0,56	0,54	0,63	0,39	0,26	0,3	0,53																																					
42	DG-19,18	0,67	0,69	0,69	0,69	0,68	0,66	0,66	0,71	0,67	0,67	0,66	0,64	0,65	0,62	0,65	0,67	0,67	0,68	0,69	0,64	0,68	0,68	0,68	0,64	0,66	0,64	0,72	0,73	0,72	0,71	0,72	0,69	0,68	0,64	0,64	0,68	0,69	0,65	0,68	0,47	0,69																																					
43	DG-19,38	0,69	0,69	0,6	0,6	0,66	0,69	0,69	0,66	0,69	0,69	0,6	0,63	0,66	0,62	0,62	0,67	0,66	0,67	0,66	0,65	0,66	0,66	0,61	0,65	0,66	0,62	0,69	0,66	0,69	0,69	0,65	0,66	0,65	0,66	0,59	0,65	0,64	0,63	0,49	0,67	0,62	0,64																																					
44	DG-19,43	0,65	0,66	0,67	0,66	0,62	0,66	0,67	0,66	0,64	0,64	0,66	0,66	0,67	0,66	0,67	0,65	0,65	0,67	0,7	0,65	0,66	0,66	0,67	0,66	0,69	0,67	0,74	0,74	0,7	0,7	0,72	0,66	0,66	0,64	0,65	0,6	0,66	0,66	0,67	0,64	0,66	0,66	0,48	0,6																																					
45	DG-20,32	0,64	0,65	0,64	0,63	0,61	0,66	0,69	0,64	0,62	0,6	0,65	0,66	0,62	0,67	0,66	0,6	0,6	0,66	0,66	0,63	0,66	0,67	0,65	0,65	0,66	0,65	0,68	0,65	0,69	0,66	0,66	0,66	0,66	0,62	0,63	0,62	0,65	0,65	0,64	0,65	0,65	0,53	0,51	0,35																																					
46	DG-20,6	0,62	0,65	0,63	0,63	0,62	0,62	0,65	0,61	0,64	0,59	0,63	0,64	0,64	0,67	0,66	0,61	0,65	0,64	0,65	0,63	0,64	0,66	0,66	0,69	0,7	0,66	0,66	0,7	0,66	0,67	0,71	0,64	0,64	0,65	0,66	0,64	0,67	0,67	0,65	0,65	0,65	0,53	0,64	0,41	0,4																																					
47	DG-20,34	0,69	0,64	0,65	0,65	0,61	0,65	0,66	0,63	0,64	0,63	0,66	0,65	0,65	0,7	0,67	0,64	0,63	0,66	0,66	0,66	0,65	0,66	0,62	0,67	0,66	0,67	0,66	0,71	0,69	0,66	0,72	0,64	0,63	0,67	0,67	0,61	0,66	0,66	0,7	0,67	0,7	0,66	0,66	0,56	0,55	0,55																																					
48	DG-32,2	0,67	0,66	0,64	0,64	0,62	0,68	0,72	0,62	0,64	0,61	0,69	0,66	0,62	0,69	0,66	0,64	0,6	0,66	0,64	0,64	0,63	0,64	0,63	0,64	0,65	0,63	0,67	0,71	0,68	0,67	0,7	0,66	0,66	0,63	0,66	0,64	0,66	0,65	0,66	0,7	0,69	0,69	0,67	0,58	0,53	0,56	0,35																																					
49	DG-32,10	0,66	0,62	0,63	0,66	0,63	0,67	0,66	0,64	0,67	0,66	0,7	0,66	0,62	0,72	0,72	0,65	0,62	0,66	0,62	0,64	0,62	0,61	0,62	0,63	0,65	0,61	0,68	0,69	0,66	0,67	0,69	0,66	0,66	0,66	0,66	0,69	0,61	0,6	0,64	0,69	0,66	0,66	0,64	0,66	0,69	0,66	0,66	0,53																																					
50	DG-32,6	0,66	0,65	0,65	0,68	0,65	0,7	0,66	0,65	0,67	0,66	0,71	0,7	0,64	0,73	0,74	0,66	0,65	0,66	0,64	0,65	0,63	0,62	0,62	0,63	0,65	0,61	0,67	0,69	0,66	0,66	0,69	0,66	0,66	0,64	0,65	0,65	0,6	0,6	0,64	0,69	0,66	0,65	0,64	0,65	0,68	0,66	0,56	0,53	0,21																																					
51	LT-2	0,66	0,63	0,64	0,66	0,62	0,7	0,69	0,65	0,66	0,66	0,69	0,7	0,61	0,71	0,7	0,66	0,63	0,66	0,63	0,65	0,62	0,62	0,61	0,62	0,64	0,6	0,68	0,66	0,69	0,69	0,69	0,64	0,63	0,64	0,62	0,65	0,59	0,58	0,64	0,67	0,65	0,67	0,63	0,66	0,69	0,69	0,63	0,52	0,26	0,26																																					
52	LT-7	0,66	0,69	0,64	0,65	0,66	0,65	0,67	0,66	0,64	0,66	0,7	0,65	0,64	0,71	0,71	0,64	0,62	0,65	0,66	0,65	0,65	0,65	0,65	0,62	0,64	0,61	0,66	0,66	0,63	0,66	0,66	0,63	0,66	0,64	0,64	0,67	0,54	0,54	0,59	0,61	0,6	0,66	0,65	0,66	0,69	0,69	0,68	0,63	0,48	0,49	0,5																																					
53	DTO-33	0,64	0,69	0,61	0,64	0,65	0,68	0,68	0,66	0,63	0,64	0,68	0,64	0,61	0,69	0,69	0,64	0,62	0,64	0,64	0,62	0,62	0,63	0,64	0,6	0,61	0,59	0,66	0,65	0,67	0,65	0,69	0,63	0,64	0,61	0,62	0,68	0,54	0,54	0,56	0,61	0,6	0,65	0,64	0,66	0,66	0,66	0,66	0,62	0,48	0,49	0,49	0,17																																					

CHAPTER V

GENETIC DISTANCE ANALYSIS OF DIFFERENT POTATO GENOTYPES (*Solanum* Species) USING SIMPLE SEQUENCE REPEATS (SSRs) MARKERS

Abstract

Knowledge of the relative genetic distance and evolutionary relationship between a crop and its closely related wild species is essential for an efficient sampling of available genetic diversity and its organization. To this end the potential of microsatellite markers for use in genetic distance and evolutionary relationship studies of 53 different potato genotypes was evaluated. Three single locus primer pairs were used to PCR amplify three single locus specific DNA. A total of 218 scorable polymorphic bands were generated. These primers detected on average between 5 to 8 polymorphic alleles at each locus. Heterozygosity values per locus ranged from 48% to 79%. A dendrogram depicting the genetic distance of the genotypes was also constructed from the phenetic analysis of the derived information. As a result three main clusters and three singletons were identified. The potential of microsatellite markers to determine genetic distances and evolutionary relationships in potato is discussed in the context of these results.

5.1 Introduction

Simple sequence repeats (SSRs) are a relatively new class of DNA markers. Jacob *et al.* (1991) were the first to coin the term SSR to such reiterated sequences of DNA. Edwards *et al.* (1992) called them short tandem repeats while Litt and Luty (1989) used the term microsatellites. SSRs or microsatellites are short nucleotide repeats of 2-6 base pair (bp) sequences (Weising *et al.*, 1991) that are ubiquitously interspersed in the eukaryotic genome (Tautz and Renz, 1984; Zietkiewicz *et al.*, 1994) and are a major source of genetic variation (Tautz, 1989). SSRs display high level of polymorphism due to variation in repeat length. Litt and Luty (1989) demonstrated the high polymorphic quality of SSRs of up to 12 alleles at some loci in the human genome. Wu and Tanksley (1993) made similar observation in rice and Rongwen *et al.* (1995) in soybean. Stuss and Plieske (1998) reported the presence of multiple

alleles in barely. The multi-allelic nature of microsatellites renders this type of marker useful for determining genetic variation in any species (Smulders *et al.*, 1997; Condit *et al.*, 1991; Plaschke *et al.*, 1995; Chavarriaga-Aguirre *et al.*, 1999).

SSRs are also useful for identity testing (Beyermann *et al.*, 1992) and genome mapping (Tautz, 1989; Wu and Tanksley, 1993; Taramino and Tingey, 1996; Roder *et al.*, 1998a). DNA markers such as SSRs with high polymorphic information content (PIC) are especially crucial for genetic comparison of organisms with a narrow genetic base (Roder *et al.*, 1998b; Smulders *et al.*, 1997; Rongwen *et al.*, 1995). Moreover, the codominant mode of inheritance of SSR markers (Beckmann and Soller, 1990) gives them an advantage over dominant marker types as easy transferable markers between different crosses (Thomas and Scott, 1993).

Microsatellites or SSR loci can be readily amplified by the PCR using primers, which are complementary to the regions flanking the repeats (Akkaya *et al.*, 1992; Lagercrantz *et al.*, 1993; Rongwen *et al.*, 1995; Senior *et al.*, 1998). The resulting PCR products are resolved electrophoretically in denaturing polyacrylamide gels (Wu *et al.*, 1994). In plants, it has been demonstrated that microsatellites are highly informative and locus specific markers. This has been reported for many plant species including potatoes (Bell and Ecker, 1994; Morchen *et al.*, 1996; Taramino and Tingey, 1996; Provan *et al.*, 1996a, b; Struss and Pliesk, 1998; Westman and Kresovich, 1999).

In 1990, Hawkes documented thoroughly the biosystematic, species organization and evolutionary relationship of the potato crop based on morphological diversity analysis. In this report it was hypothesized that there is a very great difference between the large number of wild species known and the actual number used in breeding. He noted that currently, of the total 128 wild species of potato maintained in gene banks, only 13 species have actually frequently contributed germplasm to commercial varieties thus far developed. Ross (1986) also reported that of 127 European varieties examined for their parentage 97 contain genes from one or more of these 13 species. It comes as rather a shock to be reminded that 235 species of wild and cultivated potatoes are recognized but that only 13 have actually contributed to potato cultivar development.

Ghislain *et al.* (1997) reported the discrepancy between the huge number of potato accessions maintained and the limited funding for their characterization and evaluation to be used in breeding. Unevaluated materials in the collections are useless unless their useful characters are known. Hence, this study was initiated with the main objective of investigating the genetic distances and evolutionary relationships amongst seven of the 13 frequently used potato species in potato breeding.

5.2 Materials and methods

5.2.1 Genetic materials

A total of 53 genotypes consisting of Ethiopian commercial cultivars converted from European cultivars, advanced breeding clones of CIP germplasm, wild and cultivated diploid genotypes and dihaploids of tetraploids were studied. These materials comprise seven of the 13 different potato species that are frequently used in potato breeding (Table 5.1).

5.2.2 DNA Extraction

DNA was extracted from fresh young tissues based on the modified monocot protocol of Edwards *et al.* (1991). Young plant leaf tissue collected from one plant of each clone was used to isolate DNA for fingerprinting analysis. Tissue was frozen with liquid nitrogen and ground into a fine powder using a mortar and pestle. Ten ml of extraction buffer (1 M Tris-HCl pH 8; 0.25 M EDTA and 20% SDS) was added to each sample and then incubated at 65°C for half an hour with inversion every ten minutes, thereafter, 1 ml Cetyl triethyl ammonium bromide (CTAB) (10% w/v) and 2 ml 5 M NaCl were added to the homogenate and incubated for a further hour with gentle agitation every 10 minutes. Chloroform-isoamyl alcohol (24:1) extractions were performed at room temperature with centrifugation at 10 000 rpm for 15 minutes to remove proteins. After centrifugation the DNA was percipitated by the addition 2 volumes (v/v) of ethanol and stored at 4°C. The precipitated DNA was spooled and washed with 70% ethanol to remove residual salt before redissolving in sterile double distilled water and stored at 4°C. DNA

concentration was estimated using UV spectrophotometery. Then the DNA solution was diluted to a working concentration of 100 ng μ L^{-1} and stored at 4°C. Finally the integrity and concentration of the DNA was confirmed by agarose electrophoresis and visualized with ethidium bromide staining under UV light.

Table 5.1 List of potato genotypes used for genetic analysis.

Treatment Number	Species name	Plant identity Number	Country of origin	Ploidy level	Source	Status
1	Solanum stenotomum	CPC-1839	Peru	2n=24	VOPI	Cultivated
2	*S. stenotomum*	GAN-56	Bolivia	2n=24	VOPI	Cultivated
3	*S. stenotomum*	GAN-30	Bolivia	2n=24	VOPI	Cultivated
4	*S. stenotomum*	GAN-29	Bolivia	2n=24	VOPI	Cultivated
5	*S. stenotomum*	GAN-19	Bolivia	2n=24	VOPI	Cultivated
6	*S.stenotomum*	CPC-1514	Peru	2n=24	VOPI	Cultivated
7	*S.stenotomum*	OCH-1922	Peru	2n=24	VOPI	Cultivated
8	*S. stenotomum*	OCH-1933	Peru	2n=24	VOPI	Cultivated
9	*S. stenotomum*	CPC-1793	Peru	2n=24	VOPI	Cultivated
10	*S. sparsipilum*	EBS-1890	Peru	2n=24	VOPI	Cultivated
11	*S. sparsipilum*	UGT-905	Peru	2n=24	VOPI	Wild
12	*S. sparsipilum*	OCH-2064	Peru	2n=24	VOPI	Wild
13	*S. sparsipilum*	EBS-1801	Bolivia	2n=24	VOPI	Wild
14	*S. canasense*	189219x189218	Hybrid	2n=24	VOPI	Wild
15	*S. stoloniferum*	CPC-2093	Mexico	2n=48	VOPI	Cultivated
16	*S.stoloniferum*	CPC-2092	Mexico	2n=48	VOPI	Cultivated
17	*S. stoloniferum*	CPC-28.4	Mexico	2n=48	VOPI	Cultivated
18	*S.stoloniferum*	COR-14247	Mexico	2n=48	VOPI	Cultivated
19	*S. stoloniferum*	HAW-1293	Mexico	2n=48	VOPI	Cultivated
20	*S. stoloniferum*	COR-14213	Mexico	2n=48	VOPI	Cultivated
21	*S.tuberosum ssp..andigena*	COR-14255	Mexico	2n=48	VOPI	Cultivated
22	*S. acaule*	EBS-9	Argentina	2n=48	VOPI	Cultivated
23	*S. tuberosum*	CIP-387675.8	CIP Peru	2n=48	Ethiopia	Cultivated
24	*S. tuberosum*	CIP-389701.3	CIP Peru	2n=48	Ethiopia	Cultivated
25	*S.tuberosum*	CIP-387675.20	CIP Peru	2n=48	Ethiopia	Cultivated
26	*S.tuberosum*	CIP-387676.24	CIP Peru	2n=48	Ethiopia	Cultivated
27	*S.tuberosum*	CIP-386423.13	CIP Peru	2n=48	Ethiopia	Cultivated
28	*S.tuberosum*	CIP-378501.3	CIP Peru	2n=48	Ethiopia	Cultivated
29	*S.tuberosum*	MENAGESHA	CIP Peru	2n=48	Ethiopia	Cultivated
30	*S.tuberosum*	TOLCHA	CIP Peru	2n=48	Ethiopia	Cultivated
31	*S.tuberosum*	WOCHECHA	CIP Peru	2n=48	Ethiopia	Cultivated
32	*S.tuberosum*	GENET	CIP Peru	2n=48	Ethiopia	Cultivated
33	*S.tuberosum*	KP-90108.5	CIP Peru	2n=48	Ethiopia	Cultivated
34	*S.tuberosum*	KP-90143.5	CIP Peru	2n=48	Ethiopia	Cultivated
35	*S.tuberosum*	KP-90134.5	CIP Peru	2n=48	Ethiopia	Cultivated
36	*S.tuberosum*	KP-90134.2	CIP Peru	2n=48	Ethiopia	Cultivated
37	*S.tuberosum*	KP-90138.12	CIP Peru	2n=48	Ethiopia	Cultivated
38	*S.tuberosum*	DG-18.2	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
39	*S.tubrosum*	DG-18.32	ARC_Roodplaat SA	Dihaploids,	VOPI	Reduced genome
40	*S.tuberosum*	DG-18.9	ARC_Roodplaat SA	Dihaploids,	VOPI	Reduced genome
41	*S.tuberosum*	DG-19.18	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
42	*S.tuberosum*	DG-19.38	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
43	*S.tuberosum*	DG-19.43	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
44	*S.tuberosum*	DG-20.32	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
45	*S.tuberosum*	DG-20.34	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
46	*S.tuberosum*	DG-20.6	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
47	*S.tuberosum*	DG-32.10	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
48	*S.tuberosum*	DG-32.2	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
49	*S.tuberosum*	DG-32.8	ARC_Roodplaat SA	Dihaploids	VOPI	Reduced genome
50	*S.tuberosum*	DTO-33	USA	2n=4x	VOPI	Cultivated
51	*S.tuberosum*	LT-2	Peru	2n=4x	VOPI	Cultivated
52	*S.tuberosum*	LT-7	Peru	2n=4x	VOPI	Cultivated
53	*S.tuberosum*	Buffelspoort	ARC_Roodplaat SA	2n=4x	VOPI	Cultivated

5.2.3 SSR characterization

PCR was carried out in a total reaction volume of 25 μL containing 2.5 μL 10x PCR buffer (20 mM Tris HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), 1 unit of Taq DNA polymerase (Gibco), 10-pica mole (0.5 μM) of each primer (Table 5.1) and 20 ng template DNA. Amplifications were performed in Hybaid thermal cycler using the following parameters: one cycle of 4 min denaturation at 95°C, 40 sec annealing at 55°C and 2 min extension at 72°C; 35 cycles of 20 sec denaturation at 95°C, 40 sec annealing at 55°C, and 2 min extension at 72°C followed by a final extension at 72°C for 4 min. Amplicons were separated in a vertical 10% PAGE gel in 1xTAE buffer at 70 V for 8 hours. After electrophoresis, the gel was stained with ethidium bromide and visiualized by UV illumination. The DNA profiles were documented and scored manually by assigning a value of 1 for band presence and 0 for band absence. The scores of band presence or absence were used to calculate a pairwise genetic distance matrix using the Eculidean distance formula of Excoffier *et al.* (1992).

$$E = n\,[1-(2n_{xy}/2n)]$$

Where, $2n_{xy}$ equals the number of shared bands and n equals the total number of banding positions. Finally a phenogram summarising the genetic distance between the genotypes studied was constructed based on the most commonly used clustering algorithm of unweighted pair-group method using an arithmetic average (UPGMA) cluster analysis of the genetic distance matrix by Number Cruncher Statistical System for Windows (NCSS 2000).

In total, n (n-1)/2 or 1378 pairwise comparisons were made of the SSR DNA products obtained from the 53 genotypes. The level of polymorphism of each locus was also computed using the genetic diversity index formula of Shaghai Maroof *et al.* (1994),

$PIC=1-\sum p_i^{\,2}$, where PIC is polymorphic information content and p_i is the frequency of the i^{th}

SSR allele.

Table 5.2- Microsatellite primer sequences used, number of alleles, range of allele size, and thegene diversity obtained in this study

Name	Associated gene	Simple sequence Repeat	Primer sequence (5'-3')	Number of alleles detected	Gene diversity	Range of allele size s (bp)	Total number of alleles scored
STWIN 12 G	Potato wound-induced genes WIN1 and WIN 2	(TGAAA)2(ATA)6	TGTTGATTGTGGTGATAA TGTTGGACGTGACTTGTA	5	0.48	<100 – 400 bp	59
STS1+2	Starch synthase	(TCAC)m	TCTCTTGACACGTGTCACTGAAAC TCACCGATTACAGTAGCGCAAGAGA	7	0.77	<100 – 500 bp	74
STS1+3	Starch synthase	(TCAC)m.(CTT)n	TCTCTTGACACGTGTCACTGAAAC TTGCCATGTGATGTGTGGTCTACAA	8	0.79	350 – 700 bp	85

5.3 Results

The three-microsatellite primer pairs (Table 5.2) amplified a total of 218 clearly scorable bands. The number of amplified DNA products observed, ranged between zero to two at the (TGAAA)2(ATA)6 locus, zero to four at the (TCAC)m and (TCAC)m. (CTT)n loci for the individual genotypes. The number of alleles detected at each of the three loci varied with a total of number of five alleles detected at (TGAAA)2 (ATA)6 locus, seven alleles at the (TCAC)m locus and eight alleles at the (TCAC)m (CTT)n locus.

Diversity values of 0.48, 0.77 and 0.79 were computed for the (TGAAA)2 (ATA)6, (TCAC)m and (TCAC)m (CTT)n SSRs, respectively, amongest the genotypes tested. Furthermore, the amplified SSRs loci were capable of distinguishing 47 of 53 potato genotypes studied. The six genotypes that could not be distinguished are clonal and therefore genetically identical or only slightly dissimilar. The microsatellite locus (TGAAA)2 (ATA)6 distinguished the lowest number of genotypes examined (8) as reflected by the heterozygosity or the polymorphism value (Table 5.1), while (TCAC)m and (TCAC)m (CTT)n distinguished 18 and 21 of the 53 genotypes studied, respectively. The heterozygosity of the latter loci demonstrats the high potential of these loci in detecting genotypic differences as compared to the first locus.

The pairwise genetic distance estimates computed for the 53 genotypes based on SSRs are shown in Table 5.2. The genetic distance ranged between 0.00 and 0.84. A 100 % similarity for the microsatellite loci tested was observed for DG-32.2 and DG-20.6, DG-20.34 and DG-18.9, LT-2, DG-32.8 and DG-32.10, OCH-1933 and Tolcha, Haw-1293 and CPC-1793, Buffelspoort and EBS-9 and OCH-2064 and EBS-1890. Conversely, the greatest genetic distance of 0.84 was observed between KP-90134.5 (*S. tuberosum subsp. tuberosum*) and COR-14213 (*S. stoloniferum*) (Table 5.3).

A dendrogram depicting the genetic distance between the 53 genotypes studied was constructed from the distance matrix using the UPGMA clustering method (Fig. 5.1). Goodness of fit of the dendrogram constructed was confirmed by the co-phenetic correlation r=0.83. The 53 different potato genotypes were grouped into three main clusters and three singletons at 0.535 genetic distance (Fig. 5.1).

The first cluster is comprised of five genotypes, i.e., Wochecha, Menagesha, CIP-386423.13, CIP-387675.20 and KP-90143.5. This cluster contained only genotypes belonging to the species *Solanum tuberosum subsp tuberosum*. The second cluster consisted of the lowest number of genotypes, GAN-30 (*S. stenotomum*) and COR-14213 (*S. stoloniferum*). The genetic distance between these two genotypes is 0.45. The third cluster contained the largest number of the tested genotypes. This cluster is further sub-divided into two sub-clusters, A and B. The sub-cluster A contains A_1 and A_2. Most of the genotypes in A_1 are clonal dihaploid materials namely DG-32.2, DG-20.6, DG-20.34, DG-18.9, DG-19.38, DG-19.18, DG-18.36, DG-18.2, DG-18.36, DG-32.8, DG-32.10, of *Solanum tuberosum subsp. tuberosum*, CPC-1514 (*S. stenotomum*), OCH-1922 (*S. stenotomum*), 189218 x 189219 (*S. canasense*), and COR-14247 (*S. stoloniferum*). The tetraploid COR-14247 (*Solanum stoloniferum*) included in this subgroup is relatively distant from the other genotypes. A-2 contains genotypes that are relatively distant from A-1. These genotypes are CPC-2093 (*Solanum stoloniferum*), GAN-56 (*S. stenotomum*), DG-20.32 (*S.tuberosum subsp. tuberosum*) and DTO-33 (*S.tuberosum subsp. tuberosum*). Sub-cluster B of the third main cluster is grouped into B-1, B_2, B-3 and B-4. Consequently, B-1 contains the genotype Gan-19 (*S. stenotomum*) and CPC-28.4 (*S.*

stoloniferum) that are distant to the others in this sub-group. B-2 consists of 3 genotypes, viz., Genet, CIP-389701.3, and CIP-387675.8. The latter two CIP genotypes are closer to each other than to Genet. All of these genotypes belong to *Solanum tuberosum subsp. tuberosum.* B-3- contains CIP-387676.24, Och-1933, Tolcha and KP-90138.12. All the genotypes belonging to this sub group are of *S. tuberosum subsp. tuberosum* with the exception of Och-1933 is which belongs to the species *Solanum stenotomum.* Of these groups Tolcha and Och-1933 had an identical allelic profile that gave them a 0% dissimilarity value. B-4 consists of CPC-1839 (*S. stenotomum*), Och-2064 (*S. sparsipilum*), EBS-1890 (*S. sparsipilum*), Bufflespoort (*S.tuberosum subsp. tuberosum* dihaploid), CPC-1793 (*S.stenotomum*), HAW-1293 (*S.stoloniferum*), KP-90108.5 (*S. tuberosum subsp. tuberosum*), EBS-9 (*S. acaule*), Gan-29 (*S. stenotomum*), COR-14255 (*S. tuberosum subsp. andigena*), and the two distantly linked UGT-905 and EBS-1801. The three distantly liked singletons genotypes are Awash, KP-90134.5 and KP-90134.2, belonging to *Solanum tuberosum subsp. tuberosum.*

5.4 Discussion

Out of a total number of 53 genotypes representing seven species of potato, unique DNA profiles were produced for 47 of these using three SSR loci associated with the starch synthase and potato wound-induced genes. Similar findings have been reported by other researchers (Kawchuk *et al.*, 1996; Provan *et al.*, 1996a,b; McGregor *et al.*, 2000). Taramino and Tingey (1996) noted the absence of correlation between the number of alleles generated and compound repeats. Hence, the most important care to be taken is in the selection of microsatellite loci with high level of polymorphism (Rongwen, 1995).

The genetic distance values amongst the 53 genotypes of the seven different potato species varied from 0.00 to 0.84 as indicated on Table 5.3. A 100% similarity value was observed between HAW-1293 and CPC-1793, Och-1933 and Tolcha, EBS-9 and KP-90108.5, DG-19.18, DG-19.38 and DG-18.36, DG-20.34, DG-32.8 and LT-2, and DG-32.2 and DG-20.6. While some of these genotypes, especially those designated as DG may be synonyms, it is not reasonable to arrive at a conclusion that the others are as well. The most likely explanation to this is that the number of base pairs a eukaryotic haploid

genome is claimed to contain will impede the identification of these genomes as unique. Therefore, further analysis is needed using primers that are known to detect loci in various genomic positions.

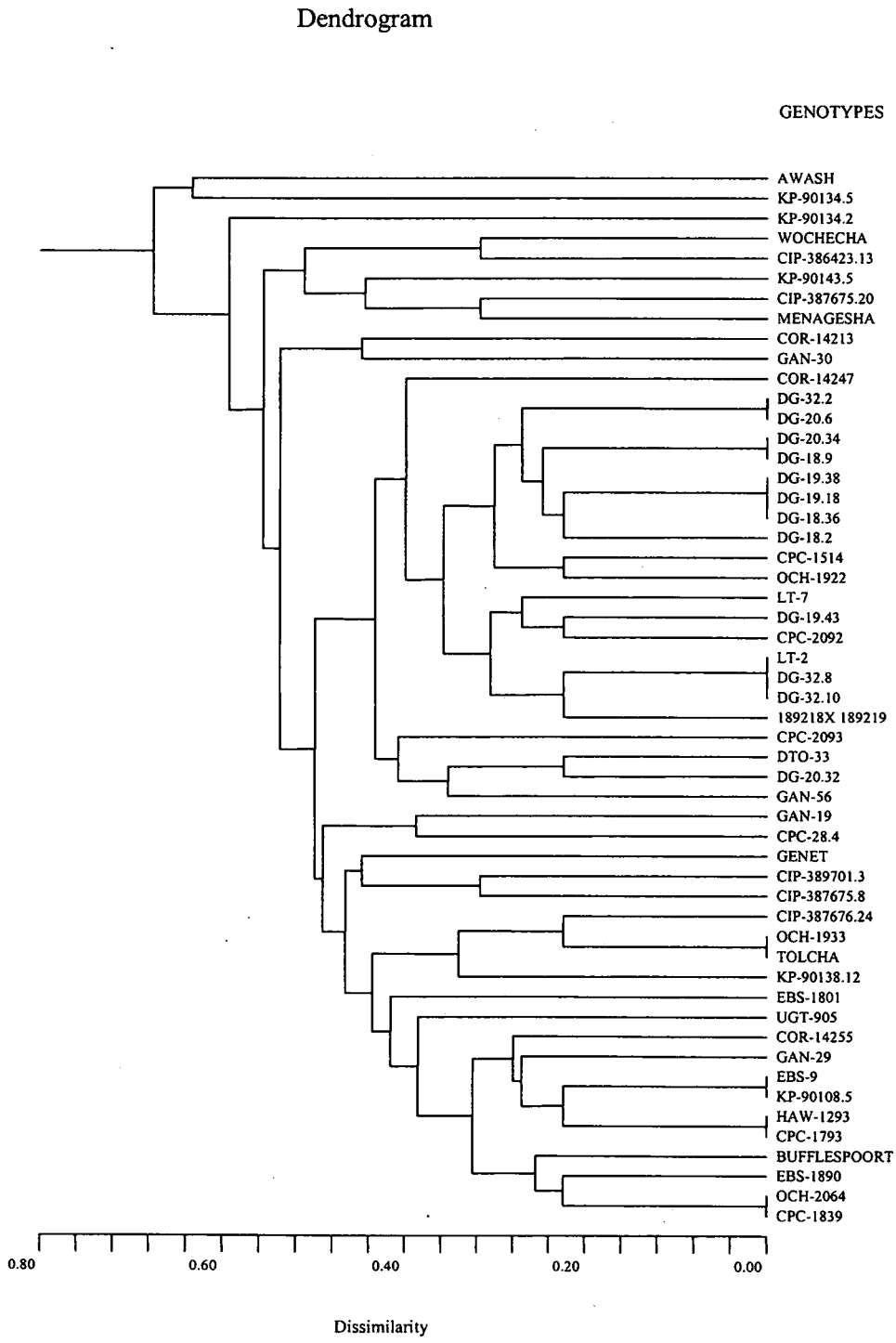

Figure 5.1 Dendrogram showing the genetic distance between 53 potato genotypes constructed based on 218 SSR Data using UPGMA clustering method. Clusters

are dentified using a 0.535 genetic distance as a cut-off point.

It is also interesting to note the close relationship between Och-1933, a cultivated diploid (*S. stenotomum)*, and the commercial cultivar Tolcha, (*S. tuberosum subsp tuberosum*). Similarly, between GAN-29 (*Solanum stenotomum*) and COR-14255 (*Solanum tuberosum subsp. andigena*).

This result clearly agrees with the current hypotheses of Cribb and Hawkes (1986) who strongly argue that *S. stenotomum* is the partial ancester of the tetraploid cultivated potato, *S. tuberosum subsp. andigena*. The identical SSR allelic profile observed between EBS-9 (*S. acaule*) and KP-90108.5 (*S. tuberosum*) for these three SSR loci may be the result of the frequent use of *S. acaule* in breeding programs for introducing traits of the potato virus X, spindle tuber viroid, nematode, frost, heat and drought resistance at CIP (Hawkes, 1990). Senior *et al.* (1998) reported on the role that selection plays in the DNA markers found in genotypes not related through descent.

It is interesting to note the distant and separate grouping of more than half the commercial cultivar and advanced breeding material from the dihaploid and diploid genotypes. Although four genomes or chromosomes are scanned under the tetrasomic tetraploid genotypes in contrast to disomic dihaploid and diploid genotypes, the primer pairs used in this study revealed clearly different allelic profiles. However, this is in agreement with the findings of Crib and Hawkes (1986) on the allotetraploid origin of *Solanum tubersum*. The grouping of the Mexican polyploid *S. stoloniferum*, *S. canasense* (189218x189219), *S. stenotomum*, *S. sparsipilum* (the putative partial ancestor of the cultivated potato *S. tuberosum subsp. andigena*), and *S.tuberosum* tetraploid and its dihaploids under the same main cluster is in agreement with the previous reports of Hosaka *et al.* (1984) and Debener *et al.* (1990).

Within species comparison for the genetic distance indicated the existence of a relatively greater diversity among the *S. tuberosum subsp tuberosum* genotypes. The genetic distance between these species ranged from 0.22 to 0.78. The most likely explanation for this variability is the result of the use of diverse genetic parental material in their development. The vegetative propagation method of this crop in fixing certain combinations of desirable genes in contrasted to sexual reproduction in which segregation is a common phenomenon, as reported by Caligari (1992) and Poehlman and Sleper (1995) also had a paramount effect. *S. stenotomum* and *S. sparsipilum* are the putative ancestor of *S.*

tuberosum andigena, and have a genetic distance distribution of 0.22 to 0.54 clearly indicates the low diversity among them. The genetic distance of 0.45 within *S. stoloniferum* is agreement with Hawkes (1990) description of this species as a very variable species with very wide distribution. Furthermore, it is interesting to note that the maximal genetic distance of 0.84 between *S. tuberosum subsp tuberosum* and *S. stoloniferum*. This result between these two geographically and ecologically distant genotypes correlates to Hawkes (1990) species organization and hypothesis that potatoes geographical and ecological isolation has a major contribution in the speciation of potatoes.

In conclusion the microsatellite markers used in this study provided a positive assessment of the ability of SSR markers to identify different potato genotypes. With careful selection of microsatellit loci for high level of polymorphism and coverage using representative loci for various genomic positions, it is very likely that this marker system will have the advantage of distinguishing closely related cultivars arising from a similar pedigree. Therefore, site-specific amplified SSRs represent an unlimited source of easily examined genetic markers for genetic distance analysis. Furthermore, this molecular data confirm Hawkes' (1990) hypothesis concerning the evolutionary relationship of potato and the number of wild and indigenous cultivars used in potato breeding as well as the report on the gene pool composition of modern potato varieties. Hence, the association observed between the studied modern varieties and other species believed to be distant from one another is not surprising as there is a continuous process of transfer of useful genes from wild species and primitive cultivars to the developed genetic backgrounds of highly selected breeding lines.

Table- 5.2 Genetic distance matrix of the 53 different potato genotypes using Euclidean distance analysis of simple sequence repeat DNA data.

No.	GENOTYPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	CPC-1839																																																				
2	UGT-906	0.45																																																				
3	CPC-1793	0.39	0.39																																																		
4	HAM-1293	0.39	0.39	0																																																
5	EBS-1890	0.22	0.39	0.32	0.32																																															
6	GAN-66	0.59	0.6	0.55	0.55	0.55																																														
7	189218X189219	0.55	0.55	0.6	0.6	0.6	0.39																																												
8	CPC-2092	0.55	0.55	0.6	0.6	0.6	0.6	0.32																																											
9	GAN-30	0.32	0.45	0.6	0.6	0.39	0.6	0.63	0.63																																										
10	OCH-1922	0.39	0.39	0.32	0.32	0.32	0.45	0.39	0.39	0.6																																								
11	CPC-2093	0.63	0.55	0.59	0.59	0.59	0.39	0.45	0.45	0.55	0.6																																							
12	CPC-28.4	0.6	0.6	0.45	0.45	0.45	0.55	0.6	0.6	0.6	0.45	0.6																																						
13	COR-14213	0.55	0.45	0.6	0.6	0.6	0.6	0.63	0.63	0.45	0.6	0.45	0.6																																					
14	GAN-19	0.55	0.55	0.6	0.6	0.6	0.59	0.63	0.55	0.55	0.6	0.55	0.39	0.45																																				
15	GAN-29	0.39	0.39	0.32	0.32	0.32	0.55	0.6	0.39	0.6	0.32	0.59	0.45	0.6	0.39																																				
16	COR-14255	0.39	0.39	0.32	0.32	0.32	0.55	0.6	0.6	0.6	0.32	0.59	0.45	0.39	0.6	0.32																																		
17	COR-14247	0.55	0.55	0.6	0.6	0.6	0.6	0.45	0.45	0.63	0.39	0.45	0.59	0.55	0.55	0.6	0.6																																	
18	CPC-1814	0.45	0.45	0.39	0.39	0.39	0.6	0.45	0.45	0.55	0.22	0.55	0.6	0.55	0.55	0.39	0.39	0.45																																	
19	CIP-397675.8	0.45	0.45	0.39	0.39	0.39	0.59	0.45	0.45	0.55	0.39	0.45	0.39	0.45	0.45	0.39	0.39	0.45	0.45																															
20	MENAGESHA	0.59	0.59	0.55	0.55	0.55	0.63	0.59	0.6	0.59	0.55	0.59	0.45	0.59	0.6	0.45	0.55	0.67	0.59	0.6																														
21	CIP-389701.3	0.55	0.55	0.39	0.39	0.6	0.67	0.55	0.55	0.63	0.6	0.55	0.6	0.55	0.55	0.6	0.6	0.55	0.45	0.32	0.59																														
22	KP-90199.6	0.32	0.32	0.22	0.22	0.22	0.6	0.45	0.45	0.45	0.22	0.55	0.39	0.45	0.45	0.22	0.22	0.45	0.32	0.32	0.6	0.45																													
23	GENET	0.55	0.55	0.6	0.6	0.6	0.67	0.55	0.45	0.63	0.6	0.63	0.6	0.55	0.55	0.39	0.39	0.63	0.45	0.45	0.6	0.45	0.45																													
24	KP-90143.5	0.63	0.63	0.59	0.59	0.59	0.67	0.55	0.45	0.71	0.6	0.63	0.59	0.71	0.63	0.6	0.59	0.63	0.55	0.55	0.39	0.63	0.55	0.55																													
25	CIP-388423.13	0.59	0.59	0.55	0.55	0.55	0.71	0.59	0.6	0.67	0.55	0.67	0.55	0.67	0.59	0.45	0.55	0.67	0.59	0.6	0.45	0.59	0.6	0.6	0.39																													
26	WOCHECHA	0.59	0.59	0.55	0.55	0.55	0.71	0.59	0.59	0.67	0.55	0.59	0.55	0.59	0.59	0.55	0.55	0.59	0.59	0.39	0.55	0.6	0.6	0.59	0.6	0.32																													
27	CIP-397675.20	0.6	0.6	0.45	0.45	0.45	0.55	0.59	0.6	0.6	0.45	0.59	0.45	0.6	0.39	0.32	0.45	0.59	0.6	0.6	0.32	0.59	0.39	0.6	0.6	0.55	0.63																													
28	KP-90134.6	0.71	0.77	0.67	0.67	0.74	0.81	0.71	0.63	0.77	0.67	0.77	0.74	0.84	0.77	0.67	0.74	0.77	0.63	0.71	0.59	0.63	0.71	0.63	0.45	0.6	0.59	0.67																													
29	KP-90134.2	0.59	0.67	0.63	0.63	0.55	0.71	0.59	0.59	0.67	0.55	0.59	0.63	0.67	0.67	0.63	0.63	0.6	0.6	0.6	0.71	0.6	0.59	0.59	0.59	0.63	0.55	0.71	0.67																													
30	KP-90139.12	0.6	0.59	0.55	0.55	0.45	0.71	0.59	0.6	0.59	0.55	0.67	0.55	0.67	0.59	0.45	0.55	0.59	0.59	0.6	0.55	0.59	0.6	0.6	0.6	0.45	0.55	0.55	0.67	0.45																													
31	TOLCHA	0.39	0.6	0.45	0.45	0.32	0.63	0.6	0.6	0.6	0.45	0.59	0.45	0.59	0.59	0.45	0.45	0.6	0.6	0.39	0.55	0.6	0.39	0.6	0.59	0.55	0.55	0.55	0.74	0.45	0.32																													
32	CIP-397675.24	0.32	0.45	0.39	0.39	0.22	0.59	0.55	0.55	0.45	0.39	0.63	0.6	0.55	0.55	0.39	0.39	0.45	0.45	0.45	0.59	0.55	0.32	0.55	0.63	0.59	0.59	0.6	0.78	0.6	0.39	0.22																											
33	AWASH	0.63	0.71	0.59	0.59	0.59	0.74	0.77	0.71	0.63	0.67	0.77	0.67	0.71	0.63	0.59	0.67	0.71	0.63	0.71	0.6	0.63	0.59	0.63	0.63	0.67	0.74	0.6	0.59	0.67	0.59	0.59	0.55																											
34	OCH-1933	0.39	0.6	0.45	0.45	0.32	0.63	0.6	0.6	0.6	0.45	0.59	0.45	0.59	0.59	0.45	0.45	0.6	0.6	0.45	0.55	0.6	0.39	0.6	0.59	0.55	0.55	0.55	0.74	0.45	0.32	0	0.22	0.59																											
35	OCH-2094	0	0.45	0.39	0.39	0.22	0.59	0.55	0.55	0.32	0.39	0.63	0.6	0.55	0.55	0.39	0.39	0.55	0.45	0.39	0.59	0.55	0.32	0.55	0.63	0.59	0.59	0.6	0.71	0.59	0.6	0.39	0.32	0.63	0.39																											
36	EBS-6	0.32	0.32	0.22	0.22	0.22	0.6	0.45	0.45	0.45	0.22	0.55	0.39	0.45	0.45	0.22	0.22	0.45	0.32	0.32	0.6	0.45	0	0.45	0.55	0.6	0.6	0.39	0.71	0.59	0.6	0.39	0.32	0.63	0.39	0.32																											
37	EBS-1901	0.6	0.6	0.32	0.32	0.45	0.63	0.59	0.6	0.59	0.45	0.67	0.55	0.59	0.6	0.32	0.45	0.59	0.6	0.6	0.45	0.6	0.39	0.6	0.6	0.45	0.55	0.45	0.59	0.71	0.55	0.55	0.6	0.6	0.55	0.6	0.39																											
38	BUFFLESPOORT	0.32	0.39	0.32	0.32	0.32	0.55	0.6	0.6	0.39	0.32	0.59	0.45	0.6	0.6	0.32	0.32	0.6	0.39	0.39	0.55	0.6	0.22	0.6	0.59	0.55	0.55	0.45	0.67	0.63	0.55	0.45	0.39	0.67	0.45	0.22	0.22	0.45																											
39	DG-18.2	0.39	0.6	0.45	0.45	0.45	0.45	0.39	0.39	0.6	0.32	0.6	0.55	0.59	0.59	0.45	0.45	0.39	0.39	0.6	0.63	0.59	0.39	0.59	0.59	0.63	0.63	0.55	0.67	0.63	0.63	0.55	0.6	0.74	0.55	0.39	0.39	0.55	0.32																											
40	DG-18.86	0.45	0.45	0.39	0.39	0.39	0.39	0.32	0.32	0.55	0.22	0.45	0.6	0.55	0.55	0.39	0.39	0.32	0.32	0.45	0.59	0.55	0.32	0.55	0.55	0.59	0.59	0.6	0.71	0.59	0.59	0.6	0.45	0.71	0.6	0.45	0.32	0.6	0.39	0.22																											
41	DG-18.9	0.6	0.6	0.45	0.45	0.45	0.45	0.39	0.39	0.59	0.32	0.6	0.55	0.59	0.59	0.45	0.45	0.39	0.22	0.6	0.63	0.6	0.39	0.6	0.59	0.63	0.63	0.55	0.67	0.55	0.63	0.55	0.6	0.67	0.55	0.6	0.39	0.55	0.45	0.32	0.22																											
42	DG-18.19	0.45	0.45	0.39	0.39	0.39	0.39	0.32	0.32	0.55	0.22	0.45	0.6	0.55	0.55	0.39	0.39	0.32	0.32	0.45	0.59	0.55	0.32	0.55	0.55	0.59	0.59	0.6	0.71	0.59	0.59	0.6	0.45	0.71	0.6	0.45	0.32	0.6	0.39	0.22	0	0.22																											
43	DG-18.38	0.45	0.45	0.39	0.39	0.39	0.39	0.32	0.32	0.55	0.22	0.45	0.6	0.55	0.55	0.39	0.39	0.32	0.32	0.45	0.59	0.55	0.32	0.55	0.55	0.59	0.59	0.6	0.71	0.59	0.59	0.6	0.45	0.71	0.6	0.45	0.32	0.6	0.39	0.22	0	0.22	0																											
44	DG-18.43	0.6	0.59	0.55	0.55	0.45	0.55	0.39	0.22	0.59	0.45	0.6	0.55	0.67	0.59	0.45	0.55	0.6	0.6	0.6	0.55	0.59	0.6	0.6	0.6	0.55	0.59	0.55	0.67	0.55	0.45	0.45	0.6	0.67	0.45	0.6	0.6	0.55	0.55	0.45	0.39	0.45	0.39	0.39																											
45	DG-20.32	0.63	0.63	0.59	0.59	0.59	0.39	0.32	0.45	0.63	0.6	0.45	0.6	0.63	0.63	0.59	0.59	0.55	0.55	0.55	0.6	0.63	0.55	0.63	0.55	0.67	0.67	0.6	0.71	0.67	0.67	0.59	0.63	0.71	0.59	0.63	0.55	0.67	0.59	0.6	0.45	0.6	0.45	0.45	0.6																											
46	DG-20.6	0.6	0.6	0.45	0.45	0.45	0.32	0.39	0.39	0.6	0.32	0.39	0.45	0.6	0.6	0.45	0.45	0.39	0.39	0.6	0.55	0.59	0.39	0.59	0.59	0.63	0.63	0.45	0.74	0.63	0.63	0.55	0.6	0.67	0.55	0.6	0.39	0.55	0.45	0.32	0.22	0.32	0.22	0.22	0.45	0.39																											
47	DG-20.34	0.6	0.6	0.45	0.45	0.45	0.45	0.39	0.39	0.59	0.32	0.6	0.55	0.59	0.59	0.45	0.45	0.39	0.22	0.6	0.63	0.6	0.39	0.6	0.59	0.63	0.63	0.55	0.67	0.59	0.63	0.55	0.6	0.67	0.55	0.6	0.39	0.55	0.45	0.32	0.22	0	0.22	0.22	0.45	0.6	0.32																											
48	DG-32.2	0.6	0.6	0.45	0.45	0.45	0.32	0.39	0.39	0.6	0.32	0.39	0.45	0.6	0.6	0.45	0.45	0.39	0.39	0.6	0.55	0.59	0.39	0.59	0.55	0.63	0.63	0.45	0.74	0.63	0.63	0.55	0.6	0.67	0.55	0.6	0.39	0.55	0.45	0.32	0.22	0.32	0.22	0.22	0.45	0.39	0	0.32																											
49	DG-20.10	0.6	0.6	0.45	0.45	0.45	0.45	0.22	0.22	0.59	0.32	0.39	0.45	0.59	0.59	0.45	0.45	0.39	0.39	0.39	0.55	0.6	0.39	0.6	0.6	0.55	0.55	0.55	0.67	0.55	0.55	0.45	0.6	0.74	0.45	0.6	0.39	0.55	0.45	0.32	0.22	0.32	0.22	0.22	0.32	0.39	0.32	0.32	0.32																											
50	DG-32.8	0.6	0.6	0.45	0.45	0.45	0.45	0.22	0.22	0.59	0.32	0.39	0.45	0.59	0.59	0.45	0.45	0.39	0.39	0.39	0.55	0.6	0.39	0.6	0.6	0.55	0.55	0.55	0.67	0.55	0.55	0.45	0.6	0.74	0.45	0.6	0.39	0.55	0.45	0.32	0.22	0.32	0.22	0.22	0.32	0.39	0.32	0.32	0.32	0																											
51	LT-2	0.6	0.6	0.45	0.45	0.45	0.45	0.22	0.22	0.59	0.32	0.39	0.45	0.59	0.59	0.45	0.45	0.39	0.39	0.39	0.55	0.6	0.39	0.6	0.6	0.55	0.55	0.55	0.67	0.55	0.55	0.45	0.6	0.74	0.45	0.6	0.39	0.55	0.45	0.32	0.22	0.32	0.22	0.22	0.32	0.39	0.32	0.32	0.32	0	0																											
52	LT-7	0.59	0.59	0.55	0.55	0.55	0.55	0.39	0.22	0.67	0.45	0.6	0.55	0.67	0.59	0.45	0.55	0.6	0.6	0.6	0.45	0.59	0.6	0.6	0.39	0.55	0.63	0.45	0.59	0.63	0.55	0.55	0.59	0.67	0.55	0.59	0.6	0.55	0.55	0.45	0.39	0.45	0.39	0.39	0.32	0.39	0.45	0.45	0.45	0.32	0.32	0.32																											
53	DTO-33	0.59	0.59	0.55	0.55	0.55	0.32	0.22	0.39	0.59	0.45	0.39	0.45	0.59	0.59	0.55	0.55	0.6	0.6	0.6	0.55	0.59	0.6	0.59	0.59	0.63	0.63	0.55	0.74	0.63	0.63	0.55	0.59	0.74	0.55	0.59	0.6	0.63	0.55	0.45	0.39	0.45	0.39	0.39	0.45	0.22	0.32	0.45	0.32	0.32	0.32	0.32	0.45																											

CHAPTER VI

SUMMARY

Genetic analysis of 53 potato genotypes representing seven of the 13 potato species that are frequently used in the varietal development of potato was examined using morphological and molecular markers with main objective of analyzing their genetic distance and evolutionary relationships. These genotypes were obtained from the Ethiopian National Potato Research Program and the ARC-Roodeplaat Vegetable and Ornamental Plant Institute of South Africa. These species are collections from the major diversity centres of potato, i.e., Peru, Argentina, Mexico and Bolivia. They include both wild and cultivated diploid and tetraploid genotypes.

Of the 53 genotypes only 15 genotypes consisting 11 advanced breeding clones and Ethiopian converted commercial tetraploid varieties were examined morphologically. As a result a genetic distance ranging from 0.46 to 1.68 was computed. This clearly indicates the phenotypic diversity existing among them and the diverse genetic background of their parental materials. A direct positive correlation was found between stem height and weight of tuber and days to flowering and days to harvesting as previously reported and negative associations between number of tubers per plant and average weight of a tuber and leaflet width and days to flowering.

AFLP and SSR analysis of these genotypes together with 38 other genotypes of different species supported the phenotypic diversity result with a genetic distance value ranging from 0.28 to 0.70 for AFLP and 0.28 to 0.78 for SSR. As expected the genetic distance computed using molecular data are more conservative in their estimate than those computed using morphological data. Furthermore, AFLP and SSR data revealed that *Solanum stenotomum* and *Solanum sparsipilum* might be the putative ancestor of the cultivated potato *Solanum tuberosum subsp. andigena* as previously hypothesized. However, the grouping of species based on DNA does not always indicate ancestry but is rather a result of introduced traits from wild species. This data clearly indicated those species that have contributed genes to potato cultivars thus far developed. Hence, with the use of morphological markers that are heritable and stable genetic distance could be successfully studied. This, however, does not

rule out the major drawbacks of morphological markers caused by the low level of heritable and stable characters.

In conclusion, this study has revealed the relevance of employing morphological markers together with molecular markers to determine genetic distances for potato breeding. Although there were differences in the ranges of genetic distances calculated using the different markers, there was a remarkable correlation between the relationships of the different genoptypes based on these distances. Hence, DNA marker systems with high multiplex ratio like AFLP and high diversity index like SSR can be an excellent component to the morphological and conventional markers that are currently used to estimate genetic diversity or distance and evolutionary relationships for potato.

OPSOMMING

Genetiese analise was uitgevoer op 53 aartappel genotipes verteenwoordigend van sewe uit 13 aartappel spesies wat in variëteit ontwikkeling gebruik word. Die doel van hierdie studie was die vergelyking van genotipes op morfologiese en molekulêre vlak, die bepaling van genetiese afstand tussen genotipes en daaropvolgende evolusionêre verwantskappe tussen hulle te ondersoek. Plant materiaal is ontvang vanaf die Ethiopiese-Nasionale-Aartappel-Navorsing-Program en die LNR-Roodeplaat Groente en Sierplant Instituut van Suid-Afrika en is verteenwoordigende versamelings van die hoof diversiteits bronne van aartappel naamlik Peru, Argentinië, Mexiko en Bolivia, insluitend wildetipes, gekweekte diploïedes en tetraploïedes.

Net 15 van die 53 genotipes, afkomstig van ontwikkelde teellyne en Ethiopiese tetraploïede variëteite was morfologies ondersoek. Daar is vasgestel dat die genetiese afstand tussen genotipes wissel tussen 0.46 tot 1.68. Dit dui op 'n breë diversiteit en asook die ouermateriaal waaruit hulle ontwikkel is. 'n Positiewe korrelasie is gevind tussen stamhoogte en gewig van knolle asook die hoeveelheid dae tot bloei en oes van knolle. 'n Negatiewe korrelasie is gevind tussen die hoeveelheid knolle per plant en gemiddelde gewig van knolle asook blaartjie wydte en dae tot bloei.

AFLP en SSR analise van hierdie genotipes tesame met 38 ander genotipes verteenwoordigend van verskillende spesies het die hoeveelheid fenetiese diversiteit ondersteun met 'n genetiese waarde van 0.28 tot 0.70 vir AFLPs en 0.28 tot 0.78 vir SSR analise. Soos verwag, is die genetiese afstande afkomstig vanaf molekulêre data meer konserwatief as ooreenkomstige waardes deur morfologie bepaal. AFLP en SSR data het ook verder getoon dat *Solanum stenotomum* en *Solanum sparsipilum* die moontlike voorouer van die gekweekte variëteit *Solanum tuberosum subsp. Andigena* is. Alhoewel, die verwantskappe aangetoon deur DNA analise nie altyd 'n aanduiding van afkoms is nie maar eerder a.g.v. ingeteelde eienskappe vanaf wildetipe spesies. Hierdie data gee dus 'n duidelike aanduiding van die spesies gebruik in die ontwikkeling van gekweekte ontwikkelde lyne. Dus, met behulp van stabiele morfologiese eienskappe kan betroubare genetiese

afstande tussen genotipes bepaal word. Nieteenstaande, is die gebruik van morfologie bemoeilik deur lae vlakke van duidelike oorerwing en stabiliteit.

Ten slotte, hierdie studie dui die belangrikheid van die gebruik van morfologiese eienskappe tesame met molekulêre eienskappe om genetiese afstande in aartappels te bepaal. Alhoewel daar gevind is dat verskillende merkers verskillende genetiese afstande getoon het, is gevind dat daar 'n merkwaardige ooreenstemming is tussen die verwantskappe soos deur die verskillende merkers aangetoon. DNA merker sisteme soos AFLPs, met veelvoudige lokus herkenning, en SSRs, met hoë diversiteits indeks, is ideaal geskik vir gebruik saam met morfologiese en konvensionele merkers soos tans gebruik om genetiese afstande en verwantskappe te bepaal in aartappels.

Chapter VII

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