MERIEM NEFZAOUI

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF CACTUS PEAR ACCESSIONS FROM MEDITERRANEAN AND BRAZIL COLLECTIONS

RECIFE

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Thesis presented within the post-graduation program in agronomy "Plant breeding" at the "Universidad Federal Rural de Pernambuco" as a requirement to obtain the Master degree in agronomy, plant breeding

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ABSTRACT

Phenotypic and molecular characterization of cactus pear accessions from Mediterranean and Brazil collections

Around 2.5 billion people – 30 percent of the world's population – live in the dry areas, which cover more than 40 percent of the world's land surface. Scarce natural resources, land degradation and frequent droughts severely challenge food production in these areas. Both North Africa (Morocco, Algeria, Tunisia) and the North East of Brazil fall under arid and semi-arid climate. Cacti have developed phenological, physiological and structural adaptations for growth and survival in arid environments where they have multiple functions (food, feed, soil conservation, etc.). Cacti are well positioned to cope with future global climate change; they can generate, under arid conditions, a carbon sequestration equivalent to 30 tons of CO_2 $ha^{-1}year^{-1}$. Cactus pear, the most commonly cropped belongs to the genus *Opuntia* and compared to other species, *Opuntia ficus-indica* is the most spread over all continents. The continuous morphological variation within the genus, the lack of clear descriptors for each species, and the relative ease of cross hybridization has led to an erroneous species designation. To overcome these problems, molecular markers might be useful tools to help unravel uncertainties in classification that are not addressed by morphological characterization. The objective of this contribution is to assess the genetic diversity of two cactus collections using morphological and molecular traits. The in situ collections are located at IPA in Northeast of Brazil with 300 accessions oriented toward forage production and at INRA Agadir station with 20 accessions representative of the Mediterranean Basin. Phenotypic characterization was achieved using FAO Cactusnet descriptor while the molecular characterization used the SSR technique and 8 recently recommended primers (Opuntia 3, Opuntia 5, Opuntia 9, Opuntia 11, Opuntia 12, Opuntia 13, Ops 9 and Ops 24). Phenotypic data have been submitted to principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) using XLSTAT 2015 package. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram based on Nei's genetic distance has been used for molecular, and the relationship between morphological and molecular traits was assessed by Mantel test. Results show that accessions may be discriminated by the morphological descriptors. Many of these morphological descriptors are significantly correlated as the number of cladodes and the number of fruits (r=0.73), the number of cladodes and the plant diameter (r=0.73), the length of the cladode

and the plant height (r=0.7), the length of the spine and the number of areoles (r=0.67). PCA and AHC are good tools to segregate accessions using a reduced number of morphological descriptors. The cladode shape and the number of spines and areoles are the recommended descriptors, and are capable de discriminate accessions with a suitable accuracy. SSR analysis revealed 72 alleles with an average allele number of 9 per locus. All microsatellites used were found to be discriminative with a mean value of Polymorphic Information Content (PIC) estimated at 0.458. Genetic dissimilarities estimated between the accessions varied widely, suggesting that an important genetic variability exist in the collection. All the markers used were either informative or highly informative and can be recommended to detect genetic diversity in *Opuntia* species; the most discriminant markers are Ops 24 and Opuntia 9 and the less discriminant is Opuntia 5. The relationship between phenotypic traits and the allele based genetic distances from the SSR analysis was highly significant (r=0.4, p=0.01) and obtained for the first time while using SSR for molecular characterization. Consequently, SSR technique is one of the best tools to assess the level of genetic diversity in *Opuntia* germplasm collections; it complements phenotypic characterization and it is recommended for planning breeding programs and to revise the current taxonomical classification.

Keywords: Cactus pear, genetic diversity, phenotypic characterization, SSR markers.

RESUMO

Caracterização fenotípica e molecular de acessos de Palma provenientes de coleções do Mediterrâneo e do Brasil

Cerca de 2,5 milhões de pessoas - 30 por cento da população mundial - vivem nas áreas secas que cobrem mais de 40 por cento da superfície terrestre do mundo. Os recursos naturais escassos, a degradação da terra e secas frequentes desafiam severamente a produção de alimentos nessas áreas. Tanto o Norte da África (Marrocos, Argélia, Tunísia) quanto o Nordeste do Brasil se encontram nestas condições. Cactus desenvolveram adaptações fenológicas, fisiológicas e estruturais para o crescimento e a sobrevivência em ambientes áridos onde eles possuem múltiplas funções de uso (alimento, pasto, conservação do solo, etc). Cactus são bem posicionados para lidar com futuras alterações climáticas globais já que eles podem gerar, sob condições áridas, um sequestro de carbono equivalente a 30 toneladas de CO_2 por hectare ao ano. A palma é o cactus mais comumente cultivado, pertence ao gênero *Opuntia* e em comparação com outras espécies, *Opuntia ficus-indica* é a mais espalhada por todos os continentes. A variação morfológica dentro do gênero, a falta de descritores claros para cada espécie, e a facilidade relativa de hibridação cruzada levou a uma designação de espécies errada. Para superar estes problemas, os marcadores moleculares podem ser ferramentas úteis para ajudar a desvendar incertezas na classificação que não são abordadas pela caracterização morfológica. O objetivo desta contribuição é avaliar a diversidade genética de duas coleções de palma utilizando características morfológicas e moleculares. As coleções in situ estão localizadas no IPA no Nordeste do Brasil com 300 acessos orientados para a produção de forragem e no INRA - estação de Agadir com 20 acessos representante da Bacia do Mediterrâneo. A caracterização fenotípica foi realizada usando descritores da FAO CactusNet enquanto que a caracterização molecular foi efetuada através da técnica SSR com 8 marcadores recomendados recentemente (Opuntia 3, Opuntia 5, Opuntia 9, Opuntia 11, Opuntia 12, Opuntia 13, Ops 9 and Ops 24). Os dados fenotípicos foram submetidos à análise de componentes principais (PCA) e ao Agrupamento Hierárquico Aglomerativo (AHC) usando o pacote XLSTAT 2015. O dendrograma obtido pelo UPGMA (método de média aritmética não ponderada), com base na distância genética de Nei, foi usado para a parte molecular. Já a relação entre as características morfológicas e moleculares foi avaliada pelo teste de Mantel. Os resultados mostram que os acessos podem ser discriminados pelos descritores morfológicos. Muitos destes descritores são significativamente correlacionados como o número de cladódios e o número de frutos (r=0.73), o número de cladódios e o

diâmetro da planta (r=0.73), o comprimento do cladódio e a altura da planta (r=0.7), o comprimento do espinho e o número de auréolas (r=0.67). PCA e AHC são boas ferramentas para distinguir acessos utilizando um número reduzido de descritores morfológicos. A forma do cladódio, o número de espinhos e auréolas são os descritores recomendados, sendo capazes de discriminar acessos com precisão adequada. A análise SSR revelou 72 alelos com um número médio de alelos de 9 por locus. Todos os microssatélites utilizados se revelaram discriminativos com um valor médio de conteúdo de informação polimórfica (PIC) de 0,458. As similaridades genéticas estimadas entre os acessos variaram muito, o que sugere que existe uma importante variabilidade genética na coleção. Todos os marcadores utilizados foram informativos ou altamente informativos, podendo ser recomendados para detectar a diversidade genética em espécies de *Opuntia,* sendo que os mais são Ops 24 e Opuntia 9 e Opuntia 5 é o menos discriminante. A relação entre as características fenotípicas e as distâncias genéticas baseadas nos alelos da análise SSR foi altamente significativa (r=0.4, p=0.01) e obtida pela primeira vez na caracterização molecular pela técnica SSR. Consequentemente, esta última é uma das melhores ferramentas para avaliar o nível de diversidade genética nas coleções de germoplasma *Opuntia*; complementa caracterização fenotípica e recomenda-se para o planejamento de programas de melhoramento genético e induz a rever a classificação taxonômica atual.

Palavras-chave: Cactus pear, diversidade genética, caracterização fenotípica, marcadores SSR.

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1. Introduction

Around 2.5 billion people – 30 percent of the world's population – live in the dry areas, which cover more than 40 percent of the world's land surface. Scarce natural resources, land degradation and frequent droughts severely challenge food production in these areas. Approximately 1/3 of the population living in drylands depends on agriculture for their food security and livelihoods – often as their only source of income. Drylands are home to the poorest and most marginalized people in the world, with 16 percent of the population living in chronic poverty. Productivity in dryland regions face a multitude of challenges – persistent water scarcity, frequent droughts, high climatic variability, various forms of land degradation, including desertification, and loss of biodiversity. Climate change is projected to affect the people living in dry areas and marginal lands the worst. In the developing world, dryland productivity is further hampered by many socioeconomic factors, such as limited access to technology, poor market linkages, weak institutions, lack of partnerships, and marginalization of rural people (CRP Dryland Systems, 2015)

Both North Africa (Morocco, Algeria, Tunisia) and the Northeast of Brazil fall under arid to semi-arid climate. Indeed, North Africa is marked by an acute water scarcity, combined with a highly variable Mediterranean climate. While the average world per capita share of fresh water is 7000 cubic meter (m^3), all three North African countries are below the water poverty threshold of 1000 m^3 (Nefzaoui et al., 2012). The scarcity of natural water resources, combined with the highly variable and generally very low rainfall in most of the region explain in part the low agricultural productivity, especially of key crop commodities (wheat, barley, pulses) and the reliance of North African countries on food imports to meet their growing national demands (Nefzaoui et al., 2012). In Brazilian semi-arid, maize grain productivity is approximately 600 kg/ha/year, and in years with severe drought, which are frequent, maize productivity will be close to zero (Dubeux Jr. et al., 2015). Similarly in North Africa wheat and barley yields under rainfed conditions in arid and semi-arid regions does not exceed 500 kg/ha/year (Nefzaoui et al., 2012).

Cacti have developed phenological, physiological and structural adaptations for growth and survival in arid environments in which severe water stress hinders the survival of other plant species. Among these adaptations stand out the asynchronous reproduction and CAM metabolism of cacti, which combined with structural adaptations such as succulence allow them to continue the assimilation of carbon dioxide during long periods of drought reaching acceptable productivity levels even in years of severe drought (Nobel, 2009). Under

rainfed conditions and in North Africa regions with low rainfall (200 mm/year), biomass yield of *Opuntia ficus- indica* reaches 40 tons/ha/year (Le Houérou, 2002). Dubeux et al (2015) stated that compared to maize, cactus has the potential to produce at least 20 times more forage per unit land area under rainfed conditions of the Northeast of Brazil.

CAM plants (Agaves and Cacti) can use water much more efficiently with regard to CO2 uptake and productivity than do C3 and C4 plants (Nobel, 2009). Biomass generation per unit of water is on an average 5 to 10 times greater than C4 and C3 plants. In contrast to C3 and C4 plants, CAM plants net CO_2 uptake occurs predominantly at night. As stated by Nobel (2009), the key for the differences between nocturnal gas exchange by CAM plants and C3 and C4 plants is temperature. Temperatures are lower at night, which reduces the internal water vapor concentrations in CAM plants, and results in better water use efficiency. This is the key reason that makes CAM species the most suited plants for arid and semi-arid habitats. In addition, C3 and C4 plants suffer irreparable damage once they lose 30 % of their water content. On the other hand, many cacti can survive an 80 to 90 % loss of their hydrated water content and still survive. This is due to the ability of CAM plants to store a lot of water; to shift water around among cells to keep crucial metabolism active; and to tolerate extreme cellular dehydration (Nobel, 2009). Cacti, thus,are well positioned to cope with future global climate change. *Opuntia ficus-indica*, for example, can generate a carbon sequestration of 20 tons of dry matter (equivalent to 30 tons of CO2) per ha and per year under sub-optimal growing conditions similar to those in North Africa arid regions (Nobel, 2009).

Cacti and *Opuntia* spp. in particular can prevent or reverses desertification through different ways: cacti are drought tolerant species, they are used in watershed management and in water harvesting and its efficient use, in wind and water erosion control, in rangeland and marginal land rehabilitation, in cropland management and crop diversification to contribute alleviating poverty and to reach better livelihood of the rural poor in dryland areas (table 1)

The utilization by man of the cactus *Opuntia* was recorded in Mexico in pre-Hispanic times, where it played a major role in the agricultural economy of the Aztec empire; with maize (*Zea mays*) and agave (*Agave* spp.), *opuntia*s are the oldest cultivated plants in Mexico (Reynolds and Arias, 2001).

Table 1. Estimated land areas utilized for raising cacti, mainly *Opuntia ficus indica,* for forage and fodder (Nefzaoui, 2009)

Region or country	Surface area, x 1000 ha
Brazil	600
Other South American countries	75
Mexico	230+ 3 million ha Wild
Other North American countries	16
South Africa	2
Italy	70
Tunisia	600
Algeria	150
Other West Asia and North Africa countries	300
Total	2 million ha cultivated + 3 million ha wild

Cacti are easy to establish and they have very large spectrum of uses. Cacti and *Opuntia* spp. present various alternatives to its exploitation (Nefzaoui, 2014):

- As fruit: A cultivation policy must be defined aiming to achieving high yields and high quality; to achieve both objectives a sustainable horticultural system is required. The potential market for this product is extensive but little exploited, so better marketing strategies and post-harvest technology are required. Due to their management requirements, *Opuntia* spp. require extensive labor, which is an important variable in developing countries (Inglese et al., 2002).

- As forage: Since they grow in severely degraded land, their use is important because of their abundance in areas where few crops can grow. Also present high palatability, digestibility, and reduce the need for supplying water to animals; however, they must be combined with other foods to complete the daily diet, because they are poor in proteins, although rich in carbohydrates and calcium (Nefzaoui and Ben Salem, 2002; Dubeux Jr. et al., 2015).

- As vegetable (nopalitos): They are consumed fresh mainly in Mexico and by Mexicans living in the United States of America; however, Mexican exports to Europe and Asia are increasing, which shows an expanded demand in

non-traditional markets, which should be adequately examined (Saenz Hernandez et al., 2002).

- As cochineal: Carminic acid is obtained, which is a natural red dye accepted by health authorities worldwide, with variable yields according to the production system used, both concerning plant density and irrigation and fertilization systems. Cochineal constitutes a significant alternative because of its profitability and intensive use of labor, but the market for this product has large price fluctuations, which makes investment decisions difficult (Flores-Flores and Tekelenburg, 1999).

- Industrialization: it is feasible to industrialize cladodes, fruit, and "nopalitos". This potential market deals mainly with concentrated foods, juices, liquors, semi-processed and processed vegetables, food supplements and the cosmetic industry; it is feasible, but it requires work and investment to develop the market (Saenz Hernandez et al., 2002)

- Medicinal Applications: This is a new area of research and promising results are obtained. Cactus cladodes, fruits, and flowers have been traditionally used as natural medicines in several countries. Cladodes are still used in folk medicine for the treatment of gastric ulcer and as therapeutic agents for its healing activity. They are also well-known the properties of the infusions of cactus dried flowers to prevent prostate cancer. Remarkable progress has been made in disease prevention over the past decades considering fruit, vegetables and herbs incorporation to the diet. Scientific investigations confirmed that cactus products may be efficiently used as a source of several phytochemicals of nutraceutical importance, such as mucilage, fibers, pigments and vitamins (Nazareno, 2013)

Although cactus pear originates from arid and semi-arid areas in Mexico, it is presently cultivated worldwide; specifically *O.ficus indica* which is cultivated in over 20 countries for its fruits and as feed for livestock (Inglese et al., 2002). As stated by Casas and Barbera (2002), its dispersal around the world was facilitated by the inclusion of fresh cladodes on European ships in the late 15th century.

2. Literature review

2.1. The *Cactaceae* family and related taxonomy problems

The classification of the *Cactaceae* family remains uncertain up today (Caruso et al., 2010). Since the mid-1990s, the system produced by the International *Cactaceae* Systematics Group (ICSG) of the "International Organization for Succulent Plant Study" has been used as the basis of many published classifications. Detailed treatments produced in the 21st century have divided the family into around 125–130 genera and 1,400–1,500 species, which are then arranged into a number of tribes and subfamilies (Bárcenas et al., 2011).

The ICSG classification of the family recognizes four subfamilies: Pereskoideae (consisting only of the genus *Pereskia*), *Opuntioideae*, *Maihuenioideae* (consisting only of the genus *Maihuenia*) and *Cactoideae*. Molecular phylogenetic studies suggest that *Pereskia* is not monophyletic (i.e. its species are not the complete set of descendants of a common ancestor), although the three other subfamilies are.

Five tribes have been recognized within the subfamily *Opuntioideae*: *Tephrocacteae, Pterocacteae, Austrocylindropuntieae, Cylindropuntieae* and *Opuntieae*. All but the first, *Tephrocacteae*, were shown to be "essentially monophyletic" in molecular phylogenetic study in 2009. Thus the classification of the Cactaceae family may be summarized as follows (Figure 1)

Early European botanists called cactus "*Ficus indica*" (Donkin, 1977), although some found this to be an unsuitable name, as the plant did not resemble the Indian fig (possibly *Ficus benghalensis* L.) already known (Anderson, 2001). On the other hand, Miller combined these two "names" to come up with *Opuntia ficus-indica* in 1768 (Griffith, 2004). The number of species belonging to the *cactaceae* family is still uncertain and some authors report more than 1600 (Gibson and Nobel, 1986; Barthlott and Hunt, 1993). The number of species belonging to the *Opuntia* genus is estimated to 300 and spread over all continents (Scheinvar, 1995). The exact number of species within *Opuntia* genus is still unknown and figures vary according to authors.

The taxonomy of cacti is difficult for a number of reasons: their phenotypes, which vary greatly according to ecological conditions; their polyploidy, with a great number of populations that reproduce vegetatively and sexually; and the existence of numerous hybrids, as almost all species blossom during the same period of the year and there are no biological barriers separating them (Mondragon-Jacobo, 2001). There's also a limited number of morphological descriptors, a high phenotypic plasticity, high level of intra- and interspecific

hybridization as well as allopolyploidy versus autopolyploidy (Mondragon and Chessa, 2013; Chessa et al., 2013).

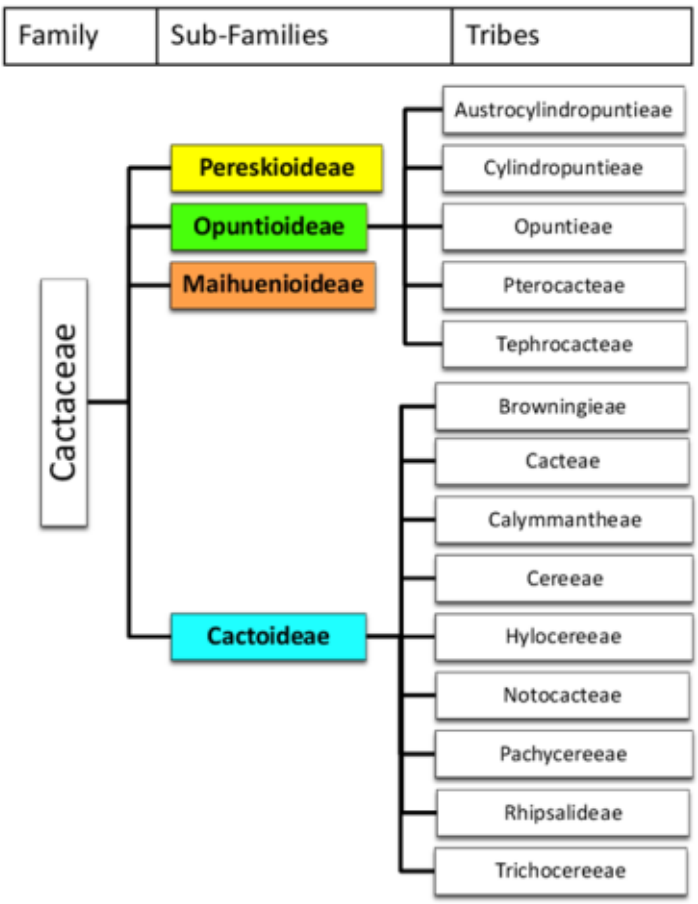


Figure 1. Classification of the Cactaceae family (adapted from Griffith and Porter, 2009)

Phenotypic variability is most frequently observed in fruit size and colour, cladode size, morphology, and phenology (fruit ripening time) (Pimienta-Barrios and Muñoz-Urias, 1995). According to Gibson and Nobel (1986), variability of both wild and domesticated cactus pear populations is thought to have occurred via natural hybridization associated with poliploidy and geographic isolation.

The presence of spines in the cladodes is an inadequate feature to discriminate *Opuntia ficus-indica* from other arborescent O*puntia*s (Nieddu and Chessa 1997; Kiesling 1998; Felker et al. 2005). Within the genus, the growth habit, the presence of spines, the number of spines per areole, and the number of areoles may differ drastically in different growing regions (Rebman and Pinkava, 2001)..

2.2. The *Opuntia* genus

*Opuntia*s are often divided into *cylindropuntias* and *platyopuntias* (Gibson and Nobel 1986). *Cylindropuntias* are shrubby species with cylindrical stems (or joints). *Platyopuntias*, which have flattened stems called cladodes (Gibson and Nobel 1986), include agronomically important species that are cultivated as both fruit and forage crops. Cultivated *opuntias* include *O. megacantha, O. streptacantha, O. albicarpa, O. amyclaea, O. robusta, O. hyptiacantha,* and *O. cochenillifera* (syn. *Nopalea cochenillifera*, primarily grown in Mexico as a forage crop) among others (Pimienta-Barrios 1994; Scheinvar1995; Kiesling 1998; Mondragon-Jacobo 2001). The most diffused and economically important species is *O. ficus indica*. This specie, commonly referred to as cactus pear, prickly pear, Indian fig, Barbary fig, etc., was probably domesticated about 9,000 years ago in central Mexico and diffused in several warm regions of the world by European travelers beginning in the late 15th century (Kiesling1998; Griffith 2004).

The species *O. ficus-indica* has diffused into Argentina, California, Chile, Israel, and South Africa where naturalized stands and commercial plantations for fruit occur. Other plantations can also occur in Brazil, Colombia, Peru, Spain, Greece, Turkey, Italy, Jordan, Egypt, Tunisia, Algeria, and Morocco (Inglese et al., 2002).

Deducting from historical sources the cactus pears present in these areas may have had a common origin; domesticated cactus pears brought from Mexico after the discovery of America and dispersed by the colonial activity of Italy, Spain and other European countries and the influence of Arab countries in North Africa and the Middle East countries. Field observations support the hypothesis that the original pool was restricted mostly to domesticated accessions, which segregated and adapted to these new environments (Mondragon and Chessa, 2013). As stated above, several accessions are cultivated in different growing regions, but little is known about their ancestries and level of genetic diversity. Therefore, characterization of genetic resources of these plants is a prerequisite for breeding strategies aimed at improving sustainability of the crop in several conditions and for improving quality of the different products obtainable (Mondragon and Chessa, 2013).

The most important taxonomical species that produce edible fruits in both cultivated and wild populations are *O. ficus indica*, *O. albicarpa*, *O. streptacantha* and *O. robusta*. *Opuntia ficus indica* is the most commercially used around the world for both fruit and forage production. The Italian varieties (Bianca, Gialla and Rossa), having good quality fruit and high yields are predominant in the Mediterranean Basin (Figures 2 and 3).

The most distinguished Mexican varieties are “Blanca chapeada”, “Blanca reyna”, “Amarilla naranjona”, “Amarilla huesona”, “Blanca burrona”, “Blanca cristallina”, “Pelon-liso”, “Charola”, and “Cardona” (Pimienta-Barrios and Munoz-Urias, 1995) (Figure 4).

Opuntia megacantha

Opuntia streptacantha

Opuntia amyclaea

Opuntia hyptiacantha

Opuntia cochenillifera (syn. Nopalea cochenillifera)

Opuntia echios

Figure 2. Examples of *Opuntia* species

Opuntia ficus indica (var. Algerian)

Opuntia ficus indica (var. American Giant)

Opuntia ficus indica (var. Monterey)

Opuntia ficus indica (Var. Roly Poly)

Opuntia ficus indica (Var. Messina)

Opuntia ficus indica (Var. Rossa)

Opuntia ficus indica (Var. Gialla)

Opuntia ficus indica (var. Bianca)

Figure 3. Examples of *Opuntia ficus indica* varieties

Figure 4. Major registered commercial varieties in Mexico (Mondragon-Jacobo and Chessa, 2013)

2.3. Major cactus collections and germplasm enhancement and breeding

The germplasm will be the cornerstone of all future applications of cactus pear; intensive exploration, effective in-situ and ex-situ conservation, dynamic evaluation on new sites and vigorous projects of germplasm improvement are needed to realize the full potential of this valuable resource (Mondragon-Jacobo and Chessa, 2013). Mexico, Italy and Brazil are sources of germplasm and know-how of cactus pear cultivation for fruit, vegetable and fodder production (Mondragon-Jacobo and Chessa, 2013).

During the last decade, ICARDA with the support of the universities of Sassari and Palermo (Italy) transferred selected material for both fruit and fodder production to create collections in Libya, Tunisia, Morocco, India, Iran, Pakistan, Lebanon and Jordan (Nefzaoui et al., 2012). In the western hemisphere live collections are present in Argentina, Chile, Brazil and Mexico, while the collection of Kingsville, TX is no longer active, but the USDA is trying to assemble a new one under the umbrella of the National Clonal Repositories system in California.

Cactus pear breeding has been attempted since the late XIX century with mixed results mainly due to: biological complexity of *Opuntia*s – all *Opuntia*s with horticultural value are polyploid and present apomixis- and long-term juvenility, associated to limited output of breeding programs and costly projects, features that are directly related to funding. As a result

actual breeding programs are irregular, short lived and poorly funded. Three programs supported by the Mexican, Italian and Brazilian governments have been conducting breeding, herein we briefly describe them:

Mexico

Starting in 1995 the program conducted hybridizations and selection using the best Mexican genotypes for fruit production. The program is located in San Luis de la Paz, Guanajuato and is supported by the Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias, the objectives are to obtain multipurpose varieties, improve fruit quality and adaptation, in this publication the first three improved varieties are reported (Mondragon-Jacobo and Chessa, 2013).

Being the Center of origin for cacti, Mexico has the largest in-situ and ex-situ collections. Chapingo University (Universidad Autonoma de Chapingo) has the "National Opuntia Depository" with a field collection containing around 410 accessions of domesticated cactus pear. The mandate of this depository is to protect the national wealth *Opuntia*, to promote and conduct research on *Opuntia* germplasm, to support conservation and utilization, and to provide reference material and data for legal rights (Mondragon-Jacobo and Chessa, 2013). The table 2 compiles the Mexican Germplasm banks of cactus pear.

Early 2011 the Mexican government launched the National Center of Genetic Resources (CENARGEN) in Tepatitlan, Jal., which will serve as national repository, the facilities are designed for long term storage of all crops and related organisms relevant to the national agriculture, and cactus pear is included.

Mexico INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) has a strong breeding program that includes 200 accessions for fruit, forage and vegetable. INIFAP produced 2500 individual plants derived from controlled crosses (Mondragon-Jacobo and Chessa, 2013).

Table 2. Inventory of Mexican Germplasm Banks of cactus pears, updated to 2011 (Mondragon-Jacobo and Chessa, 2013)

Use	CRUCEN	IIZD	CBTA 38	INIFAP.SLP
Fresh fruit	357	302	136	908
Fruit and forage	5			17
Forage	7	3	3	47
Vegetable	39	30	3	86
Triple use	2			2
Not reported (N.D.)				28
Animal feed				29
Ornamental				4
Condiment				15
Total	410	335	142	1021

CRUCEN. Centro Regional Universitario Universidad Autonoma de Chapingo. El Orito, Zacatecas., Zac.

IIZD. Instituto Investigaciones en Zonas Deserticas. Universidad Autonoma de San Luis Potosi. San Luis Potosi. SLP.

CBTA 38. Centro de Bachillerato tecnologico Agropecuario. Ojocaliente Zac.

INIFAP- SLP. Campo Experimental San Luis Potosi. Ojo de Agua de la Palma, SLP.

Italy

Italy maintains the largest and oldest collection –acting as a germplasm bank and breeding collection- outside Mexico. It has been established in 1992 in Oristano, Sardinia by the University of Sassari. This collection includes more than 2200 accessions gathered from different provenances (Sardinia and Sicily in Italy, Argentina, Chile, USA, Canada, France, Morocco, and South Africa). This collection compasses *Opuntia* and *Nopalea* species, wild genotypes and ecotypes from Italy, varieties selected locally, hybrids from open pollination, and hybrids from controlled crosses and embryoculture (Mondragon-Jacobo and Chessa, 2013). The Italian breeding program has been involved in germplasm collection, description and documentation since 1992. They also performed crosses and pioneered embryo culture of cactus pear. Among the products obtained are 12 selections of green, yellow and red peel (4 each) suitable to grow in the Mediterranean countries.

Brazil

Brazil reports 1417 accessions including genotypes from several countries as well as segregants and hybrids of controlled crosses. Brazil has oriented its conservation efforts towards forage accessions while the rest of the collections contain fruit, forage as well as double purpose entries.

The history of the introduction of fodder *Opuntia* into Brazil is a much debated topic, but probably it was introduced in the 18th Century, from the Canary Islands, to raise the cochineal insect (*Dactilopius cacti L.*) for dye production (Pessoa, 1967). After losing competitiveness, dye production died out, and both species of *Opuntia* (*Opuntia ficus-indica L.*) Miller and *Nopalea cochenillifera Salm-Dyck* became ornamental plants. The use of *Opuntia* as fodder in the semi-arid areas of northeast Brazil occurred only at the beginning of the twentieth century (Dos Santos and Albuquerque, 2001).

Northeast Brazil with its semiarid tropical unique agroclimate is the most important growing area for fodder *Opuntia* in the world. Palma Gigante and Palma Redonda (both *O. ficus-indica Mill.*) are widely cultivated in the Northeast Brazil.. Together with Palma Miúda (*Nopalea cochinellifera Salm-Dick*), which tolerates more humid conditions, they are the mainstay of commercial production of this crop (Dos Santos and Albuquerque, 2001). 'IPA-Clone 20' was selected from open pollinated seeds of Palma Gigante (*O. ficus-indica* Mill.). In field trials, IPA-Clone 20 produced 50% more fodder than the maternal entry (Arruda and Warumby, 1999). Mondragon-Jacobo and Chessa (2013) estimates that IPA collection comprises 1061 clones from open pollinated Palma Gigante. In addition, there is: 3 small (less than 100 accessions) germplasm banks in Petrolina-PE, Tacina-PB and Rio Grande du Norte at EMPARN; 171 clones open pollinated Palma Miúda; 159 clones from Universidad Autonoma de Chapingo, México; 17 clones from several countries by CPATSA; 5 clones from Rio Grande do Norte; 4 clones from Petrolina, utilized probably to produce cochineal dye (Arruda and Warumby, 1999).

South Africa

In South Africa, the varieties currently present originated from the introduction of 21 spineless types imported from the Burbank nursery of California in 1914. Actually, it is the unique collection of Burbank´s "improved" varieties existing today. The number of accessions available today is around 42 and was developed from the original material, either as clones or as artificial or natural hybrids (Mondragon-Jacobo and Bordelon, 1996).

North Africa

Cactus crop covers around 600.000 ha in Tunisia and 200.000 ha in Morocco and it is increasing. Cactus is used for both fruit production and as fodder. In addition small-scale transformation units are being established for both fruit and pads. Two collections are being present, one in Tunisia and the other one in Morocco. The Tunisian collection has been established in early 60's and has been recently reinforced by new introduction from Sardinia

and duplicated in two climatic regions. The total number of accessions exceeds 100 today and unfortunately no breeding program is implemented. The recently established collection in Agadir (Morocco) includes local population and around 40 accessions from Sardinia introduced by ICARDA (Nefzaoui and al., 2012).

East Africa

Tigray, a highland region shared by Ethiopia and Eritrea hosts the densest naturalized stocks in Africa. It is originated from domesticated cactus pear likely from Italy. It is a valuable as source of tolerance to drought and shallow rocky soils, but the fruit quality needs improvement (Tegegne, 2001).

2.4. Germplasm Characterization

2.4.1. Characterization of cactus pear germplasm

Characterization of germplasm is essential to provide information on the traits of accessions promoting their classification including the estimation of the genetic diversity within a cluster. To facilitate and standardize characterization of collected accessions, a descriptor list for cactus pear (Chessa and Nieddu, 1997) was developed by the FAO (Food and Agricultural Organization) and ICARDA (International Center for Agricultural Research in the Dry Areas) Technical Co-operation Network on Cactus Pear (FAO-ICARDA CACTUSNET), compiled following the international format currently endorsed by the Bioversity International.

The usefulness of both molecular and morphological data in conservation planning has been underlined by Helsen *et al.* (2009), based on the relatively high morphological divergence found on the Galapagos endemic *Opuntia* species associated with a low genetic variability, as evidence for divergent selection and adaptation to local environments. The same authors gave evidence that the current morphology-based taxonomic differentiation between the *Opuntia* taxa was not supported by molecular data (Helsen at al., 2009).

Traditionally, morphological descriptors are used to evaluate accessions and to assess their genetic diversity. Although expression of these descriptors is strongly influenced by environmental conditions and agricultural practices, morphological characterization is highly recommended as a first step prior to attempting advanced assessment through molecular markers (Hoogendijk and Williams, 2001).

Simplicity, speed and inexpensive nature make these morphological descriptors the most widely used genetic markers for germplasm characterization (Mondragon and Chessa,

2013). A set of morphological descriptors can be used to describe the Cactus Pear plant phenotype. Cladodes (pads), fruits, spines, glochids and seed traits can be measured and expressed in numeric values. According to Erre et al. (2009), the effectiveness of morphological characterization may be hampered by the high hybridization level within the species and the several environmental factors that can affect the macro-morphological plant classification. Chapman et al. (2002) hold a similar view and stated that the exclusive use of morphological traits has often led to duplication, complicating subsequent evaluation and utilization. On the other hand, Chessa et al. (1995) found that the number of spines allowed the classification of biotypes of cactus pear according to their territorial distribution.

However, germplasm characterization based on molecular traits provides more reliable information, and has attained special attention due to its increased use in crop improvement and the selection of desirable genotypes for breeding crops. Molecular fingerprinting, using RAPDs and ISSR, have been applied to the management of cactus pear collections (Wang et al., 1998; Garcia-Zambrano et al., 2006; Zoghlami et al., 2007; Luna-Paez et al., 2007) and to elucidate the hybrid origin of *Opuntia* species (Griffith, 2004). The genetic relationships among different species and the variability of collected genetic resources were investigated through AFLP (Labra et al., 2003; Garcia-Zambrano et al., 2009).

Estimates of genetic diversity and the relationships between germplasm collections from different regions are very important to identify genetically diverse, agronomically superior accessions for the improvement of cactus pear (Chessa et al., 2013).

2.4.2. Use of molecular tools to assess cactus pear variability

Phenotypic identification based only on morphological markers can be misleading; due to the complex genotype and environment interaction, that governs most of the traits of interest. Markers based on DNA polymorphism provide a superior tool for the assessment of genetic diversity over other methods (Erre and Chessa, 2013). An array of molecular marker techniques has been developed and are commonly used for genotyping individuals and inferring information on the genetic structure of germplasm collections, discovery of synonymy, and kinship. However, development and application of molecular markers is actually still limited in minor crop species, such as cactus pear. (Mondragon and Chessa, 2013).

2.4.2.1. Isozymes

Isozymes are the earliest molecular markers developed. They occur as a result of variations in nucleotide sequence that results in the substitution of one amino acid for another. Such substitution may result in the alteration of the net electrical charge on a protein. The charge difference is subsequently detected as an alteration in the migration rate of a protein through an electrical field. Electrophoretic separation is then used to measure protein mobility variation within a population (Klug and Cummings, 2002).

Chessa et al. (1997) reported the description of Italian cactus pear through isoenzymes. They analyzed 32 accessions with 13 enzyme systems in preparations of roots, cladodes, petals and pollen. It was found that only Malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and phosphoglucomutase (PGM) isozyme banding patterns on pollen allowed the grouping of different *Opuntia* varieties and biotypes. Through electrophoresis, seven enzyme systems were investigated by Uzun (1997) with three Italian cultivars, and 15 Turkish cactus pear ecotypes that showed no variation in isozyme banding patterns. According to Barbera (1995), Turkish germplasm probably came from a genetic basis narrower than the Italian. Chessa et al (1997) conclude that a unique cultivar identification using isozymes was not possible.

2.4.2.2. DNA markers

DNA polymorphisms represent differences in the DNA sequence of two individuals and are the desired markers for the identification and characterization of plants. Given that DNA is an integral part of plants and is not subject to environmental modification (Bachmann et al., 2001), nuclear and cytoplasmic (chloroplast DNA [cpDNA], and mitochondrial DNA [mtDNA]) can be analyzed for polymorphisms using various techniques.

DNA marker techniques have progressed from hybridization-based methods such as restriction fragments length polymorphism (RFLPs), to more rapid polymerase chain reaction (PCR-based DNA methods such RAPDs, simple sequence repeats (SSRs) or microsatellites, sequence-tagged sites (STS), AFLPs, inter-simple sequence repeat amplifications (ISSR) and single nucleotide polymorphisms (SNPs) (Gupta et al., 1999).

The RAPD technique

In the past two decades, some studies have been performed to characterize existing cactus germplasm collections using random molecular markers (RAPD) (Wang et al., 1998; Mondragón-Jacobo 2003; Zoghlami et al., 2007; Luna-Paez et al., 2007; García-Zambrano et

al., 2009; Souto-Alves et al., 2009). RAPDs patterns are PCR derived markers obtained by the random amplification of DNA using short nucleotide primers (generally 10 nucleotides) of arbitrary nucleotide sequence (Williams et al., 1990). The technique is relatively quick and easy to perform and uses fluorescence instead of radioactivity (Williams et al., 1992). These markers are selectively neutral, involve a large number of loci and cover a large part of the genome. They also provide more valuable information into population differentiation and help to elaborate efficient conservation strategies (Wang et al., 1998; Arnholdt-Schmitt et al., 2001, Labra et al., 2003, Chatti et al., 2003). However, most RAPD loci are assumed to possess only two alleles and segregate as dominant markers, leading to an underestimation of the genetic diversity (Lynch and Milligan, 1994; Caetano-Anolles et al., 1991). These markers were successfully applied to verify the somatic origin within some Mexican accessions (Mondragon-Jacobo, 1999). The germplasm bank collection of the Facultad de Agronomia de la Universidad Autonoma de Nuevo Leon (FAUANL) has been characterized and duplicates revealed by means of RAPDs (Garcia-Zambrano et al., 2006), not confirmed using AFLP markers (Garcia-Zambrano et al., 2009). In order to identify fruit cultivars, vegetable and ornamental accessions, Wang et al. (1998) applied RAPDs, combined with morphological and physiological data. *O. ficus indica* ecotypes from Tunisia were characterized by means of RAPDs, and 13 main groups were identified, without relation to different geographical region (Zoghlami et al., 2007).

Zoghlami et al. (2007) reported for the first time the analysis of genetic diversity within a set of 36 Tunisian *Opuntia ficus indica* (L.) Mill. ecotypes using RAPD markers. Random decamer primers were screened to assess their ability to detect polymorphisms in this plant crop. Thirty-nine RAPD markers were revealed and used to survey the genetic diversity at the DNA level and to establish relationships. Consequently, considerable genetic diversity was detected and the UPGMA analysis permitted the discrimination of all the genotypes and enabled their sorting into thirteen groups. Zoghlami et al. (2007) have demonstrated the reliability of RAPD analysis to detect DNA polymorphisms and relationships within *Opuntia ficus indica* (L.) Mill. in Tunisia. Using RAPD markers Bendhifi et al. (2013) analyzed the genetic diversity of 28 Tunisian *Opuntia ficus indica* ecotypes and showed that this technique allows distinguishing all considered cultivars and resolving homonymy problem. Using Ward's clustering method, Bendhifi et al. (2013) found that 92.58 % of the total variance was accounted within group and the remaining 7.42 % between groups. A positive and significant correlation was evidenced between morphological descriptors and RAPD markers.

Another study was made in Agadir, Morocco in which 13 cladodes of *Opuntia ficus-indica* from 13 provenances were used for RAPD research. Among 14 primers used to assess polymorphism in the tested ecotypes, 13 have revealed scorable bands and only the primer OPA-13 did not amplify. Researchers have demonstrated that RAPD patterns can be obtained from cacti using primers OPA-11 (De La Cruz et al., 1997), and OPA-12 (Tel-Zur et al., 1999). RAPD profiles have been used to verify the maternal origin of apomictic seedlings in cactus pear (Mondragón-Jacobo and Bordelon, 2002).

The AFLP technique

AFLP (Amplified fragment length polymorphism) is another DNA-based marker technique that involves the digestion of genomic DNA with two endonucleases, followed by the ligation of site specific adaptors to the DNA fragments. Thereafter DNA fragments are resolved on standard sequencing gels (Vos et al., 1995). This technique has the advantages of being highly sensitive, reproducible and widely applicable. Labra et al. (2003) used AFLP to verify the lack of genetic differentiation between *O. ficus indica* and *O. megacantha* populations. AFLP markers were also applied to investigate genetic relationship among species within three *Opuntia* collections in Tunisia (Snoussi Trifa et al., 2009). Mashope (2007) conducted a study in which nine primers were used to assess the genetic diversity within South African cactus pear germplasm. The analysis generated 346 fragments per sample, of which 168 were polymorphic. A large number of the markers produced had a polymorphic information content (PIC) value between 0.3-0.5, indicating a good discriminatory value. AFLP technique has the advantages of being highly sensitive, reproducible and widely applicable. Its limitations, however, are that it is relatively expensive, technically demanding, and a dominant marker system (Ford-Lloyed, 1996).

The SSR technique

The codominant nature of SSRs (Simple Sequence Repeat) has make them the marker of choice to unravel cases of erroneous species designation (Helsen et al., 2009) and to investigate the differentiation level among cactus pear genotypes of different origin (Caruso et al., 2010). A novel set of microsatellite loci were isolated in different species and varieties of *Opuntia* (Erre *et al.*, 2013). Five out of ten SSR loci developed were used to characterize two field collections from Italy and Argentina. The level of polymorphism and a relatively high number of alleles detected suggested that these markers can be used for both inter and intra-specific studies, as well as to provide a more reliable tool in the classification of *Opuntia* species, based on their allelic profiles (Chessa *et al.*, 2013).

The SSRs data combined with agronomic, qualitative, morphological, and phenological data will create a useful instrument to facilitate the management and use of cactus pear collections. However, it should be noted that due to the presence of polyploidy within the *Opuntia* genus, the SSR may have a limited capacity to represent true genetic distances between cultivars, owing to the difficulty of identifying the allelic profile at locus.

A better understanding of the effectiveness of the different molecular markers is considered a priority step toward management of cactus pear collection and a prerequisite for more effective breeding program (Mondragon-Jacobo and Chessa, 2013).

Caruso et al. (2010) analyzed eight highly polymorphic SSR loci that allow them to investigate the level of genetic diversity among cactus pear species, cultivars, and accessions from different regions of the world. SSRs, although scored as codominant markers, were more informative than random markers; they were able to produce useful information regarding the level of diversity among the most diffused cultivars, and may have revealed the level of hybridization between *O. ficus indica* and its related species. Caruso et al. (2010) stated that with their small sample of progeny resulting from a cross between *O. ficus indica* 'Bianca' and a clone of *O. amyclaea*, observed a random combination of parental alleles, which is typical of autopolyploid species. This finding is also shared by Doyle and Egan (2010). Consequently, microsatellites could be used to analyze a greater number of individuals originating from controlled crosses with different parentals to investigate the molecular evolution of polyploidy in *Opuntia*s at a deeper level. In addition, SSRs may serve as a quick and reliable tool to discriminate *Opuntia* apomictic seedlings from zygotic ones (Mondragon-Jacobo and Bordelon 2002; Reyes-Aguero et al. 2006).

The work of Caruso et al. (2010) as well as previous work based on molecular variation (Wang et al.1998; Labra et al. 2003; Griffith 2004), clearly supports the fact that the present classification of cultivated varieties and wild genotypes based on morphological parameters is misleading. Consequently, molecular tools are definitely the most appropriate tools for the assessment of the level of genetic diversity in *Opuntia* germplasm collections. Such analysis should be a prerequisite for planning breeding programs that capture most of the existing variability among cactus pear. The use of these markers is strongly suggested to reclassify the cactus pear cultivated accessions, which exhibit a high level of variation regardless of the current taxonomical classification and probably should be classified as the same species, as suggested by Kiesling (1998). Caruso et al. (2010) findings showed that although there are differences in fruit color, the SSR profiles of these genotypes were strongly similar.

3. Objectives and Hypotheses

3.1. Objectives

The objective of this research is to compare phenotypical and molecular-based methods in assessing genetic diversity of cactus pear from two *ex-situ* collections, located in the Mediterranean Basin (Agadir, Morocco) and the Northeast of Brazil.

3.2. Hypotheses

Phenotypic identification based only on morphological markers can be misleading due to the complex genotype and environment interaction that governs most of the traits of interest. Markers based on DNA polymorphism provide a superior tool for the assessment of genetic diversity over other methods. An array of molecular marker techniques has been developed and are commonly used for genotyping individuals and inferring information on the genetic structure of germplasm collections, discovery of synonymy, and kinship.

4. Material and methods

4.1. General description of the experimental design

The diagram 1 shows a global view of the research conducted.

The work has been conducted on two in-situ collections, namely the IPA Arcoverde (Brazil) collection with 300 accessions and the INRA collection in Agadir (Morocco) with 20 accessions. All the accessions have been submitted to morphological characterization using the FAO Guideline (Chessa and Nieddu, 1997).

Using XLSTAT 2015 package (https://www.xlstat.com/en/solutions/biomed), the morphological data have been submitted to principal component analysis (PCA) and agglomerative hierarchical clustering (AHC). The molecular characterization using SSR methodology has been conducted on 50 accessions distributed as following:

- All the 20 accessions from the Mediterranean/Moroccan collection, and
- 30 accessions from IPA Arcoverde collection chosen at random from clusters resulting from the Agglomerative Hierarchical Clustering (AHC) analysis.

Finally, the relationship between morphological and molecular data was performed using the Mantel test (Mantel, 1967)

These methodologies are developed in more details in the following sections.

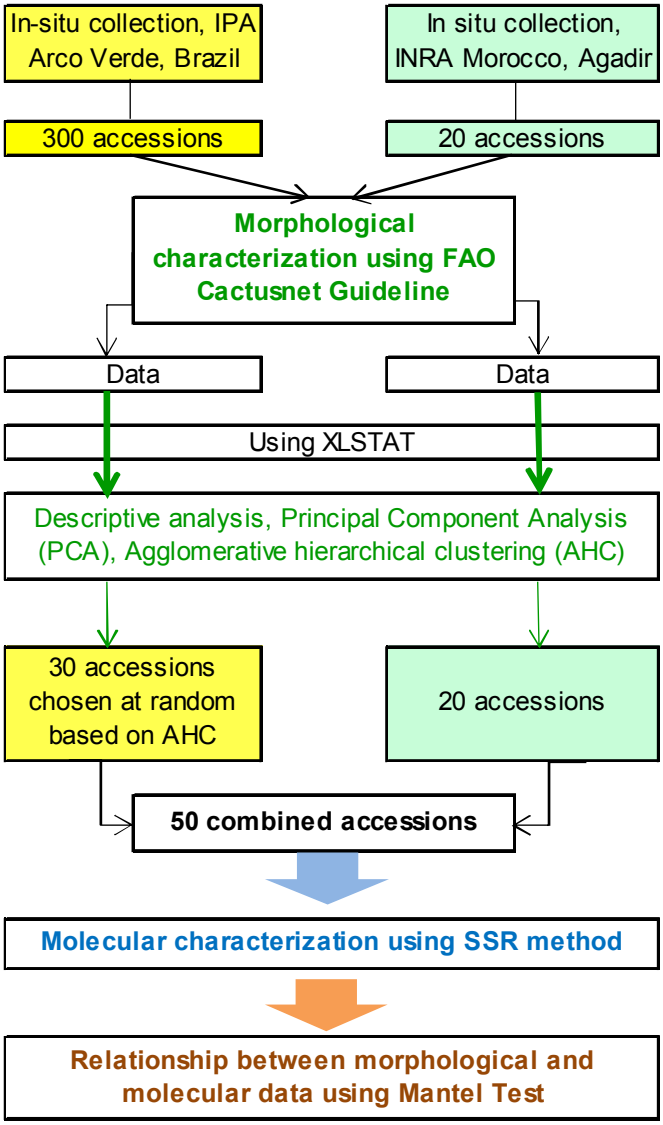


Diagram 1. Overview of the experimental design

4.2. Plant material

As stated above, the research was conducted on two *in situ* collections that differ at least from two points of view: the climate and the selection targets. The Brazilian collection is located in tropical semi-arid agro-ecological zone and is oriented toward forage production and resistance to pests (cochineal). IPA collection includes a large number of varieties introduced from many countries and mainly three commercial varieties (“Gigante" ,“Redonda" and “Miúda") that have been used to develop more than 1000 clones. This collection is the base for a vigorous plant breeding program, conducted by UFRPE and IPA.

The Moroccan collection includes 4 local accessions and the others are provided by the international collection hosted by Italy in Sardinia and Palermo. The agroclimate in Agadir is of temperate Mediterranean type with cold winters and the selection target is oriented toward quality fruit and forage production. All accessions from INRA Morocco belong to *Opuntia ficus indica* (Mill) while the Arcoverde collection is from different species and includes a large number of crosses.

4.3. Morphological characterization

The morphological characterization has been conducted using the "Descriptor for Cactus Pear" produced by the FAO-ICARDA Cactusnet (Chessa and Nieddu, 1997).

Morphological parameters measured for the whole plant:

- Nclad_P: Number of cladodes per plant
- Nyclad_Y: Number of young cladodes of the current year
- Nfruit_P: Number of fruits per plant.
- Pheig: Plant height (cm)
- PDEW: Plant diameter East-West (cm)
- PDNS: Plant diameter North-South (cm)

Morphological parameters measured for the cladode:

- Lclad: Cladode lenght (cm)
- Wclad: Cladode width (cm)
- Thclad: Cladode thickness (mm)
- CladshIx: Cladode shape index (Lc/wclad)
- Na: Number of areoles per cladode
- Da: Distance between areoles (mm)
- Nspin_a: Number of spines per areole
- Llspin: Length of the longest spine (mm)

4.4. Molecular characterization

4.4.1. Sample preparation for DNA extraction

To remove mucilage, a piece was cut from a fresh cladode far from the glochids, and the cuticle was well peeled using a scalpel. At this step, all the spongy internal tissue, which has a lower number of cells and therefore lower yields of DNA were removed (Figure 5).

This process was done for each variety/accession on the same day. Each piece of cuticle was put into a 2 ml Eppendorf tube and placed into the lyophilizer (Thermo / Savant Modulyo-220 Freeze Dryer) for 3 days at -54°C, 0.04 mbar (Figure 6).

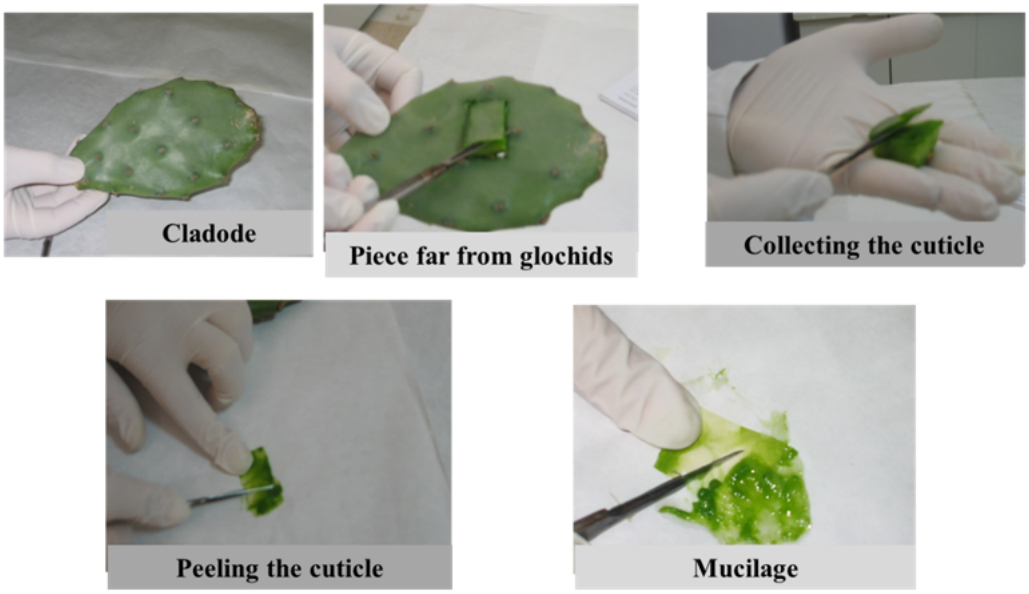


Figure 5. Steps for plant material preparation

Figure 6. Lyophilization of plant material

4.4.2. Molecular analysis

4.4.2.1. DNA extraction from freeze-dried samples

Before starting DNA extraction, two steel beads were placed at the bottom of each tube (Figure 7, annex 3) containing the lyophilized material which was subsequently submitted to mechanical grinding for 15-20 minutes using a mechanical grinder (Qiagen Tissue Lyser/Retsch) (Figure 8, annex 3). A fine green-colored powder was obtained. Then, DNA extraction was performed using the technique of Cetyl Trimethyl Ammonium Bromide (Saghai-Maroof et al., 1984), modified by (Udupa et al., 1998).

The 2 X CTAB solution was kept warm to 65 °C in a water bath (GFL No. 1083). 750 µL of pre-warmed 2 x CTAB solution were added to each 2 ml capacity microfuge tube containing the lyophilized samples, making sure to mix gently afterwards. At this step, cell membrane is disrupted and DNA is released by this cationic surfactant. Then, an additional volume of 750 µL was added. The tubes were incubated at 65 °C for 45-60 min and volume was adjusted to 1.5 ml by adding additional amount of 2 x CTAB solution, mixing gently, every 15-20 min. This solution contains a detergent (Cetyltrimethyl Ammonium Bromide) that binds with DNA and enables its separation from proteins, preventing its degradation. Once the incubation finished, 500 µL of 'chloroform:isoamylalcohol' mix (24:1) were added filling the tubes completely and then mixing vigorously for 15 min. This component allows precipitation of proteins, polysaccharides and extraction of chlorophyll pigments.

A centrifugation was performed at 13000 rpm for 15 min at room temperature in an Eppendorf centrifuge 5415D. At this stage, the solution in each tube is composed of three phases, an aqueous phase which represents the DNA at the top, a yellowish opaque emulsion where polysaccharides and proteins are aggregated and finally an organic phase containing pigments and chloroform at the bottom of the tube (Figure 9, annex 3).

The supernatant of 1 ml of each tube was collected after centrifugation and transferred to a new tube of 2 ml capacity microfuge tube. 666 µL of isopropanol was added and mixed well. The tubes were incubated at room temperature for at least 30 minutes.

Another centrifugation was performed at 13000 rpm for 10 min in an Eppendorf Centrifuge 5415 R for 4° C and then DNA was collected. The supernatant was discarded and pellet washed with 1 ml of 70% ethanol for at least 5 min. Residual CTAB, salt and contaminants were e removed by this solution. A centrifugation at 13000 rpm was launched at 4 ° C for 5 min and the supernatant discarded. After air-drying the pellet at room temperature, 100 µL of sterile distilled water were added to the tubes and placed at 4 ° C overnight.

Next day, the pellet was dissolved by gentle tapping and a current centrifugation was carried out at 13000 rpm for 5 min to collect undissolved debris. The supernatant was removed from each tube and transferred into new tubes of 1.5 mL capacity. These are then labeled as "Stock DNA" and registered with the number representing each accession.

4.4.2.2. DNA assessment by electrophoresis on agarose gel

After DNA extraction, a quality test by agarose gel (1%) electrophoresis was performed to confirm the presence, quality and quantity of DNA.

The agarose gel in 1xTBE buffer melted by boiled in the microwave and then cooled down to 65 °C on stirrer. Then, the gel was poured on to gel casting tray. The samples for loading were prepared by mixing 3 µL DNA, 4 µL sterile distilled water and 3 µL loading buffer (agarose blue).

These components were mixed together in an eppendorf tube then centrifuged for 15 seconds. A volume of 5 µL was taken from the size marker MIII (Figure 10, Annex 3) and deposited in the specific well. (Figure 11a, Annex 3). Once solidified, the gel was run at 60 V and followed by 80 V for 2 hours (Figure 11b, Annex 3). After migration, the gel was immersed in a tank filled with a solution of ethidium bromide to 1 mg / mL for 30 min, followed by a washing with distilled water for 20 min. The bands were then visualized under UV light using a transilluminator (Molecular Imager, Bio Rad Gel Doc XR+) (Figure 11c, Annex 3).

4.4.2.3. DNA dilution

For each sample, 10 µL of DNA stock was diluted with 90 µL sterile double distilled water

4.4.2.4. Polymerase Chain Reaction amplification by SSR markers (microsatellites)

Solutions used, their concentrations and volumes are shown in Annex 2. DNA amplification reaction were carried out in a final volume of 10 µl containing 1 µl of template DNA, and 9 µl of the PCR master mix composed of 4.375 µl of sterile distilled water, 2 µl of 5 X PCR buffer (GoTaq DNA Polymerase), 0.6 µl of 15mM MgCl2, 1 µl of 0.2 mM dNTPs, 1 µl of 10 pmole/µl of forward and reverse primers and 0.025 µl (0.125 U) of Taq DNA Polymerase (Roche) (Figure 12, annex 3). The enzyme (Taq polymerase) was added at the end. The resulting PCR master mix was mixed well and briefly spin down in a microfuge. 9 µL of the PCR master mix were distributed into each PCR tube (Figure 13, Annex 3). Then a volume of 1 µL of diluted DNA (~30-40 ng) was added to each tube containing the PCR master mix. The PCR reaction was carried out in a master cycler gradient thermocycler (Eppendorf 5331) (Figure 14, annex 3) and the PCR program was adjusted by changing

annealing temperature according to Tm of the eight microsatellites primers used for the amplification (Table 3).

Table 3: Microsatellite markers used to analyze the diversity of 50 varieties of *Opuntia ficus indica* Mill.

Microsatellite loci	Forward primer 5'→3'	Reverse primer 5'→3'	Tm en °C
Opuntia 9	CTAGGCTTCATCCCACATTAGG	TCCAAATTCACCTCCTCTGC	59
Opuntia 12	TAATCTTATTCTCAGGTCAGTTACGGTATCTTGTTATTCGTTCG		54
Opuntia 5	TATGCACAAAGCACCATGC	CCAACCATACCAACTGTACTGAC	58
Opuntia 11	CCTACACCTGCTGCCAATC	CGAGACAAACATCAGAGGAG	59
Opuntia 13	CCAAATACCCAGCCCATAC	CGAGAACCTAACTTCCGATG	58
Opuntia 3	GTGAGTGCCCAGATGAAACT	TCCTCAACTTTATTGTAGCAAGAG	57
Ops 9	AACTGCCTCACACGAGTTCC	GCTACGAAATCTGCCGAGTC	60
Ops 24	TCCTTCCATTTCCACCACAC	CAAGACCCCTCATTCCAAAG	58

The following temperature cycles were used:

An initial denaturation step at 94 ° C for 5 minutes followed by 35 cycles of (1) Denaturation of the double-stranded DNA during 60 s at 95 °C; (2) annealing of primers to DNA during 60 s at 55 °C (changes according to TM of the primers).; (3) elongation step during 90 s at 72 °C. The last cycle was followed by a final incubation for 5 min at 72 °C and PCR products were stored at 4 °C before analysis (Figure 15, annex 3).

The DNA amplification products were loaded on a 6% native acrylamide gel in 1 x TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM EDTA)

The two glass plates of the vertical electrophoresis unit were cleaned with NaOH and rinsed with distilled water to remove impurities. The gel solution (150 ml per gel) was prepared as follows: 22.5 ml of 40% acrylamide solution and 15 ml of TBE (5X) and distilled water was added to complete 150ml. The following polymerization agents were added: 110 µl of TEMED and 400µl of 25% APS. The combs were fixed to each plate and gel polymerization took approximately 30 minutes (Figure 16a, annex 3). A pre-run was performed in the presence of 1X TBE buffer for 15 min at 150 V.

The amplified product (7 µL) of the PCR were loaded after mixing with ge loading blue ().100bp size marker was used as size standard (Figure 17, annex 3) (Figure 16b, annex

3). The electrophoresis was performed initially at 150 V for 15 min and later on at 180V. (Figure 16c, annex 3).

After the completion of electrophoresis, the gel was stained ethidium bromide solution (0.1 µg/mL) for 4 min. The stained gel was rinsed in distilled water briefly and then visualized under ultraviolet light (Figure 16e, annex 3) water (Figure 16d, annex 3).

4.5. Data analysis

4.5.1. Morphological characterization

Morphological data from accessions were submitted to:

(i) descriptive statistics (average, standard deviation, coefficient of variation), correlation analysis between different descriptors.

(ii) Pearson principal component analysis (PCA) using XLSTAT 2015 package, to will allow visualization of the differences among the individuals and identify possible groups.

(iii) Agglomerative Hierarchical clustering (AHC) analysis using XLSTAT 2015 package. The Ward's linkage has been used as a clustering method while the Euclidean distance was used the genetic distance (GD) between accessions (Mohammad and Prasanna, 2003). The Euclidean distance between two individuals I and j, having observations on morphological characters (p) denoted by x1, x2, …xp and y1, y2, …yp for i and j, respectively, can be calculated by the following formula (Mohammad and Prasanna, 2003):

$$d(i,j)= [(x_1-y_1)^2 + (x_2-y_2)^2 + \dots (x_p-y_p)^2]^{1/2}$$

The obtained GD matrix was then used to produce the dendrogram with the distribution of accessions based on the morphological descriptors.

4.5.2. Molecular characterization

The number of alleles per locus was counted from the gel profile analysis. The genetic diversity index (**H**) was calculated for all the loci studied according the formula of Nei (Nei, 1987):

$$H = \frac{n(1 - \sum pi^2)}{(n-1)}$$

where "n" is the number of analyzed genotypes and "p_i" is the frequency of i^{th} allele.

The polymorphism information content (PIC) for each marker was also determined, using the following equation of Botstein et al. (1980).

$$\widehat{PIC}_l = 1 - \sum_{u=1}^{k} \tilde{p}_{lu}{}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\tilde{p}_{lu}^2 \tilde{p}_{lv}^2$$

where p_i is the frequency of the i^{th} allele in the set of 50 genotypes. Those parameters served to evaluate the information given by the microsatellites markers.

Assessment of the genetic distance

The binary matrix was obtained from the reading of the electrophoretic patterns corresponding to all the microsatellites analyzed. Amplified fragments for each locus were scored as present allele (1) or absent allele (0). The binary matrix was used to calculate the genetic distance between each pair of accessions using the formula of Jin and Chakraborty (1993).

$$D_{SAB} = 1 - [2\, P_{SAB} / P_{SAX} + P_{SAY}]$$

Where D_{SAB} is the average proportion of alleles shared between populations X and Y.
P_{SAB}, P_{SAX} and P_{SAY} are calculated by all possible combinations of accessions.

A dendrogram was constructed from the genetic distance matrix using the Unweighted Pair Group Method Average (UPGMA) clustering (Sokal and Michener, 1958). These analyses were performed using the software MEGA6 (Tamura et al., 2013).

4.5.3. Relationship between morphological and molecular characterization

The Mantel test (Mantel, 1967) was used to calculate the associations between the similarity matrix of genetic distances resulting from SSR molecular analysis (matrix A) and the matrix of distances calculated from morphological data (matrix B). The p-value has been calculated using the distribution of r(AB) estimated from 10000 permutations. Calculations were made using the XLSTAT 2015 package.

5. Results and discussion

5.1. Phenotypic characterization of the Mediterranean/Moroccan accessions

5.1.1. Phenotypic traits of the Whole plant

The list of accessions and their pictures are indicated in table 4 (annex 1) and pictures 1 (annex 1). Some of these phenotypic traits are highly variable (table 5) as for example the number of fruits and young cladodes per plant and the plant diameters. For most of the phenotypic traits, the coefficient of variation are higher than 50 %. This large variation depends also of the accessions.

Table 5. Phenotypic traits of the whole plant of the Mediterranean/Morocco accessions (Nclad_P: Number of cladodes per plant; Nyclad_Y: Number of young cladodes of the current year; Nfruit_P: Number of fruits per plant; Pheig: Plant height (cm); PDEW: Plant diameter East-West (cm); PDNS: Plant diameter East-West North-South)

Accession		Plant phenotypic traits					
		Nclad_P	Nyclad_Y	Nfruit_P	Pheig, cm	PDEW, cm	PDNS, cm
Bianca Bonacardo (M01)	Average	68,4	9,75	58	117	136	136
	STD	54,65	7,14	64,17	41,47	105,97	70,57
	CV, %	79,90	73,23	110,64	35,44	77,92	51,89
Bianca Macomer (M02)	Average	106,8	2,5	384,6	159	247	234
	STD	9,2	0,58	21,09	17,46	10,95	24,34
	CV, %	8,61	23,20	5,48	10,98	4,43	10,40
Nudosa (M03)	Average	74,4	7	76,67	136	207	192
	STD	29,1	3,39	62,07	23,82	58,27	52,63
	CV, %	39,11	48,43	80,96	17,51	28,15	27,41
Local ecotype, Morocco (MO4)	Average	62,5	22,5	61,2	124	175	178
	STD	41,57	32,36	62,61	27,02	36,91	42,81
	CV, %	66,51	143,82	102,30	21,79	21,09	24,05
Lahmayma, Morocco (MO5)	Average	76	8,2	107,75	125	166	171
	STD	47,3	5,85	42,58	36,4	57,81	75,61
	CV, %	62,24	71,34	39,52	29,12	34,83	44,22
Région d'Eljadida 1, Morocco (M06)	Average	61,6	11,2	67	122	184	178
	STD	22,52	3,56	54,85	15,25	31,9	32,9
	CV, %	36,56	31,79	81,87	12,50	17,34	18,48
Région d'Eljadida 2, Morocco (M07)	Average	55,8	10,8	38,4	100	173	142
	STD	35,9	4,66	30,51	35,88	53,81	37,01
	CV, %	64,34	43,15	79,45	35,88	31,10	26,06
Bianca Roccapalumba (M08)	Average	61,4	17	92	116	177	169
	STD	20,7	25,46	65,45	16,73	38,34	32,48
	CV, %	33,71	149,76	71,14	14,42	21,66	19,22
Rossa	Average	73,8	9,4	190,2	111	189	176

Accession		Plant phenotypic traits					
		Nclad_P	**Nyclad_Y**	**Nfruit_P**	**Pheig, cm**	**PDEW, cm**	**PDNS, cm**
Roccapalumba (M09)	STD	23,69	6,43	62,37	13,42	37,15	22,75
	CV, %	32,10	68,40	32,79	12,09	19,66	12,93
Rossa San Cono (M10)	Average	79,4	11,8	244,4	108	195	197
	STD	38,47	20,29	197,18	13,04	44,44	38,5
	CV, %	48,45	171,95	80,68	12,07	22,79	19,54
Rojalisa (M11)	Average	38,2	3,4	18	115	149	148
	STD	12,11	1,52	20,41	14,14	18,84	56,63
	CV, %	31,70	44,71	113,39	12,30	12,64	38,26
Gialla Roccapalumba (M12)	Average	104	3	276	125	197,5	205
	STD	43,83	-	199,49	12,25	26,3	31,09
	CV, %	42,14		72,28	9,80	13,32	15,17
Trunzara Rossa San Cono (M13)	Average	142,25	9	229,75	143,75	205	187,5
	STD	58,04	5,29	129,32	19,31	31,09	46,28
	CV, %	40,80	58,78	56,29	13,43	15,17	24,68
Bianca San Cono (M14)	Average	137,25	2	241	130	212,5	208,75
	STD	52,8	1	107,77	27,08	20,62	37,05
	CV, %	38,47	50,00	44,72	20,83	9,70	17,75
Algerian (M15)	Average	89	1	177,33	113,33	200	181,67
	STD	14,73	0	42,36	15,28	34,64	24,66
	CV, %	16,55	0,00	23,89	13,48	17,32	13,57
Seedless Roccapalumba (M16)	Average	67,67	1	228,33	90	176,67	148,33
	STD	25,15	-	103,36	8,66	20,21	27,54
	CV, %	37,17		45,27	9,62	11,44	18,57
Morado (M17)	Average	74,33	6,33	170	108,33	151,67	152,67
	STD	63,01	2,89	191,44	37,53	45,37	56,58
	CV, %	84,77	45,66	112,61	34,64	29,91	37,06
Gialla San Cono (M18)	Average	71	2	118,67	111,67	175	136,67
	STD	30,51	1	85,34	16,07	22,91	20,82
	CV, %	42,97	50,00	71,91	14,39	13,09	15,23
Seedless Santa Margherita (M19)	Average	86,33	-	279	108,33	190	175
	STD	60,28	-	210,44	20,82	61,44	56,79
	CV, %	69,83		75,43	19,22	32,34	32,45
Israele Monastra (M20)	Average	24,33	3,33	29	180	126,67	110
	STD	17,04	1,53	-	80	45,37	62,45
	CV, %	70,04	45,95		44,44	35,82	56,77
Total average	**Average**	**58,72**	**14,19**	**124,36**	**88,07**	**129,95**	**121,43**
	STD	**49,93**	**27,48**	**105,68**	**42,79**	**66,56**	**65,14**
	CV, %	**53,93**	**37,97**	**95,47**	**36,40**	**50,83**	**53,24**

5.1.2. Phenotypic traits of the cladodes

The average length of the cladode is 36.5 cm and varies between 45 cm for 'Israele Monastra' and 31 cm for 'Gialla San Cono'. The average width of the cladode is 19.2 cm and varies between 24 cm for 'Nudosa' and 16 cm for Israele Monastra. The average thickness of the cladodes is 17.3 mm with 21.7 mm for Israele Monastra and 14 mm for 'Bianca Roccapalumba'. The CV of cladodes' traits are acceptable and vary between 7.89 % and 21.29 %. The Cladodes morphological traits seem to be more accurate to measure as supported by the relatively lower coefficient of variation (table 6). The lowest CVs are observed with the cladode shape traits with CV values varying between 5 to 20 % with an average value 17 % for the length and 15 % for the width. These values are higher than those reported for Mexican fruit accessions by Cervantes-Herrera et al (2006) with CV values of 2.7 %, 2.6 % and 11 % for cladode length, wwidth and thickness, respectively. Arba (2006) with Mediterranean fruit accessions reported CV values of 5 %, 7.7 % and 40 %, for cladode length, width and thickness, respectively.

The ANOVA calculation (table 7) indicates that the difference between accessions' groups is significant for all morphological traits except for the number of young cladodes per year (NYcladodes), the plant diameters in both directions East-West (PDEW) and South-North (PDNS), and the length of the longest spine (Llspines). Erre and Chessa (2013) using stepwise analysis found that among 38 descriptors, only cladode shape and spines are useful to discriminate Italian cactus pear accessions.

Table 6. Phenotypic traits of the cladodes of the Mediterranean/Morocco accessions

Accession		Phenotypic traits of the cladodes							
		Lclad	**Wclad**	**Thclad**	**CladshIx**	**Na**	**Da**	**Nspin_a**	**Llspin**
Bianca Bonacardo (M01)	Average	42	20	18,6	2,11	109,08	38,4	1,6	1
	STD	7,58	1,87	3,51	0,38	0,84	2,3	0,55	0,31
	CV, %	18,05	9,35	18,87	18,01	10,24	5,99	34,38	31,00
Bianca Macomer (M02)	Average	44,4	20	19,8	2,22	104,33	41,6	1,8	1,1
	STD	2,61	1,73	2,95	0,19	1,52	4,16	0,45	0,1
	CV, %	5,88	8,65	14,90	8,56	20,00	10,00	25,00	9,09
Nudosa (M03)	Average	42,6	24	18,2	1,78	136,81	41,8	2	1,16
	STD	4,56	3,54	2,49	0,09	1,14	3,27	0	0,3
	CV, %	10,70	14,75	13,68	5,06	13,26	7,82	0,00	25,86
Local ecotype, Morocco (M04)	Average	39,8	21	20,4	1,9	135,13	36,2	1,6	1,16
	STD	6,3	2,74	4,72	0,21	2,51	6,76	0,55	0,19
	CV, %	15,83	13,05	23,14	11,05	23,68	18,67	34,38	16,38
Lahmayma, Morocco (M05)	Average	35,2	18,8	14,2	1,88	109,49	33,4	1,2	0,9
	STD	5,59	3,19	3,27	0,17	2,05	5,55	0,45	0,2
	CV, %	15,88	16,97	23,03	9,04	18,98	16,62	37,50	22,22
Région d'Eljadida 1, Morocco (M06)	Average	39,2	20,8	14,8	1,9	120,73	37,6	1,5	0,98
	STD	4,32	2,95	5,17	0,28	0,55	2,61	0,58	0,15
	CV, %	11,02	14,18	34,93	14,74	5,85	6,94	38,67	15,31
Région d'Eljadida 2, Morocco (M07)	Average	33,8	20,2	13,4	1,68	106,81	35,2	1,4	0,78
	STD	2,77	2,49	2,88	0,15	2,17	3,7	0,55	0,19
	CV, %	8,20	12,33	21,49	8,93	21,27	10,51	39,29	24,36
Bianca Roccapalumba (M08)	Average	34,4	19,2	14	1,77	111,88	33,6	1	0,73
	STD	7,8	2,59	3,74	0,2	2	4,67	0	0,25
	CV, %	22,67	13,49	26,71	11,30	18,18	13,90	0,00	34,25
Rossa Roccapalumba (M09)	Average	34,8	21	24,2	1,66	113,68	36,2	1	0,8
	STD	6,1	3,54	8,38	0,13	3,13	6,46	0	0,28
	CV, %	17,53	16,86	34,63	7,83	30,10	17,85	0,00	35,00
Rossa San Cono (M10)	Average	35	19,6	16	1,79	102,78	36,2	1	1,02
	STD	4,53	1,14	3	0,25	1,14	4,21	0	0,33
	CV, %	12,94	5,82	18,75	13,97	11,88	11,63	0,00	32,35
Rojalisa (M11)	Average	32,2	19,6	18,4	1,65	130,39	28,4	-	-
	STD	2,95	2,7	2,41	0,14	1,87	1,52	-	-
	CV, %	9,16	13,78	13,10	8,48	14,38	5,35		
Gialla Roccapalumba (M12)	Average	33,25	16,25	16	2,08	74,27	31,25	1	0,67
	STD	5,56	2,75	2	0,44	1,63	3,3	0	0,15
	CV, %	16,72	16,92	12,50	21,15	14,82	10,56	0,00	22,39
Trunzara Rossa San Cono (M13)	Average	36,5	17,75	18,75	2,06	111,10	33,5	1,67	1
	STD	5,2	0,96	3,4	0,34	0,82	1,29	0,58	0,1
	CV, %	14,25	5,41	18,13	16,50	7,45	3,85	34,73	10,00
Bianca San Cono (M14)	Average	30,5	16,5	14,5	1,85	93,39	31,25	1	0,8
	STD	5,45	1,91	1,29	0,24	3,3	5,12	0	0,42

Accession		Phenotypic traits of the cladodes							
		Lclad	**Wclad**	**Thclad**	**CladshIx**	**Na**	**Da**	**Nspin_a**	**Llspin**
	CV, %	17,87	11,58	8,90	12,97	26,94	16,38	0,00	52,50
Algerian (M15)	Average	34	18,67	15	1,82	73,36	31,67	1	0,9
	STD	4,58	2,31	5,2	0,08	3,51	5,51	-	-
	CV, %	13,47	12,37	34,67	4,40	27,70	17,40		
Seedless Roccapalumba (M16)	Average	32,33	13,33	20,33	2,44	61,72	24	3	0,4
	STD	6,11	2,08	3,51	0,47	1,53	1	-	-
	CV, %	18,90	15,60	17,27	19,26	9,98	4,17		
Morado (M17)	Average	32,67	18	12,67	1,81	58,55	34,67	1	0,65
	STD	2,08	1	4,93	0,04	1,53	2,08	0	0,21
	CV, %	6,37	5,56	38,91	2,21	14,34	6,00	0,00	32,31
Gialla San Cono (M18)	Average	31	18	16,67	1,74	57,83	33	1	0,9
	STD	3	2,65	6,51	0,27	3,06	7,21	0	0,14
	CV, %	9,68	14,72	39,05	15,52	27,01	21,85	0,00	15,56
Seedless Santa Margherita (M19)	Average	35,67	19	18,67	1,88	63,54	38,67	1	0,67
	STD	2,52	0	4,73	0,13	1	5,03	0	0,31
	CV, %	7,06	0,00	25,33	6,91	10,00	13,01	0,00	46,27
Israele Monastra (M20)	Average	45	16	21,67	2,83	80,51	31	-	-
	STD	8,54	1,73	7,37	0,57	1,73	3	-	-
	CV, %	18,98	10,81	34,01	20,14	14,42	9,68		
Overall	**Average**	**36.47**	**19.20**	**17.32**	**1.92**	**97.68**	**34.78**	**1.39**	**0.92**
	STD	**6.30**	**3.06**	**4.84**	**0.34**	**43.70**	**5.60**	**0.53**	**0.27**
	CV, %	**17.29**	**15.93**	**27.94**	**17.94**	**44.74**	**16.10**	**37.70**	**29.17**

Lc : Cladode length (cm); lc : Cladode width (cm); Ec: Cladode thickness (mm); I Fc: Cladode shape index (Lc/lc); Na/10cm² : Number of areoles per 10 cm²; Da : Distance between areoles (mm); Ne/a : Number of spines per areole; Lepl : Length of the longest spine (cm)

Table 7. ANOVA results of the phenotypic data of the Mediterranean/Morocco accessions

		Sum of Squares	df	Mean Square	F	Sig.
Ncladodes	Between Groups	61,805.669	19	3,252.930	2.287	.007**
	Within Groups	91,034.367	64	1,422.412		
	Total	152,840.036	83			
NYcladodes	Between Groups	2,045.047	18	113.614	.775	.718NS
	Within Groups	7,477.239	51	146.613		
	Total	9,522.286	69			
Nfruits	Between Groups	833,809.393	19	43,884.705	3.957	.000***
	Within Groups	654,381.367	59	11,091.210		
	Total	1,488,190.759	78			
Pheight	Between Groups	29,452.230	19	1,550.117	2.041	.018*
	Within Groups	49,375.417	65	759.622		
	Total	78,827.647	84			
PDEW	Between Groups	63,561.176	19	3,345.325	1.548	.099NS
	Within Groups	140,470.000	65	2,161.077		
	Total	204,031.176	84			
PDNS	Between Groups	67,675.772	19	3,561.883	1.701	.059NS
	Within Groups	136,126.417	65	2,094.253		
	Total	203,802.188	84			
Lcladode	Between Groups	1,536.026	19	80.843	2.914	.001**
	Within Groups	1,803.150	65	27.741		
	Total	3,339.176	84			
Wcladode	Between Groups	390.167	19	20.535	3.375	.000***
	Within Groups	395.433	65	6.084		
	Total	785.600	84			
Thcladode	Between Groups	772.540	19	40.660	2.214	.009**
	Within Groups	1,193.883	65	18.367		
	Total	1,966.424	84			
CladodeSI	Between Groups	3.206	19	.169	3.134	.000***
	Within Groups	3.500	65	.054		
	Total	6.706	84			
Nareole	Between Groups	62,935.594	19	3,312.400	2.112	.011**
	Within Groups	122,322.683	78	1,568.240		
	Total	185,258.277	97			
Dareole	Between Groups	1,417.053	19	74.582	3.988	.000***
	Within Groups	1,215.700	65	18.703		
	Total	2,632.753	84			
Nspines	Between Groups	9.691	17	.570	3.570	.000***
	Within Groups	6.867	43	.160		
	Total	16.557	60			
Llspines	Between Groups	3.210	17	.189	1.310	.233NS
	Within Groups	6.200	43	.144		
	Total	9.410	60			

5.1.3. Principal component analysis (PCA) of phenotypic data of the Mediterranean/ Moroccan accessions

Correlation matrix

The resulting correlation matrix and Bartlett's sphericity test are shown in tables 8 and 9 respectively.

The number of cladodes per plant (Nclad_P) is significantly correlated to the number of fruits per plant and to plant diameters. The positive and significant correlation between the number of cladodes and the number of fruits seems to be logic while the significant correlation with the plant diameters according to cardinal points is more difficult to explain. Available literature highlights the importance of cladode orientation during planting and is related to productivity in fruit and/or biomass. The number of young cladodes is significantly correlated to the cladode width and the number of areoles; indeed an increase in the number of cladodes may increase the surface area of cladodes and consequently the number of areoles. The relationship between the number of young cladodes and the cladode width is difficult to explain. The number of fruits per plant (Nfruit_P) is positively correlated to the number of cladodes per plant which is logic but also to the plant diameter. The plant height (Pheig) is significantly and positively correlated to the cladode length, to the cladode shape index and to the length of the longest spine. The length of the cladode is significantly correlated to distance between areoles and the length of the longest spine.

Table 8. Correlation matrix (Pearson (n)) of the morphological descriptors for the Mediterranean/Moroccan accessions

Variables	Nclad_P	Nyclad_Y	Nfruit_P	Phig	PDEW	PDNS	Lclad	Wclad	Thclad	CladshIx	Na	Da	Nspin_a	Llspin
Nclad_P	**1**													
Nyclad_Y	-0.202	**1**												
Nfruit_P	**0.731**	-0.353	**1**											
Phig	0.090	-0.133	0.028	**1**										
PDEW	**0.732**	-0.142	**0.735**	0.101	**1**									
PDNS	**0.728**	0.006	**0.705**	0.140	**0.905**	**1**								
Lclad	-0.212	0.198	-0.133	**0.701**	-0.008	0.032	**1**							
Wclad	-0.158	**0.494**	-0.310	0.025	0.170	0.226	0.400	**1**						
Thclad	-0.146	-0.023	0.091	0.307	-0.013	-0.083	0.442	0.026	**1**					
CladshIx	-0.112	-0.293	0.118	**0.574**	-0.198	-0.232	**0.517**	**-0.569**	0.414	**1**				
Na	-0.120	**0.571**	-0.414	0.204	0.083	0.225	0.397	**0.714**	0.159	-0.294	**1**			
Da	0.117	0.372	0.091	0.235	0.340	0.379	**0.591**	**0.799**	0.042	-0.253	0.392	**1**		
Nspin_a	-0.089	-0.110	-0.007	0.010	0.051	-0.078	0.313	-0.151	0.382	**0.478**	0.137	-0.158	**1**	
Llspin	0.059	0.346	-0.201	**0.487**	0.226	0.295	**0.611**	**0.708**	0.139	-0.141	**0.671**	**0.645**	-0.048	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table 9. Bartlett's sphericity test* (PCA of Mediterranean/Morocco accessions)

Chi-square (Observed value)	277.116
Chi-square (Critical value)	114.268
DF	91
p-value	< 0.0001
alpha	0.05

(*) H0: There is no correlation significantly different from 0 between the variables.
Ha: At least one of the correlations between the variables is significantly different from 0.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 0.01%.

Eigenvalues

Our data shows that 81.7 % of the total variance is explained by the first 4 factors/components (Table 10; Figure 18). Factor 1, 2, 3 and 4 explain 29.4, 24.5, 19.7 and 8.1 % of the total variability, respectively. The first 3 factors explain 73.6 % of the total variance; this result is satisfactory when compared to those of Peña-Valdivia et al (2008) who reported that the first three PC explained 46 % of the total variability. Bendhifi et al. (2013) using 10 morphological traits reported with Tunisian accessions that 93.5 % of the total variance is explained by the first three principal components.

Table 10. Eigenvalues (PCA of Mediterranean/Morocco accessions)

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Eigenvalue	4.1	3.4	2.8	1.1	0.7	0.6	0.6	0.3	0.2	0.1	0.0	0.0	0.0	0.0
Variability (%)	29.4	24.5	19.7	8.1	5.2	4.4	4.2	2.0	1.2	0.7	0.3	0.2	0.1	0.0
Cumulative %	29.4	53.9	73.6	**81.7**	86.9	91.3	95.5	97.5	98.7	99.4	99.7	99.9	100.0	100.0

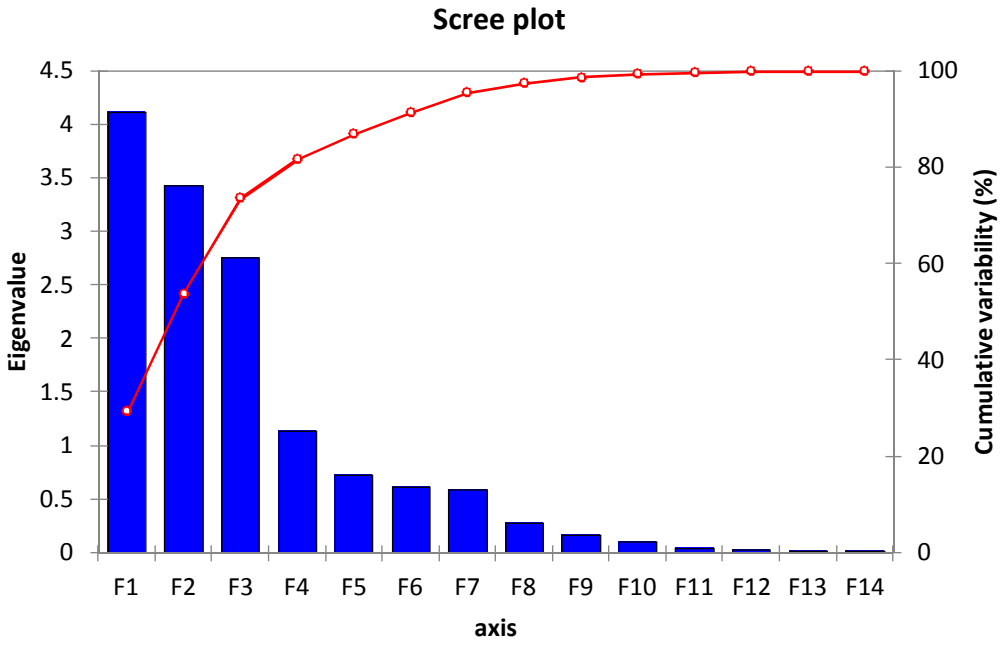


Figure 18. Scree plot of Eigenvalue and cumulative variability

The contribution of the variables to the principal components is presented in table 11. The component 1 is influenced mainly by the number of young cladodes, cladode length and width, number of areoles, distance between areoles and the length of the longest spine. The component 2 is influenced mainly by the number of cladodes, the number of fruits per plant and the plant diameters according the cardinal points. This is clearly represented in the projection of the morphological parameters in the axes 1 and 2 (Figure 19).

Table 11. Contribution of the variables (%) (PCA of Mediterranean/Morocco accessions)

	F1	F2	F3	F4
Nclad_P	0.093	22.789	0.117	0.041
Nyclad_Y	6.720	2.970	4.818	3.576
Nfruit_P	0.354	23.153	2.576	0.186
Phig	3.553	0.021	18.016	20.734
PDEW	2.780	22.279	0.263	2.785
PDNS	4.543	20.844	0.009	0.868
Lclad	10.116	2.443	15.017	1.253
Wclad	18.622	0.592	4.300	0.250
Thclad	0.659	0.931	13.838	11.169
CladshIx	1.637	0.984	29.584	0.746
Na	15.001	1.789	0.500	8.086
Da	16.977	0.487	0.060	2.506
Nspin_a	0.045	0.597	10.574	45.744
Llspin	18.900	0.120	0.327	2.056

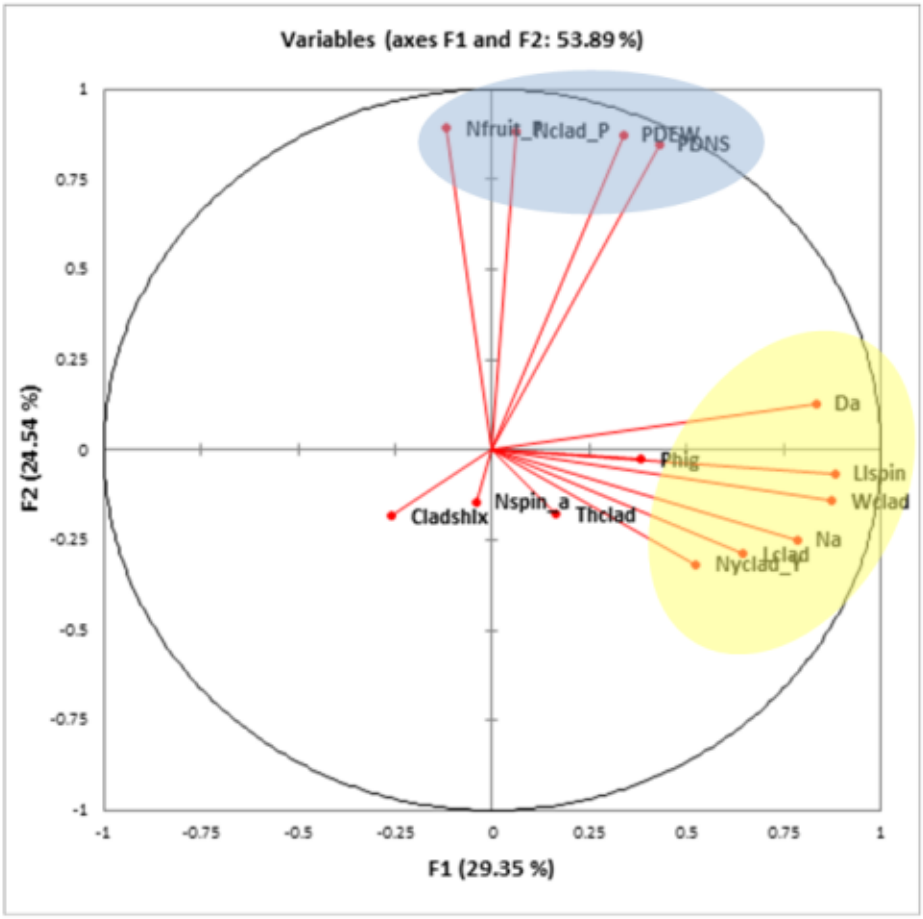


Figure 19. Projection of morphological descriptors in the axes 1 and 2 of the PC

This information is highly valuable and needs further investigation to revise the type of morphological descriptors that are appropriate for a better and more efficient morphological characterization. The number of morphological descriptors may be reduced without potential risk of reducing the accuracy of the phenotypic characterization. Depending of the selection target (fruit versus forage), further investigation is needed to test which descriptors can be removed from each group. Other descriptors do not seem to influence the morphological characterization as the cladode thickness, the number of spine, the plant height and the cladode shape index. Erre and Chessa (2013) reported that non of the plant characteristics is useful to discriminate *Opuntia* accessions, except cladode traits (length, width, thickness) and cladode shape (cladode length/cladode width). On the other hand, Peña-Valdivia et al. (2008) stated that the descriptor absence or presence of spines is a good criteria to discriminate between cactus pear accessions.

Another important output of PCA is the projection of accessions on principal axes F1 and F2 (Figure 20) that shows that they are distributed to the following groups:

- Group 1: Bianca San Cono, Gialla Rocccapaalumba, Seedless Santa Margherita, and Algerian;
- Group 2: Trunzara Rossa San Cono, Rossa San Cono and Rossa Roccapalumba. These 3 accessions have a common characteristic, the red color of the fruit;
- Group 3: Nudosa, Local Morocco (Eljadida 1), Local Morocco, Bianca Bonacardo;
- Group 4: Bianca Roccapalumba, Local Morocco (Lahmayma), Rojalisa, Local Morocco (Eljadida 2), Gialla San Cono, Morado;
- Group 5: includes 3 acccessions that are different from all the other, namely Seedless Roccapalumba, Israele Monastra and Bianca Macomer.

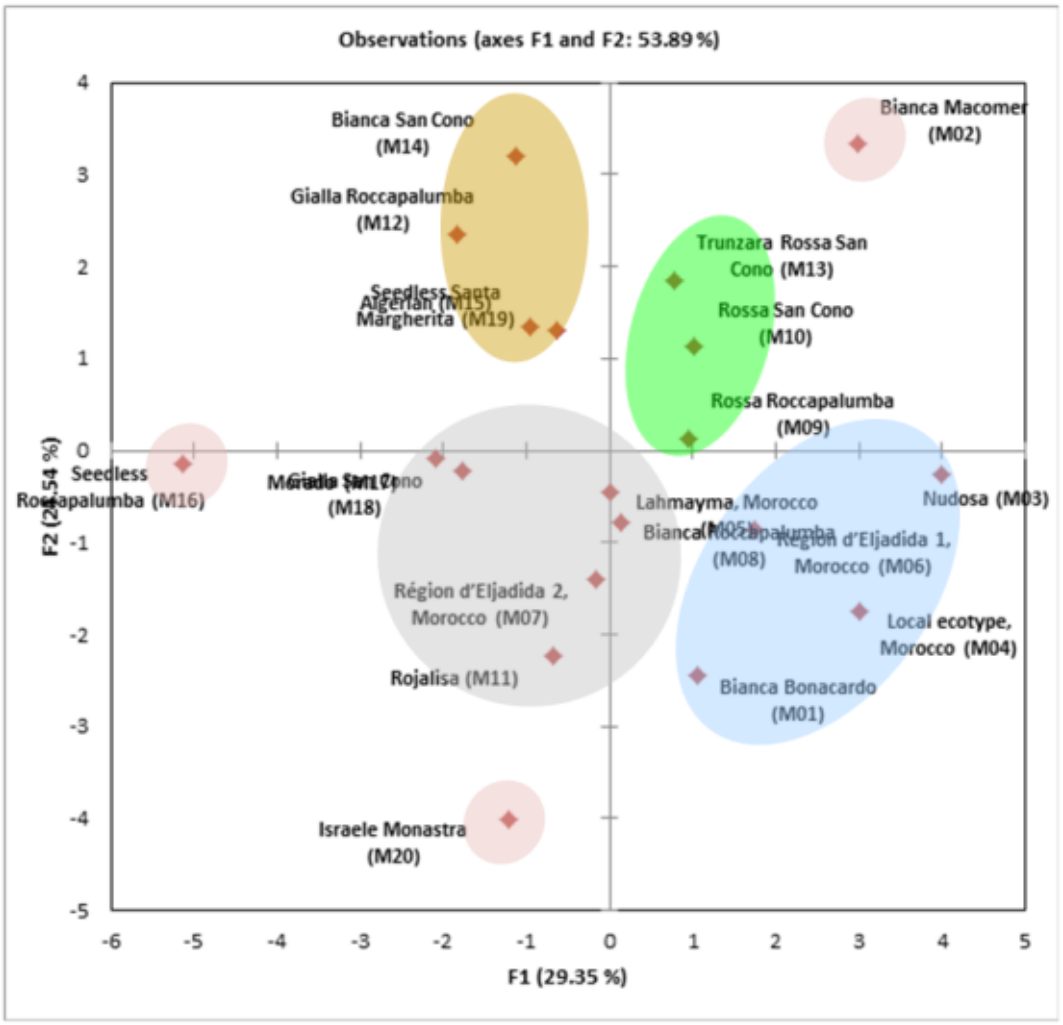


Figure 20. Distribution of Mediterranean/Morocco accessions on axes F1 and F2

5.1.4. Agglomerative hierarchical clustering (AHC) of Mediterranean/Morocco accessions

The table 12 shows the proximity matrix using the Euclidian distance. The highest value of the Euclidian genetic distance is 8.69 and corresponds to Israele Monastra and Bianca San Cono; while the lowest value (2.053) is obtained for Gialla Roccapalumba and Bianca San Cono. This distribution is in full concordance with the results obtained with Pearson principal component analysis (figure 20).

The dendrogram resulting from this matrix distributes the accessions to 4 clusters (Table 13, Figure 21):

- Cluster 1 with 14 accessions: Bianca Bonacardo (M01), Nudosa (M03) , Local ecotype, Morocco (M04), Lahmayma, Morocco (M05), Région d'Eljadida 1, Morocco (M06), Région d'Eljadida 2, Morocco (M07), Bianca Roccapalumba (M08), Rossa Roccapalumba (M09), Rossa San Cono (M10), Rojalisa (M11), Algerian (M15), Morado (M17), Gialla San Cono (M18) and Seedless Santa Margherita (M19)
- Cluster 2 with 4 accessions: Bianca Macomer (M02), Gialla Roccapalumba (M12), Trunzara Rossa San Cono (M13) and Bianca San Cono (M14)
- Cluster 3 with 1 accession: Seedless Roccapalumba (M16)
- Cluster 4 with 1 accession: Israele Monastra (M20)

Table 12. Proximity matrix (Euclidean distance) of the Mediterranean/Moroccan accessions based on phenotypic characters

	M01	M02	M03	M04	M05	M06	M07	M08	M09	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
M01	0																			
M02	6.808	0																		
M03	4.350	4.890	0																	
M04	3.553	6.648	3.947	0																
M05	3.271	6.394	4.733	4.140	0															
M06	2.830	5.829	3.102	2.990	2.121	0														
M07	3.644	7.825	5.209	4.525	2.333	2.642	0													
M08	4.078	7.358	5.480	3.958	2.065	2.638	2.056	0												
M09	4.323	6.147	4.767	4.139	3.856	3.897	4.231	3.941	0											
M10	4.334	5.432	4.580	4.007	2.525	2.756	3.513	2.866	3.210	0										
M11	4.148	8.284	5.750	5.003	3.118	3.952	3.147	3.761	4.152	4.663	0									
M12	5.877	5.820	6.799	6.654	3.633	5.004	5.105	4.449	4.747	3.667	5.530	0								
M13	4.930	4.368	4.966	4.876	3.745	4.221	5.402	4.794	4.427	3.635	5.735	3.662	0							
M14	6.511	5.952	6.698	6.885	3.908	5.254	5.409	4.848	5.190	3.857	5.842	2.053	3.305	0						
M15	4.908	6.025	5.610	5.838	2.594	3.838	3.713	3.752	4.117	2.791	4.290	2.425	3.893	2.731	0					
M16	7.542	9.280	9.516	8.900	7.005	7.928	7.240	7.526	7.614	7.773	6.951	5.969	7.145	7.118	6.626	0				
M17	4.680	7.797	6.846	6.330	2.907	4.361	3.078	3.295	4.940	3.876	4.507	3.560	5.402	4.425	2.918	6.498	0			
M18	4.530	7.483	6.373	6.038	3.038	4.392	3.339	3.927	4.284	3.843	3.749	3.813	5.021	4.342	2.296	6.456	2.382	0		
M19	4.633	5.755	5.788	5.625	3.588	4.185	4.140	3.874	3.214	2.908	5.332	2.989	4.260	4.073	2.915	6.677	3.038	3.308	0	
M20	5.460	8.649	8.147	7.396	6.555	6.922	7.558	7.436	7.688	8.056	6.731	7.526	7.398	8.687	7.480	7.870	7.300	6.904	7.538	0

Bianca Bonacardo (M01);Bianca Macomer (M02);Nudosa (M03);Local ecotype, Morocco (M04);Lahmayma, Morocco (M05);Région d'Eljadida 1, Morocco (M06);Région d'Eljadida 2, Morocco (M07);Bianca Roccapalumba (M08);Rossa Roccapalumba (M09);Rossa San Cono (M10);Rojalisa (M11);Gialla Roccapalumba (M12);Trunzara Rossa San Cono (M13);Bianca San Cono (M14);Algerian (M15);Seedless Roccapalumba (M16);Morado (M17);Gialla San Cono (M18);Seedless Santa Margherita (M19);Israele Monastra (M20)

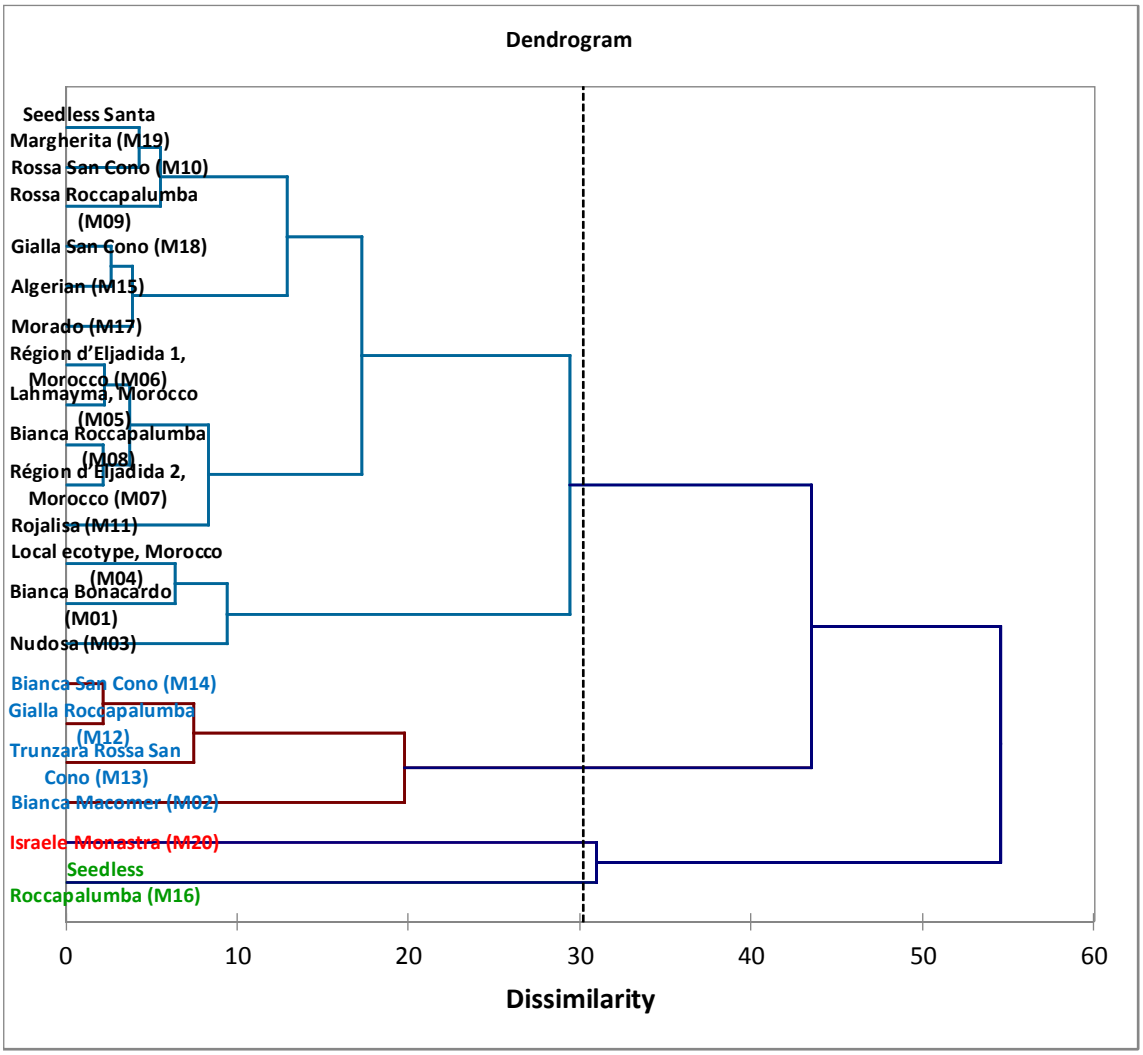


Figure 21. Dendrogram of Mediterranean/Morocco accessions based on phenotypic characterization (class 1 in black, class 2 in blue, class 3 in red and class 4 in green)

Table 13. Distribution of accession by Class resulting from the AHC analysis of Mediterranean/Morocco accessions

Cluster 1	Cluster 2	Cluster 3	Cluster 4
Bianca Bonacardo (M01) Nudosa (M03) Local ecotype, Morocco (M04) Lahmayma, Morocco (M05) Région d'Eljadida 1, Morocco (M06) Région d'Eljadida 2, Morocco (M07) Bianca Roccapalumba (M08) Rossa Roccapalumba (M09) Rossa San Cono (M10) Rojalisa (M11) Algerian (M15) Morado (M17) Gialla San Cono (M18) Seedless Santa Margherita (M19)	Bianca Macomer (M02) Gialla Roccapalumba (M12) Trunzara Rossa San Cono (M13) Bianca San Cono (M14)	Seedless Roccapalumba (M16)	Israele Monastra (M20)

The results obtained by the AHC show the same trend as those found by PCA and therefore confirm these findings.

The variance decomposition for the optimal classification (Table 14) show that the %variance within and between classes are 56 % and 44 %, respectively, indicating a relatively high heterogeneity between clusters.

Table 14. Variance decomposition for the optimal classification (AHC analysis of Mediterranean/Morocco accessions)

	Absolute	Percent
Within-class	7911.647	**56.12%**
Between-classes	6185.316	**43.88%**
Total	14096.963	100.00%

Using 65 morphological traits, Peña-Valdivia et al. (2008) showed that multivariate analysis clearly separated the 46 *Opuntia* accessions in two groups by presence or absence of spines on the cladodes. Only width and length of cladodes, areole width, number of areoles per cladode face, and leaf length were significantly different between the two groups. According to Pimienta-Barrios and Muñoz-Urias (1995), cladode size among domesticated

types is a character associated with ploidy; large cladodes correspond to domesticated polyploidy types. Contrasting to this, Mondragón-Jacobo and Pérez-González (2001) presume that intra-varietal differences in cladode size might correlate with soil nutrient. More likely cladode size depends on both environment and genotype. Indeed according to Nobel (1988), cladode thickness, color, arrangement and number of spines per areole depend on the environment and *Opuntia* genotype. *Opuntia* morphology might result of intra- and inter-specific hybridization (Peña-Valdivia et al., 2008). Gibson and Nobel (1986) pointed out that hybridization is a contributing factor for morphological variation in *Opuntia.* Also, Mondragón-Jacobo and Pérez-González (2001) indicated that partial or total crossing is common between cultivated *Opuntia* types. In our case the effect of environment may be discarded within each collection because they are submitted to the same environment.

The presence or absence of spines is controversial. Indeed spineless accessions grown under stressful environment (heat, drought) may turn spiny (Nefzaoui, personal communication). Rebman and Pinkava (2001) stated that within the genus, the growth habit, the presence of spines, the number of spines per areole, and the number of areoles may differ drastically in different growing regions. Nieddu and Chessa (1997) relate this to a different expression of the genes encoding these characters as well as to epigenetic and environmental factors (Labra et al., 2003).

Spinescence was considered to be a distinctive characteristic in the assignment of a genotype to a certain species in both early and later taxonomical classifications (Scheinvar 1995; Reyes-Aguero et al. 2006). However some phenotypic characters, including the presence/absence of spines, often considered for their agronomic value, show a great variability in progenies and can be very different from those of mother plants (Nieddu et al., 2006).

Quantitative parameters (cladodes length, fruit weight, fruit taste, number of fruit by cladodes, seed number, pulp weight and the peel weight) allowed discrimination of Tunisian cactus cultivars (Ben Dhifi et al., 2013).

5.2. Phenotypic characterization of the IPA accessions

The phenotypic characterization was conducted on 279 accessions from IPA collection in Arcoverde. The list of IPA accessions and their pictures are indicated in table 15 (annex 1) and pictures 2 (annex 1). The complete data of phenotypic traits measured on the 279 accessions are indicated in table 16 (annex 1).

Some of these traits or descriptors are highly variable with a coefficient of variation as high as 150 to 210.9 % (Table 17, annex 1). The number of spines, the length of spines, the number of spines per areole and the number of glochides are having the highest coefficient of variation.

Table 17. Samples descriptive statistics of IPA accessions

Variable	n	Minimum	Maximum	Mean	Std. deviation	CV %
P_Height	279	0.420	2.060	1.097	0.263	24.0
P_Width	279	0.520	2.320	1.388	0.321	23.1
Cl_lenght	279	19.000	59.000	36.176	5.889	16.3
Cl_width	279	10.000	34.000	20.645	3.167	15.3
CladshIx	279	0.792	4.100	1.779	0.344	19.3
Cl_thickness	279	10.000	31.000	21.982	3.796	17.3
N_areole	279	30.000	168.000	117.842	21.151	17.9
N_spines	279	0.000	5.000	0.993	1.439	**144.9**
Spine_size	279	0.000	26.000	3.308	5.244	**158.5**
NSpine_areole	279	0.000	4.000	0.348	0.541	**155.5**
N_glochides	279	0.000	7.000	0.900	1.898	**210.9**

5.2.1. Principal Component Analysis (PCA)

The plant height is significantly ($P<0.05$) and positively correlated to the plant width ($r=0.55$), the cladode length ($r=0.42$), the cladode width ($r=0.13$), the cladode shape index ($r=0.26$) and the number of areoles (0.13); the plant height is significantly and negatively correlated to the cladode thickness ($r=-0.17$), the number of spines ($r=-0.22$), the spine size ($r=-0.22$) and the number of spine by areole ($r=-0.2$) (table 18). The plant width is significantly ($P<0.05$) and positively correlated to the cladode length ($r=0.31$) and the cladode shape index ($r=0.21$) and negatively correlated to the number of spines ($r=-0.12$). The cladode length is significantly ($P<0.05$) and positively correlated to the cladode shape index ($r=0.55$), the number of areoles ($r=0.2$) and the number of glochides ($r=0.14$). The cladode width is negatively correlated to the cladode shape index ($r=-0.51$) and positively correlated to the number of spines, the spine size, the number of spine by areole and the number of glochide. The cladode shape index is negatively correlated to the cladode thickness, the number of spines, the number of spine per areole and the number of glochides. The cladode thickness is positively correlated to the number of spines ($r=0.19$), the spine size ($r=0.15$) and the number of glochides ($r=0.19$). The number of spines is positively correlated to the spine size ($r=0.91$), the number of spine by areole ($r=0.95$) and the number of glochides ($r=0.22$). The spine size is positively correlated to the number of spine by areole ($r=0.88$) and the number of glochides ($r=0.24$).

The correlation between cladode length and width has been reported by Neder et al (2013) investigating the correlation between morphological traits and biomass yield of IPA accessions. As stated by Neder et al.(2013), these morphological traits are closely correlated to the green and dry biomass yield.

De Amorim et al. (2015) used slightly different morphological traits (cladode area index, plant height, plant width, number of cladodes) to investigate morphological and productive characterization of Nopalea accessions in Alagoas, Rio largo (Brazil). He stressed that the number of cladodes and the cladode area index may be used as criteria for selection of superior varieties in breeding programs.

Table 18. Correlation matrix (Pearson (n)) between morphological descriptors of IPA (Brazil) accessions

Variables	P_Height	P_Width	Cl_lenght	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
P_Height	**1**										
P_Width	**0.548**	**1**									
Cl_lenght	**0.419**	**0.307**	**1**								
Cl_width	**0.126**	**0.213**	**0.398**	**1**							
CladshIx	**0.256**	0.074	**0.545**	**-0.513**	**1**						
Cl_thickness	**-0.168**	-0.077	-0.050	**0.141**	**-0.182**	**1**					
N_areole	**0.129**	0.059	**0.201**	0.067	0.106	0.051	**1**				
N_spines	**-0.221**	**-0.119**	-0.035	**0.160**	**-0.171**	**0.194**	0.037	**1**			
Spine_size	**-0.213**	-0.103	-0.032	**0.203**	**-0.191**	**0.147**	-0.004	**0.911**	**1**		
NSpine_areole	**-0.202**	-0.069	-0.035	**0.179**	**-0.180**	**0.196**	0.052	**0.946**	**0.878**	**1**	
N_glochides	0.054	0.000	**0.141**	**0.159**	0.000	0.082	-0.076	**0.220**	**0.237**	**0.199**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Eigenvalues

The contribution of the factors to the variability is 29.286, 19.383, 13.213, 9.695 and 8.981 % for F1, F2, F3, F4 and F5, respectively. The cumulative variability of 80 % is reached with factor F5 (Table 19, Figure 22).

Table 19. Eigenvalues obtained for IPA accessions

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	3.221	2.132	1.453	1.066	0.988	0.809	0.726	0.409	0.122	0.048	0.024
Variability (%)	29.286	19.383	13.213	9.695	8.981	7.356	6.599	3.717	1.108	0.440	0.221
Cumulative %	29.286	48.669	61.883	71.578	**80.559**	87.915	94.514	98.231	99.339	99.779	100.000

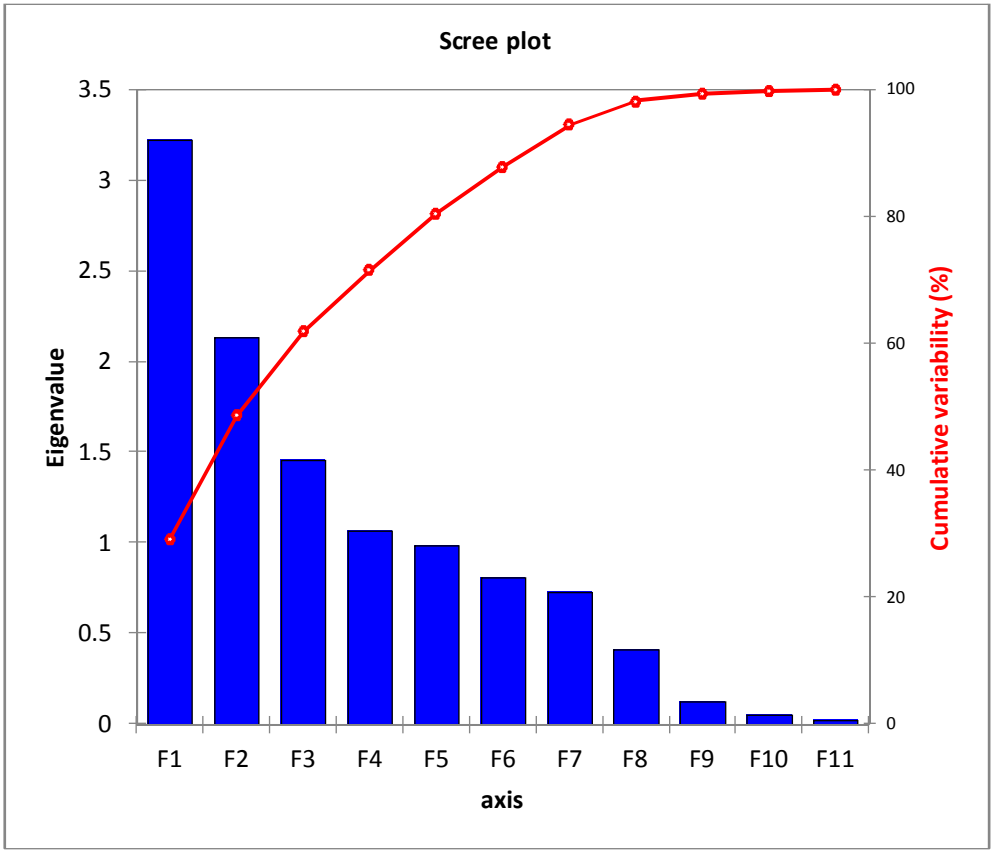


Figure 22. Scree plot of eigenvalues and cumulative variability of IPA accessions

The factor F1 is influenced mainly by the number of spines (N-spines), the spine size and the number of spine by areole. Therefore we may assume that F1 reflects the "vector spines". The factor F2 is influenced by the plant height (P_height), the plant width (P_width) and the length of the cladode (Cl_lenght). Thus, globally F2 reflects the vector cladode. Cladode width (Cl_width) and cladode shape index (Cladshix) are the variables that mostly contribute to F3. These findings confirm the results already observed for the

Mediterranean/Moroccan accessions (Table 20, Figure 23 and 24). Paixão (2012) working with the same IPA collection, highlighted the high magnitude of plant height, width and length of the cladodes as traits to discrimante cactus accessions.

Table 20. Contribution of the variables (%) (IPA accessions)

	F1	F2	F3	F4	F5
P_Height	5.304	**21.306**	0.237	1.577	4.072
P_Width	1.923	**18.471**	4.188	1.942	13.147
Cl_lenght	1.244	**30.994**	1.710	0.675	7.413
Cl_width	2.379	11.155	**35.382**	0.237	0.941
CladshIx	5.487	4.385	**44.940**	0.014	3.029
Cl_thickness	3.321	0.075	3.998	15.112	28.483
N_areole	0.024	4.386	0.866	59.649	0.470
N_spines	**26.602**	1.601	3.562	0.004	1.927
Spine_size	**25.532**	1.711	2.364	0.443	2.135
NSpine_areole	**25.754**	1.982	2.720	0.008	2.895
N_glochides	2.430	3.934	0.035	20.338	35.489

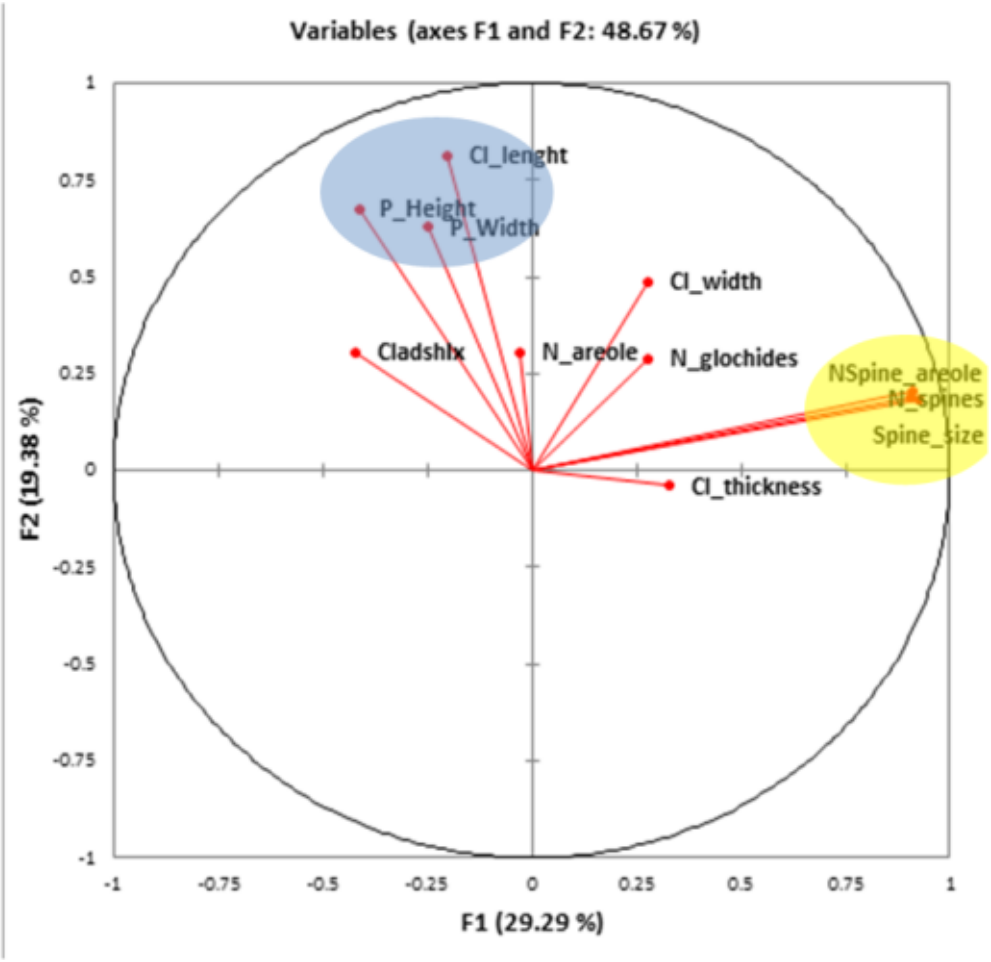


Figure 23. Projection of morphological descriptors on F1 and F2 axes

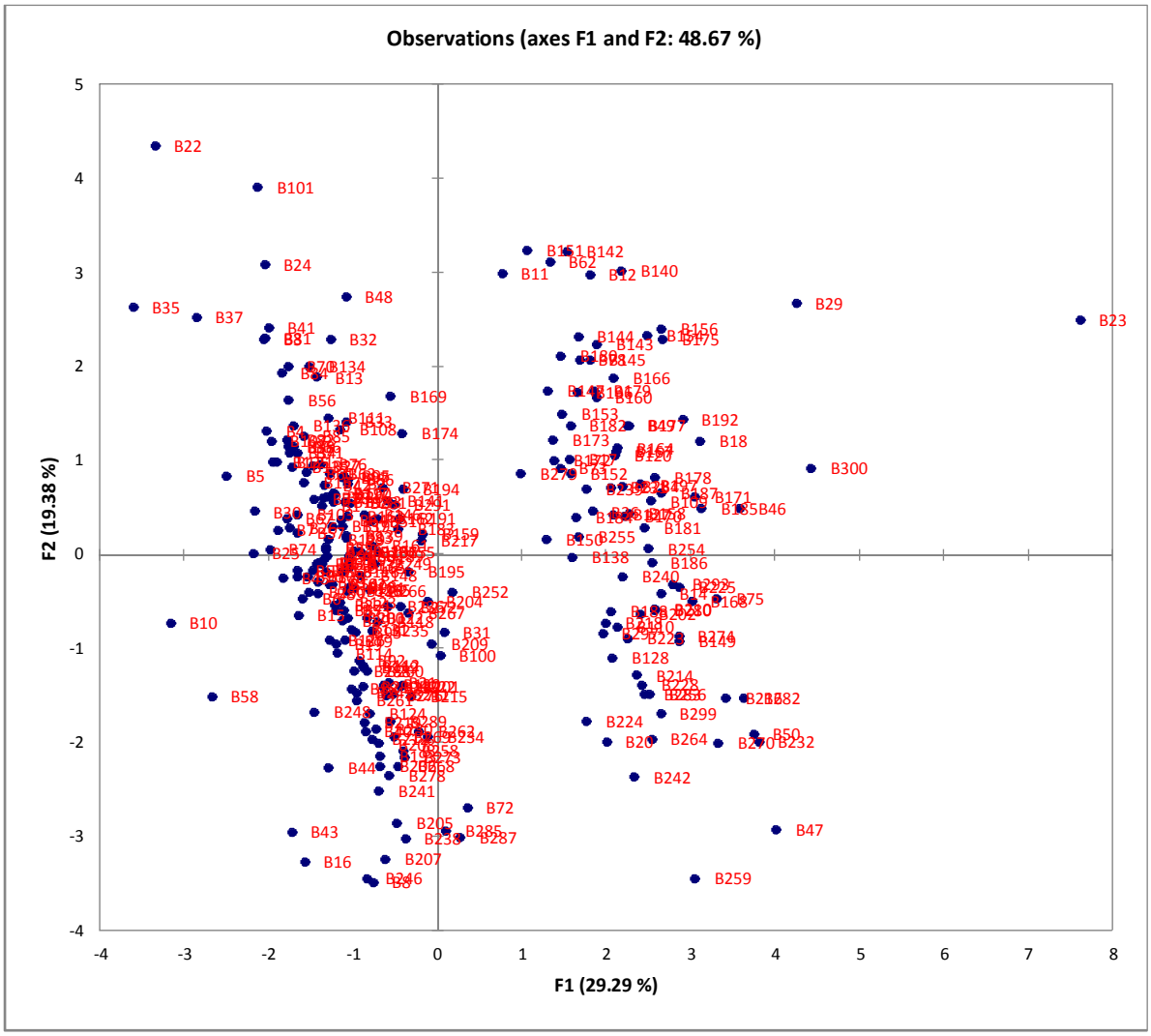


Figure 24. Projection of accessions on F1 and F2 axes

5.2.2. Agglomerative hierarchical clustering (AHC)

The AHC was conducted using the Euclidian distance to measure dissimilarity and the Ward's method for agglomeration; data were centered and reduced. Two main clusters were formed (Figure 25, Table 22).

The variance decomposition for the optimal classification (Table 21) show that the %variance within and between classes are 82.11% and 17.89% respectively, indicating a relatively high heterogeneity between clusters.

Table 21. Variance decomposition for the optimal classification (IPA accessions)

	Absolute	Percent
Within-class	443.576	82.11%
Between-classes	96.672	17.89%
Total	540.248	100.00%

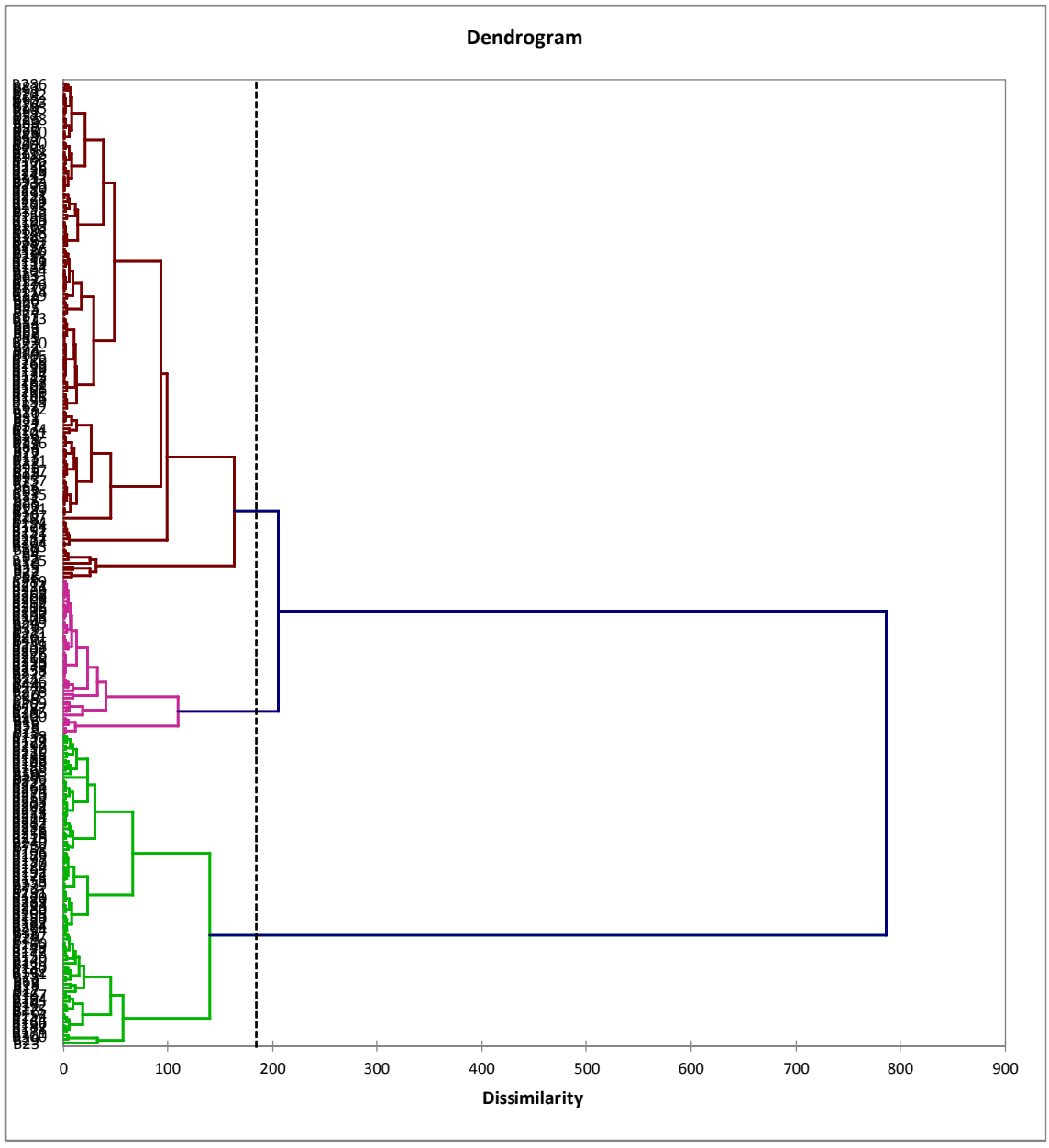


Figure 25. Dendrogram of Mediterranean/Morocco accessions based on Euclidian distance (class 1 in brown, class 2 in pink and class 3 in green)

Table 22. AHC results by class (IPA accessions)

Class	1	2	3
Objects	144	45	90
Within-class variance	318.024	934.982	402.363
Minimum distance to centroid	2.414	3.676	4.528
Average distance to centroid	14.653	23.742	16.765
Maximum distance to centroid	89.706	69.791	56.764
Accessions code number	B1, B2, B3, B4, B5, B6, B7, B10, B13, B19, B22, B24, B26, B27, B28, B30, B31, B32, B33, B34, B35, B37, B38, B41, B42, B45, B48, B51, B52, B53, B54, B55, B56, B57, B60, B61, B63, B64, B65, B66, B67, B68, B69, B70, B71, B74, B76, B77, B80, B81, B82, B83, B84, B85, B86, B87, B88, B89, B90, B91, B92, B93, B94, B95, B96, B97, B98, B99, B101, B102, B103, B104, B105, B106, B107, B108, B111, B112, B113, B114, B115, B116, B117, B118, B119, B121, B122, B123, B124, B125, B126, B129, B131, B132, B133, B134, B135, B136, B137, B139, B141, B146, B148, B155, B157, B159, B161, B162, B163, B165, B169, B174, B176, B183, B189, B190, B191, B194, B195, B198, B199, B200, B201, B204, B217, B219, B220, B233, B235, B236, B237, B249, B250, B252, B265, B266, B267, B271, B272, B281, B286, B288, B290, B291	B8, B15, B16, B20, B21, B25, B39, B40, B43, B44, B58, B72, B79, B100, B130, B193, B205, B206, B207, B208, B209, B211, B212, B215, B221, B222, B230, B234, B238, B241, B243, B246, B248, B258, B260, B261, B262, B268, B269, B273, B276, B278, B285, B287, B289	B11, B12, B14, B17, B18, B23, B29, B36, B46, B47, B49, B50, B62, B73, B75, B78, B109, B110, B120, B127, B128, B138, B140, B142, B143, B144, B145, B147, B149, B150, B151, B152, B153, B154, B156, B158, B160, B164, B166, B167, B168, B170, B171, B172, B173, B175, B177, B178, B179, B180, B181, B182, B184, B185, B186, B187, B188, B192, B196, B197, B202, B210, B214, B216, B218, B223, B224, B225, B228, B231, B232, B239, B240, B242, B254, B255, B256, B259, B264, B270, B274, B279, B280, B282, B283, B284, B293, B297, B299, B300

(*) Underlined numbers are the accessions randomly chosen to be used for molecular analysis

Most of the IPA accessions result from crosses and in the absence of full knowledge of the progeny, it is difficult to draw sound and clear conclusions of the morphological characterization and cluster and PCA results. Nevertheless, some preliminary observations may be formulated:

- Globally and based on morphological characterization, the genetic distances between most of the crosses are relatively small
- The PCA graph shows two distinct groups of accessions together with a number of accessions sparsely distributed
- The cluster analysis and the detailed resulting dendrogram may allow formulating some comments:

- One group of crosses resulting closely related to Sanrizil II – III – IV, Chile/1118, Jalpa, Copena VI, Tobarito, Marmillon/1327, Redonda and Oreja de Elefante.
- A second group of accessions closely related: cv. 1281, Marmillon/1311, Skinner court, Directeur, Algerian, Copena F1, cv. 1258 and Fausicaulis.
- A third group of crosses resulting probably from Atlixco, Moradilla and Chile/1317.
- A fourth group of accessions closely related: Nopalea Miuda, Blanco Michocan and Blanco San Pedro.
- A fifth group composed of a large number of crosses closely related to Gigante, Penca Alargado and Blanco San Pedro.
- A sixth group composed of crosses closely related to an "Unknown Mexican cv/1296, Villa nueva, Liso forrageiro, Politlan, Oaxaca, Liso M Aleman, Algeria/1267, Amarillo Milpa Alta and Marmillon/1327.

Our results confirm the findings of Paixão (2012) who used 13 quantitative traits to assess the morphological characterization of 8 *Opuntia* and *Nopalea* clones from IPA collection through multivariate analysis. This research revealed three genetically distinct groups: One group formed by the Algerian gentotypes, Chile Fruit, Copena F1, Gigante, IPA-20; a second group with Orelha de Elefante Africana and Redonda varieties; and a third group comprises Miúda variety (Neder et al., 2013).

Most of the available literature is dealing with a small number accessions compared to our experiments. In depth analysis of the cluster analysis results will probably allow a better understanding of the current genetic variability of the collection germplasm.

5.3. Relationship between phenotypic and molecular traits

The 20 accessions from the Mediterranean/Moroccan collection and the 30 accessions from IPA collection, randomly selected after cluster analysis, were combined and submitted to both phenotypic and molecular analysis and the resulting dissimilarities matrixes were compared using the Mantel test to determine potential relationship between phenotypic and molecular characteristics.

5.3.1. Phenotypic characterization

5.3.1.1. Principal component analysis

Phenotypic traits of the 50 accessions are presented in table 23 (annex 1). The PCA used is of Pearson type, with a minimum of 80 % filter factors. The summary statistics are shown in table 24 below:

Table 24. Summary statistics of IPA accessions

Variable	n	Minimum	Maximum	Mean	Std. deviation	CV%
Hp (cm)	50	7.000	180.000	112.388	32.103	28.6
Wp(cm)	50	70.000	234.000	147.572	39.813	27.0
Cl_Len(cm)	50	21.000	59.000	37.286	6.957	18.7
Cl_wid(cm)	50	10.000	27.000	19.514	3.499	17.9
Cl_thi(mm)	50	10.000	30.000	19.445	4.701	24.2
L/W index	50	1.222	4.100	1.957	0.489	25.0
Na	50	38.000	154.000	115.130	20.316	17.6
NSpine_areole	50	0.000	3.000	0.711	0.719	101.1
LlSpine (mm)	50	0.000	16.000	5.032	4.825	95.9

The correlation matrix between phenotypic traits is presented in table 25.

The Plant height is significantly correlated to the plant diameter, to the length of the cladode, to the cladode shape index and to the number of areoles. The plant diameter is significantly correlated to the number of spines per areole. The cladode length is significantly correlated to the cladode width, to the cladode shape index and to the number of areoles. The cladode width is significantly correlated to the cladode thickness and to the cladode shape index. The cladode shape index is significantly correlated to the number of areoles. The number of spine by areole is significantly linked to the length of the longest spine. On the other hand, the Sig. value for Bartlett's sphericity test (Table 26) leads us to reject the null hypothesis and conclude that there are correlations in the data set that are appropriate for factor analysis, and thus the PCA analysis may be conducted.

Table 25. Correlation matrix (Pearson (n)) between phenotypic traits of IPA accessions

Variables	Hp	Wp	Cl_Len	Cl_wid	Cl_thi	L/W index	Na	NSpine_ areole	LlSpine
Hp (cm)	**1**								
Wp(cm)	**0.66**	**1**							
Cl_Len(cm)	**0.57**	0.26	**1**						
Cl_wid(cm)	0.14	0.15	**0.37**	**1**					
Cl_thi(mm)	-0.21	-0.16	-0.00	**0.32**	**1**				
L/W index	**0.31**	-0.01	**0.53**	**-0.54**	-0.27	**1**			
Na	**0.31**	0.05	**0.44**	0.21	-0.05	**0.29**	**1**		
NSpine_areole	0.11	**0.33**	-0.05	-0.14	-0.15	0.02	-0.07	**1**	
LlSpine (mm)	0.06	0.26	-0.05	0.01	-0.14	-0.11	-0.13	**0.81**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table 26. Bartlett's sphericity test (IPA accessions)

Chi-square (Observed value)	260.796
Chi-square (Critical value)	50.998
DF	36
p-value	< 0.0001
alpha	0.05

H0: There is no correlation significantly different from 0 between the variables.
Ha: At least one of the correlations between the variables is significantly different from 0.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 0.01%.

The Eigenvalues table 26 shows that 81 % of the cumulative variance is reached with the four first factors; which is also confirmed by the Scree plot (Figure 27).

Table 27. Eigenvalues (PCA of IPA accessions)

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	2.538	2.065	1.740	0.947	0.743	0.539	0.242	0.163	0.023
Variability (%)	28.201	22.942	19.337	10.526	8.254	5.988	2.691	1.809	0.253
Cumulative %	28.201	51.143	70.480	**81.006**	89.260	95.247	97.938	99.747	100.000

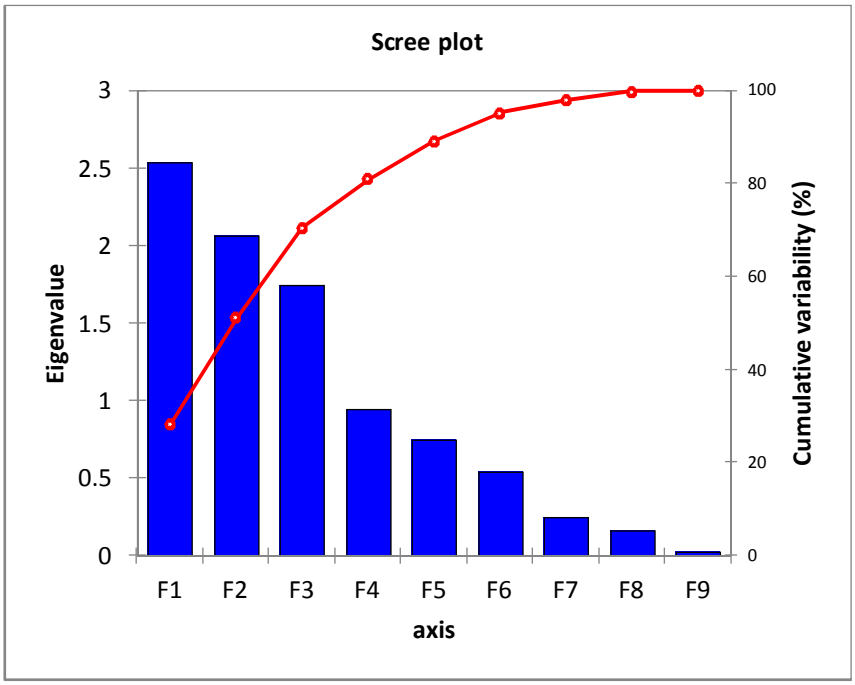


Figure 26. Scree plot for principal components

The contribution of the variables to PCs (Table 28) shows that the plant height (Hp), the plant width (Wp) and the cladode length (Cl_len) contribute to 68 % of the factor F1 while the number of spines and the length of the longest spine explain the factor F2 (Figure 27). Therefore the F1 may represent the plant dimensions while F2 is the spine load.

The projection of accessions into F1 and F2 axes (Figure 28) discriminates roughly two groups: one group dominated by the Mediterranean accessions basically fruit-oriented and another group from IPA collection oriented toward forage production. This first assessment will be reinforced by the cluster analysis.

Table 28. Contribution of the variables (%) (PCA of IPA accessions)

	F1	F2	F3	F4
Hp (cm)	**28.776**	0.173	0.662	10.508
Wp(cm)	**16.533**	4.707	5.483	24.046
Cl_Len(cm)	**23.263**	8.204	0.736	6.016
Cl_wid(cm)	0.549	3.460	**45.689**	0.535
Cl_thi(mm)	4.095	3.577	**14.715**	13.038
L/W index	**11.063**	2.155	**30.782**	5.866
Na	**10.269**	8.882	0.036	16.532
NSpine_areole	3.444	**34.621**	0.142	11.286
LlSpine (mm)	2.008	**34.220**	1.755	12.175

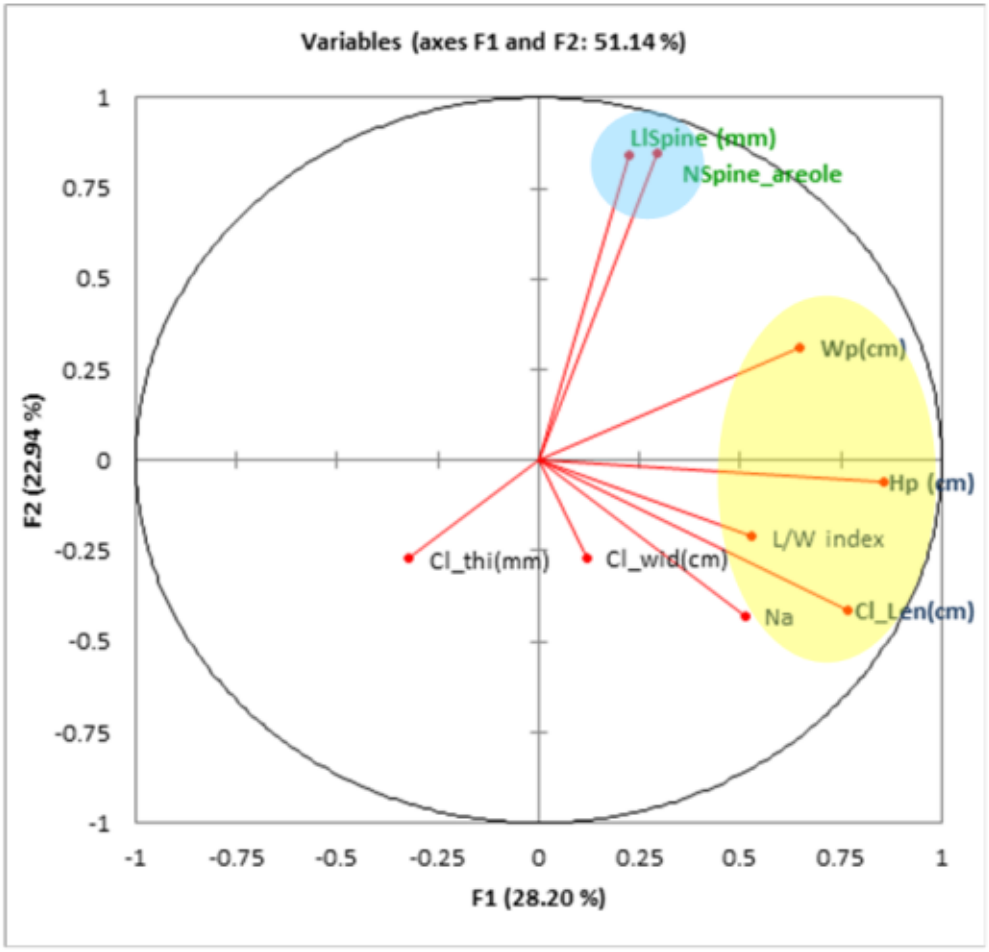


Figure 27. Discrimination of the phenotypic traits based on the two first PCs

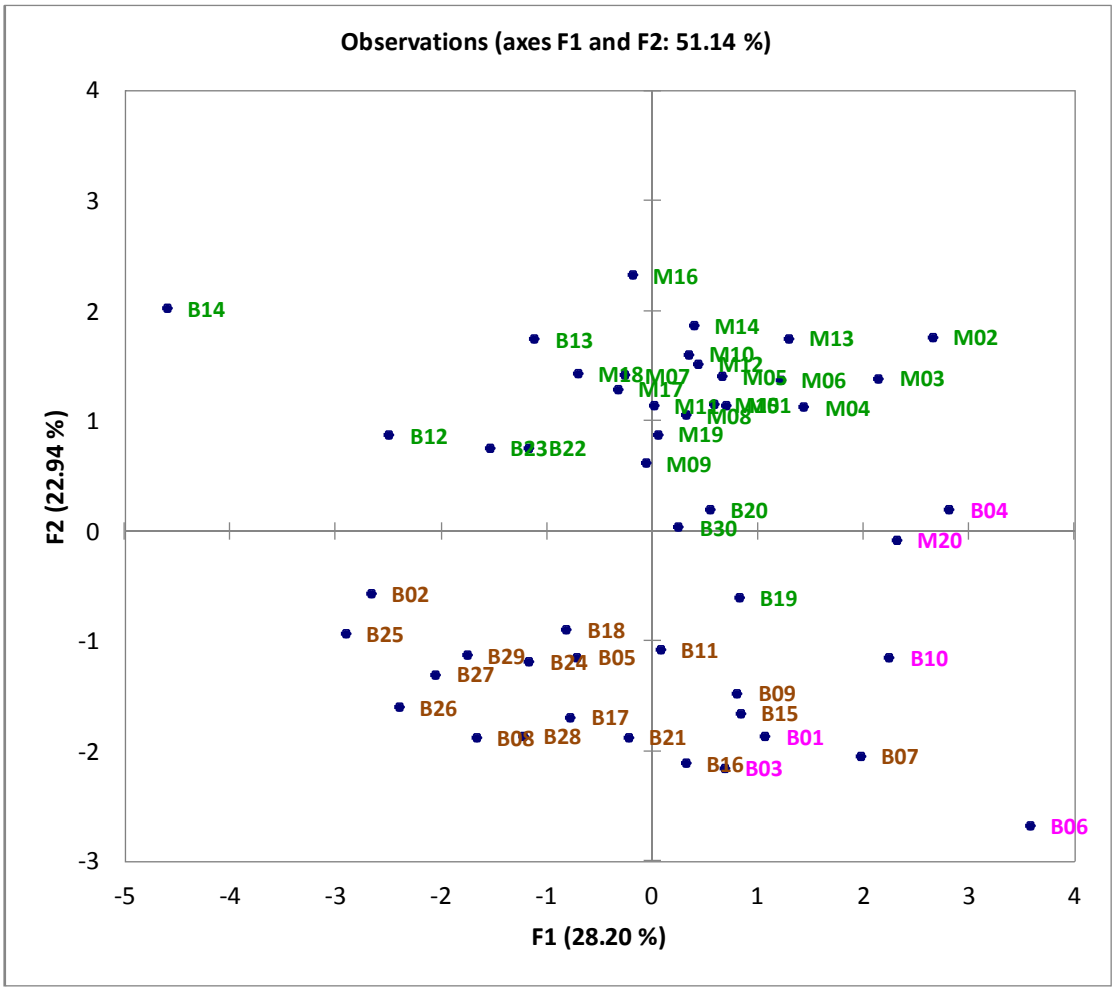


Figure 28. Discrimination of the accessions based on the two first PCs (symbols and color of the accessions according to the clusters given by the dendrogram)

The PCA analysis was conducted a second time after removal of cladode width (Cl_wid) and the cladode thickness (Cl_thi) that have low contribution in both factor 1 and 2. The new results are as follows.

Correlation matrix (Pearson (n))

The correlation values between phenotypic traits remain unchanged, while 88.6 % of the cumulative variability (Table 29) is reached by the first 4 PCs, and the analysis can be limited to the first three PCs because 78.5 % of the cumulative variability with the first three PCs. This last statement is confirmed by the curve in the Scree plot (Figure 29) and the Loading plot of the variables based on the first two PCs (Figures 30 and 31).

Table 29. Eigenvalues after reduction of the number of phenotypic traits (50 accessions from Mediterranean/Morocco and IPA collections)

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	2.455	2.028	1.013	0.709	0.391	0.243	0.160
Variability (%)	35.067	28.974	14.473	10.135	5.591	3.469	2.291
Cumulative %	35.067	64.042	**78.515**	**88.649**	94.240	97.709	100.000

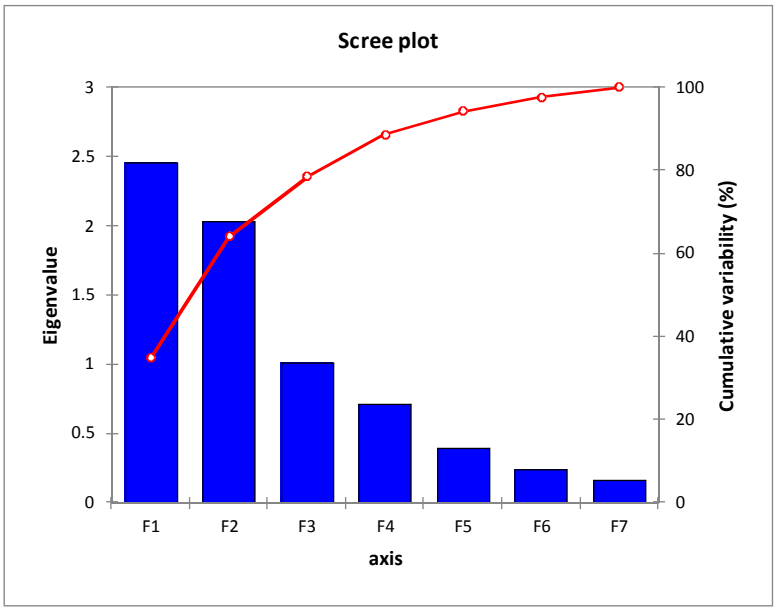


Figure 29. Loading plot of the variables based on the first two PCs after reduction of the number of phenotypic traits

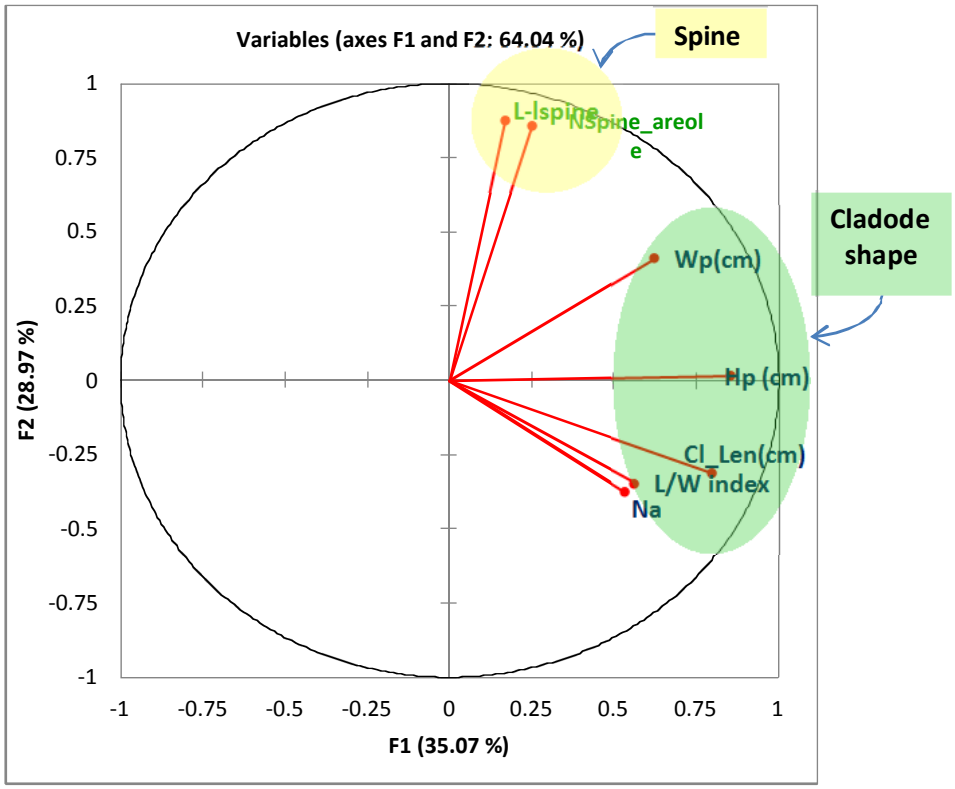


Figure 30. Loading plot of the variables based on the first two PCs after reduction of the number of phenotypic traits

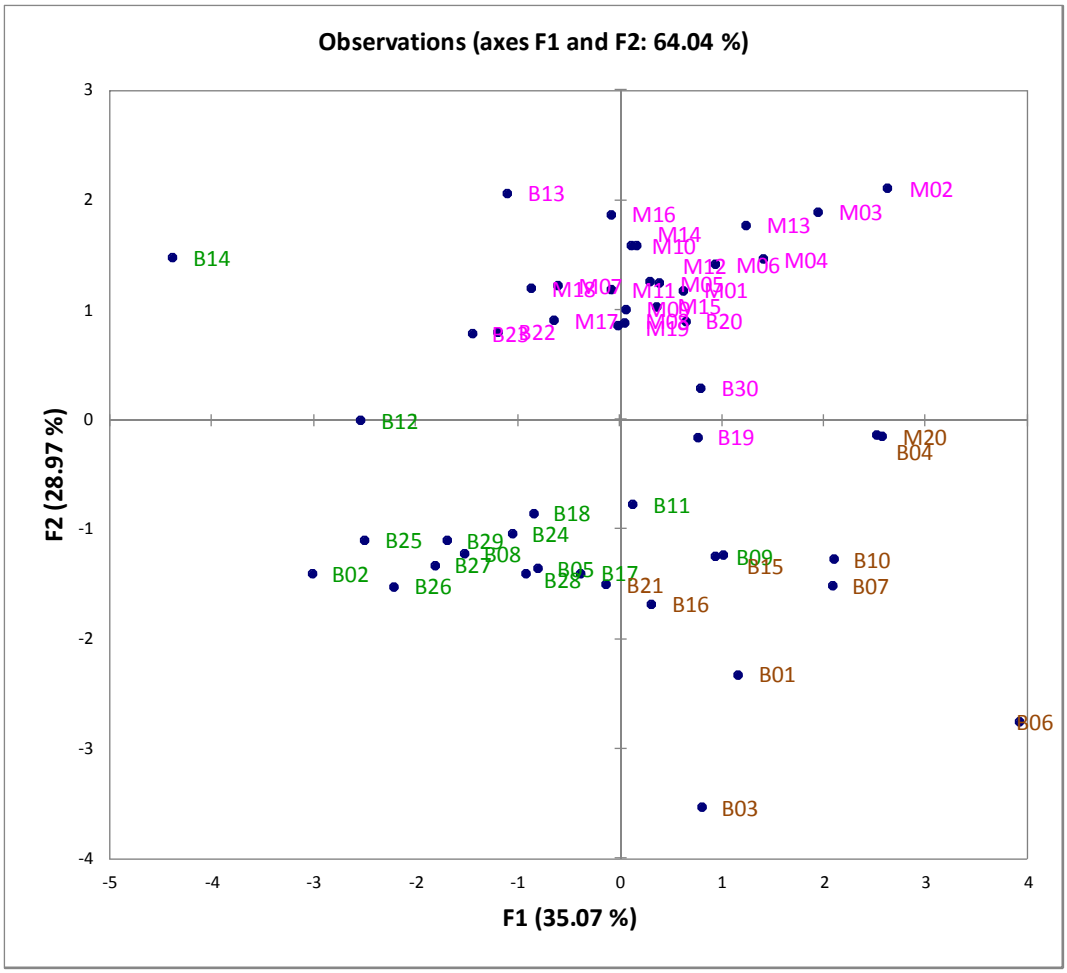


Figure 31. Discrimination of the accessions based on the two first PCs (symbols and colour of the accessions according to the clusters given by the dendrogram) after reduction of the number of phenotypic traits

5.3.1.2. Agglomerative hierarchical clustering (AHC)

The contribution of the variables (%) to the principal components shown in table 30 reveals that the component 1 is influenced mainly by the plant height, plant width, cladode length. The component 2 is considerably influenced by the number of spines per areole and the length of the longest spine. On the other hand, the variance decomposition for the optimal classification shows a very high percentage of variance within class (88.58 %) and a low percentage between classes (Table 31).

Table 30. Contribution of the variables (%) (50 accessions from Mediterranean/Morocco and IPA collections)

	F1	F2	F3	F4
Hp (cm)	29.697	0.015	12.761	0.517
Wp(cm)	15.885	8.387	32.189	0.130
Cl_Len(cm)	26.048	4.739	1.516	1.417
L/W index	12.939	5.865	27.598	**27.029**
Na	11.582	6.915	5.774	**69.966**
NSpine_areole	2.624	36.247	10.957	0.187
L-lspine	1.226	37.832	9.204	0.755

Table 31. Variance decomposition for the optimal classification (50 accessions from Mediterranean/Morocco and IPA collections)

	Absolute	Percent
Within-class	2777.251	**88.58%**
Between-classes	357.990	11.42%
Total	3135.241	100.00%

The AHC (Table 32, Figure 32) results in three distinct classes:

- Class 1 with 6 accessions
- Class 2 with 17 accessions
- Class 3 with 27 accessions

There are subgroups within each class.

The class 1 has two subgroups: a subgroup 1 composed of B10, B01, and B06 and a subgroup 2 composed of B04, B03 and M20.

The class 2 has also two major subgroups. A subgroup 1 composed of B26, B25, B29, B24, B27, B05, B28, B08, B17 and B02. A subgroup 2 includes B15, B07, B21, B16, B18, B11, and B09.

The class 3 has also two subgroups. A subgroup 1 composed of B23, B22, B13, B14, and B12 and a subgroup 2 with M16, B30, M08, M05, M15, M06, M19, M10, M11, M13, M01, M18, M17, M07, M14, M12, M04, M03, M02, M09, M20 and B19.

Table 32. AHC results by class (50 accessions from Mediterranean/Morocco and IPA collections)

Class	1	2	3
Objects	6	17	27
Sum of weights	6	17	27
Within-class variance	4194.713	2527.235	2658.518
Minimum distance to centroid	25.816	7.670	13.505
Average distance to centroid	52.704	42.518	42.282
Maximum distance to centroid	98.943	83.317	135.700
	B01, B03, B04, B06, B10, M20	B02, B05, B07, B08, B09, B11, B15, B16, B17, B18, B21, B24, B25, B26, B27, B28, B29	B12, B13, B14, B19, B20, B22, B23, B30, M01, M02, M03, M04, M05, M06, M07, M08, M09, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19

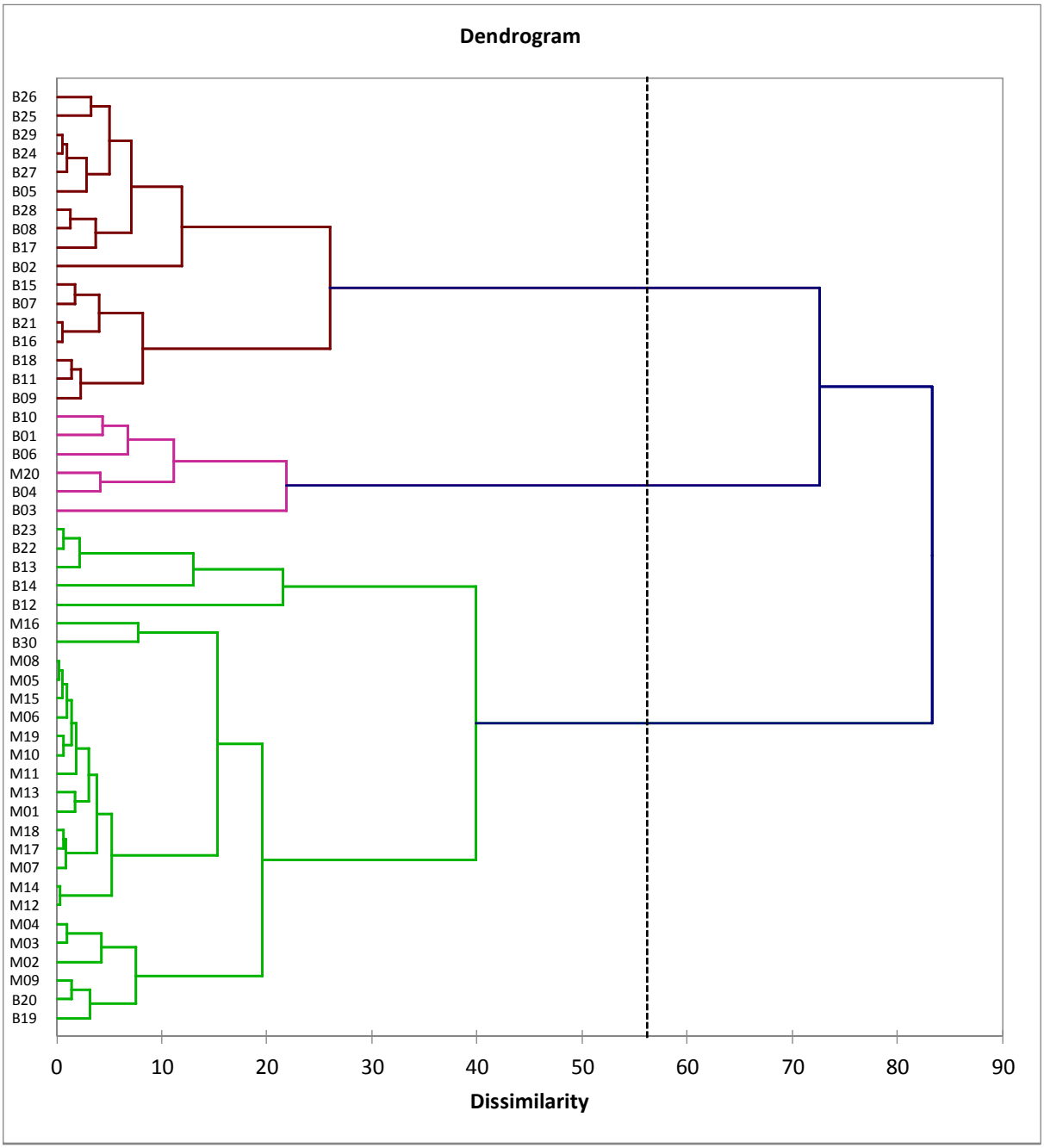


Figure 32. Ward's cluster analysis classification of the 50 accessions from the Mediterranean/Morocco collection and IPA collection

Similarly to the PCA the cluster analysis has been repeated with the reduced number of parameters and the results are as follows:

When the number of traits has been reduced the variance decomposition improves slightly with 71.43 % variance within class and 28.57 % between classes (Table 33).

The results of AHC analysis after adjustment of morphological parameters (Table 34) show globally quite similar distribution of the accessions by class (Figure 33). Again we have three classes, but with slight changes. Thus, the accessions B07, B15, B16 and B21 have been

moved from class 2 to class 1. The class 2 has been changed with the migration of B12, B13, B14, B22 and B23 from class 3 to class 2. This new distribution seems to be more close to reality; indeed the class 3 includes 19 among 20 Moroccan fruit-oriented accessions and B19 (IPA 98-T52F8), B20 (IPA 98-T19F11) and B30 (IPA 98-T42F11) from IPA collection.

Table 33. Variance decomposition for the optimal classification after adjustment of the variable number (50 accessions from Mediterranean/Morocco and IPA collections)

	Absolute	Percent
Within-class	2214.984	**71.43%**
Between-classes	885.917	**28.57%**
Total	3100.901	100.00%

Table 34. AHC results by class after adjustment of morphological parameters (50 accessions from Mediterranean/Morocco and IPA collections)

Class	1	2	3
Objects	10	18	22
Sum of weights	10	18	22
Within-class variance	3218.282	2966.778	1176.405
Minimum distance to centroid	26.560	5.204	4.764
Average distance to centroid	49.416	46.092	29.086
Maximum distance to centroid	101.754	98.849	74.812
	B01, B03, B04, B06, B07, B10, B15, B16, B21, M20	B02, B05, B08, B09, B11, B12, B13, B14, B17, B18, B22, B23, B24, B25, B26, B27, B28, B29	B19, B20, B30, M01, M02, M03, M04, M05, M06, M07, M08, M09, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19

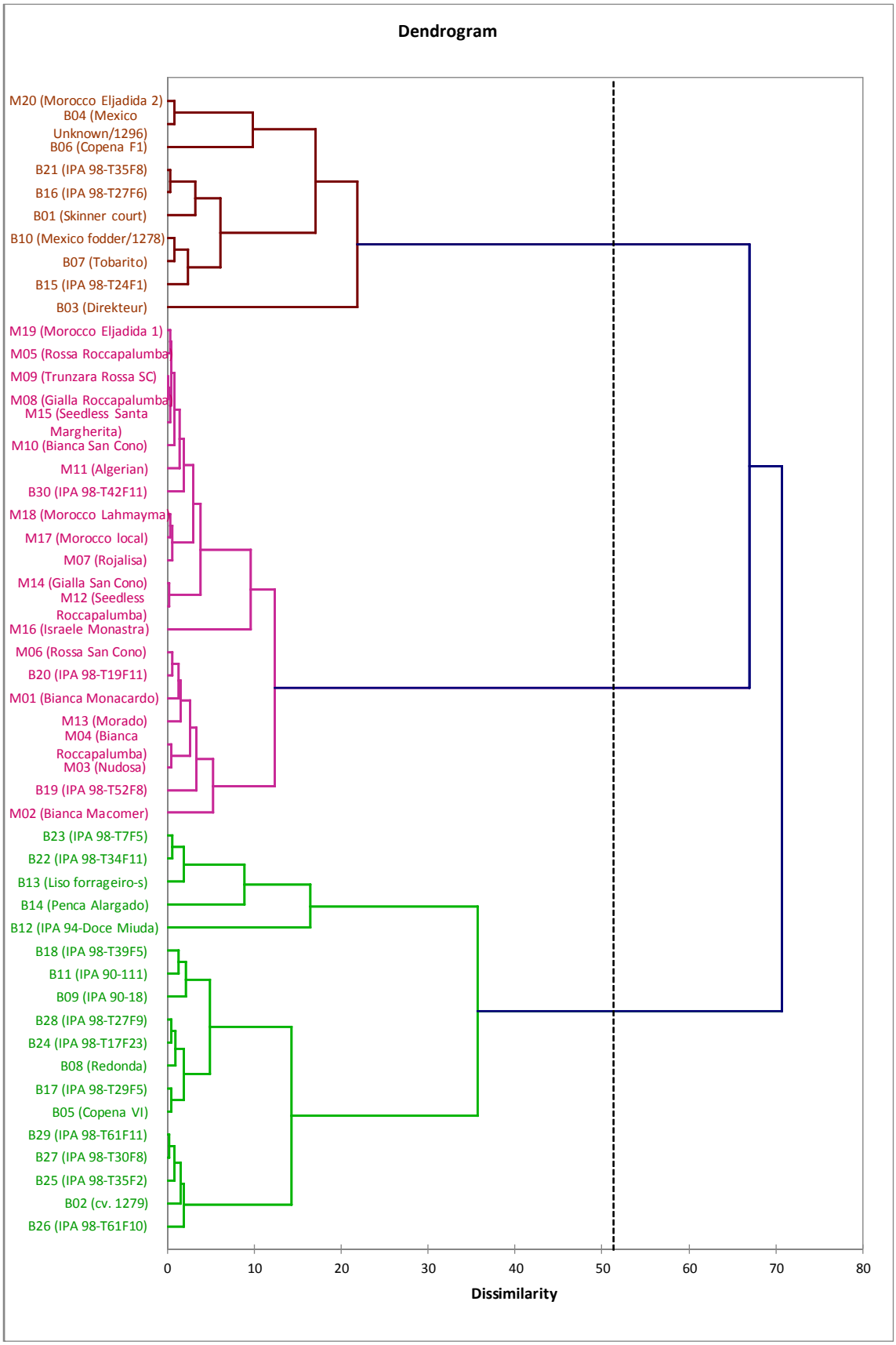


Figure 33. Ward's cluster analysis classification of the 50 accessions from the Mediterranean/Morocco collection and IPA collection after reduction of the phenotypic traits

5.3.2. Molecular characterization

5.3.2.1. DNA Quality Test

Figure 34 shows gel photograph of electrophoresis of isolated cactus DNA samples. The isolated DNA were more than 21 kb size with a fairly distinct bands, indicating their suitability for microsatellite analysis. The intensity of cactus DNA bands were compared with the standard molecular weight marker bands with known DNA concentrations to estimate the DNA concentrations.

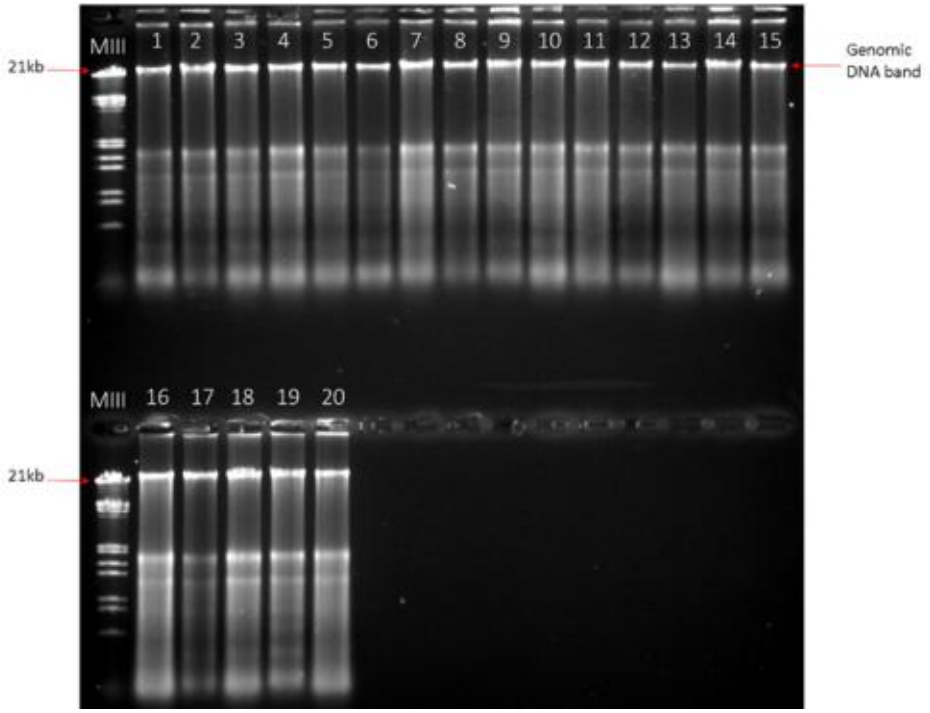


Figure 34. Agarose gel electrophoresis of DNA extracted from 20 Moroccan samples of Opuntia. Total DNA extracted from cuticles was analyzed by electrophoresis through a 1% agarose gel.

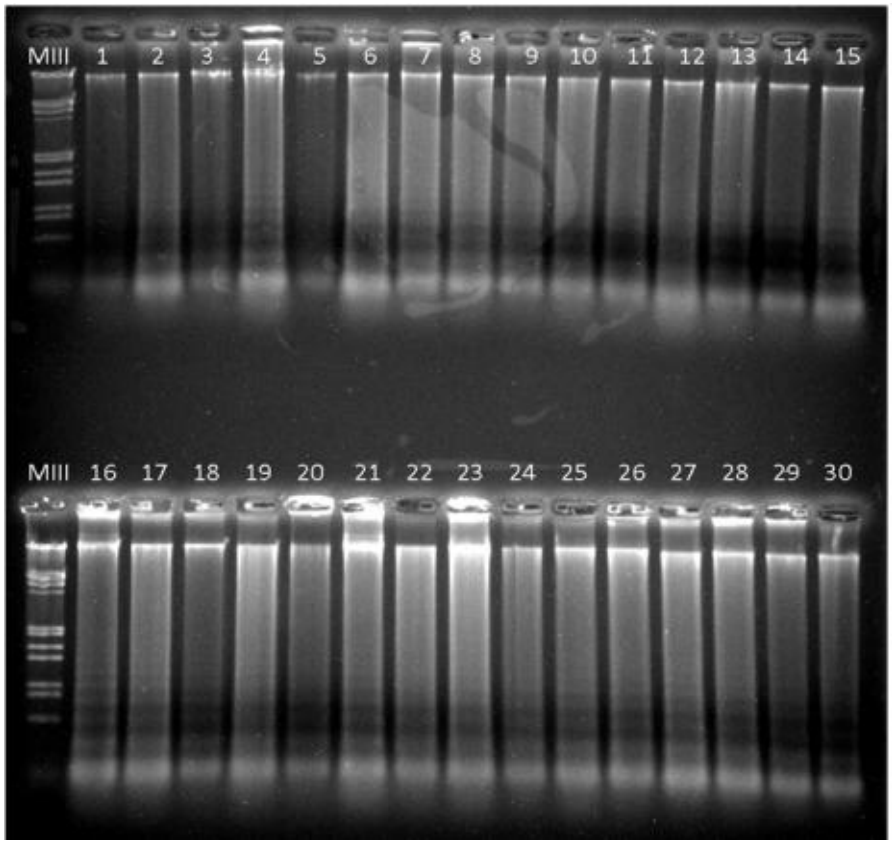


Figure 35. Agarose gel electrophoresis of DNA extracted from 30 Brazilian samples of Opuntia Total DNA extracted from cuticles was analyzed by electrophoresis through a 1% agarose gel.

5.3.2.2. Microsatellite marker analysis

The amplification profiles by the primers Opuntia3, Opuntia5, Opuntia9, Opuntia11, Opuntia12, Opuntia13, Ops9 and Ops24 M are presented in figures 36 to 44 (annex 4).

Based on he sizes of the amplied 8 microsatellite loci in 50 accessions of catctus, we identified a total of 72 alleles. Number of alleles detected at 8 loci varied grately, and ranged from 3 (*Opuntia* 5 Locus) to 11 (*Opuntia* 12 and Ops 24 locus) with an average of 9 alleles per locus (Table 35).

Genetic diversity estimated 8 microsatellite loci also varied. The lowest genetic diversity was estimated in Opuntia 5 locus (H=0.358) (Table 35). All microsatellites used were discriminative with a mean value of PIC (Polymorphism Information Content) estimated at 0.458. The PIC values vary between 0.316 (*Opuntia* 5) and 0.543 (Ops 24) with an average of 0.458. According to Botstein et al. (1980), if the PIC value is greater than 0.5 this corresponds to a very informative marker. PIC values ranging from 0.5 to 0.25 correspond to an informative marker while a PIC value less than or equal to 0.25 reflects the lack of informativeness of the corresponding marker.

Table 35. Summary stat pattern or allele based

Marker	Major Allele Frequency	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	PIC
Opuntia 3	0.7143	50.0000	49.0000	9.0000	0.9800	0.4740	0.4577
Opuntia 5	0.7800	50.0000	50.0000	3.0000	1.0000	0.3576	0.3161
Opuntia 9	0.6800	50.0000	50.0000	10.0000	1.0000	0.5208	0.5051
Opuntia 11	0.7000	50.0000	50.0000	9.0000	1.0000	0.4952	0.4805
Opuntia 12	0.7200	50.0000	50.0000	11.0000	1.0000	0.4720	0.4620
Opuntia 13	0.7143	50.0000	49.0000	10.0000	0.9800	0.4773	0.4644
Ops 9	0.7347	50.0000	49.0000	9.0000	0.9800	0.4481	0.4350
Ops 24	0.6531	50.0000	49.0000	11.0000	0.9800	0.5573	0.5432
Mean	0.7120	50.0000	49.5000	9.0000	0.9900	0.4753	0.4580

Therefore, according to our data (Table 35), all the markers used in this research were either informative or highly informative markers. The two most informative markers were *Opuntia* 9 and Ops 24. Despite of that, all these markers can be employed to detect genetic diversity in *Opuntia* species.

Our results confirm the findings of Caruso et al (2010) who using the same primers found an average number of 16.9 alleles per locus and that the most informative SSR loci are Ops24 (0.25), Ops9 (0.22), and OP13 (0.21). Since, in the present study, we used a different set of germplasm than that of Caruso et al. (2010), one could expect some difference in average number of alleles. The lower number of alleles indicate that the germplasm set used in this study could be less diverse than that of Caruso et al. (2010). Indeed, the plant material investigated by Caruso et al. (2010) included 62 wild and cultivated genotypes belonging to 16 Opuntia species collected from Sicily, Mexico, Kenya, South Africa, USA and Israel. In addition, our collection included many crosses closely related.

5.3.2.3. Genetic distances and phylogenetic tree

Population genetic parameters and cluster analysis indicated a high genetic similarity between analyzed cacti accessions. Among the 50 analysed accessions, only 17 were distinct MLGs (Multilocus Genotypes) obtained for the 8 loci studied. Overall, the cactus accessions were split into two major clusters. The first cluster included 38 accessions with most redundancies shown (8 MLGs). Four out of the 5 subclusters constituting this group showed synonymous accessions; the fourth subcluster with the largest number of individuals and especially the IPA clones and the Morocco local types, contained 29 accessions which were all genetically identical. This results is striking and may be due to the fact that these

accessions have the same ancestry which might be in this case Algerian and/or Redonda. Moreover, with these 8 primers we cannot discriminate between fruit accessions having different colors (yellow and purple fruits) which is the case for the accessions Rossa Roccapalumba, Rossa San Cono and Gialla San cono. For fodder accessions, the cultivars cv. 1296 and 1278 from Mexico are similar.

The second cluster showed smaller similarity between its 9 accessions, indicating only one synonymous case (Liso Forrajeiro and Penca Alargado).

Using the same primer, Caruso et al. (2010) could not distinguish between Sicilian cultivars 'Bianca trunzara' and 'Gialla Trunzara', which have different fruit colors. Our results confirm this statement; indeed the primers we use do not distinguish between Rossa San Cono, Rojalisa, Trunzara Rossa San Cono, Morado, Gialla San Cono and Rossa Roccapalumba. These cultivars have red or yellow color and are available in Sicily (Italy) except Rojalisa and Morado which are Mexican. Our results confirm the research implemented by Caruso et al (2010). Indeed, according to Caruso et al. (2010), the analysis of eight highly polymorphic SSR loci allowed to investigate the level of genetic diversity among cactus pear species, cultivars, and accessions from different regions of the world. SSRs, although scored as dominant markers, were more informative than random markers; they were able to produce useful information regarding the level of diversity among the most diffused cultivars, and may have revealed the level of hybridization between Opuntia ficus indica and its related species. Therefore, microsatellites could be used to analyze a greater number of individuals originating from controlled crosses with different parentals. Moreover, Mondragon-Jacobo and Bordelon (2002) assume that SSRs may serve as a quick and reliable tool to discriminate Opuntia apomictic seedlings from zygotic ones.

Under Brazilian conditions, Mergulhão et al. (2012), using ISSR and RAPD techniques and 5 varieties of *Opuntia ficus-indica* revealed high genetic similarity between the varieties assessed; however, some differences were made evident. The Chili fruit variety was isolated in one subgroup; variety Copena F1 and Clone IPA 20 were grouped together, as well as varieties Copena VI and Redonda. The primers we used are different from those used by Mergulhão et al. (2012) and therefore our results distinguish clearly between Copena VI and Redonda.

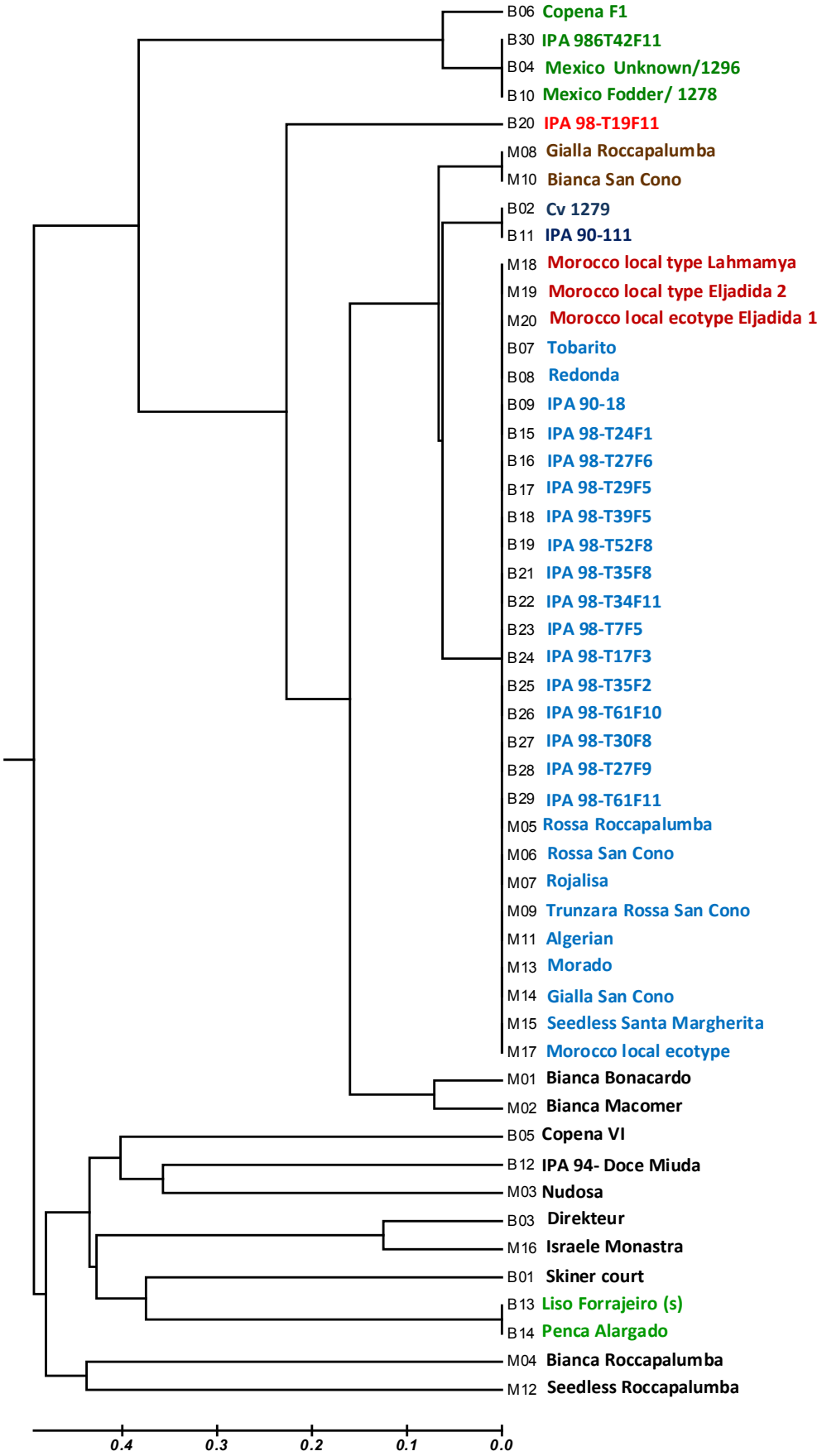


Figure 45. UPGMA tree based on shared allele distance

De Lyra et al. (2015) used the ribosomal ITS rRNA analyzed 28 *Opuntia* and *Nopalea* accessions from IPA collection and succeeded to distinguish 5 clusters. They conclude that these markers have great ability to characterize species of forage cactus, and that the current taxonomy of *Opuntia* is unsuitable which shared by many other authors (i.e. Caruso et al., 2010; Mondragon-Jacobo and Chessa, 2013).

5.3.3. Relationship Molecular and morphological characters

Mantel test using dissimilarity matrices of the morphological parameters and the allele based genetic distances from the SSR analysis has been conducted under the following two conditions:

- Option 1: Using proximity matrix (Euclidian distances) of all morphological parameters and allele based genetic distances matrix, and
- Option 2: Using proximity matrix (Euclidian distances) of adjusted morphological parameters and allele based genetic distances matrix, and

Under the option 1, the relationship between morphological and molecular characteristics expressed by the correlation r is highly significant (p=0.01) even if it is low (rAB=0.212) (Figure 46, Table 36).

Under the option 2, the relationship, expressed by the correlation (rAB) between morphological and molecular characteristics is highly significant (p=0.01) and higher than under option 1 (rAB=0.409) which may be a proof that the Euclidean distance for morphological parameters is more accurate when the number of characters is adjusted (Figure 47, Table 37).

This is probably the first time that the relationship between phenotypic traits and molecular data is demonstrated using SSR technique. The only research reported in literature is may be the one of Ben Dhifi et al. (2013) who used the Mantel test to compare RAPD matrix with phenotypic traits and found a positive correlation (r=0.159, P=0.003) between the two types of markers.

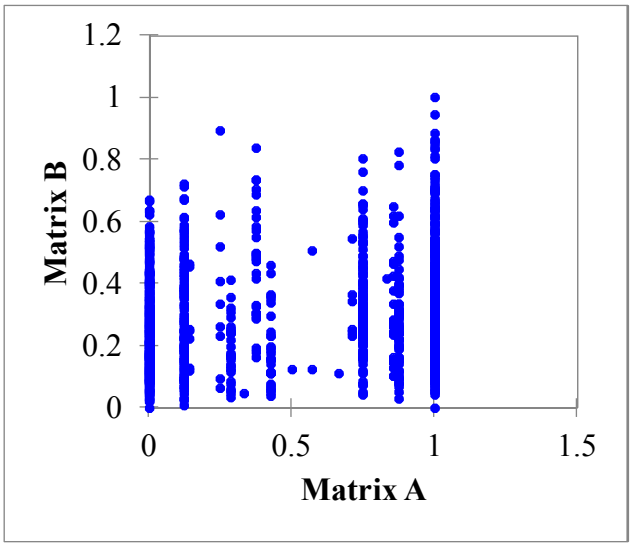


Figure 46. Correlation between proximity matrix (Euclidian distances) of all morphological parameters and allele based genetic distances matrix

Table 36. Correlation between proximity matrix (Euclidian distances) of all morphological parameters and allele based genetic distances matrix

r(AB)	**0.211**
p-value (Two-tailed)	< 0.0001
alpha	0.01

H0: The matrices are not correlated, Ha: The matrices are correlated
As the computed p-value is lower than the significance level alpha=0.01, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The p-value has been calculated using the distribution of r(AB) estimated from 10000 permutations

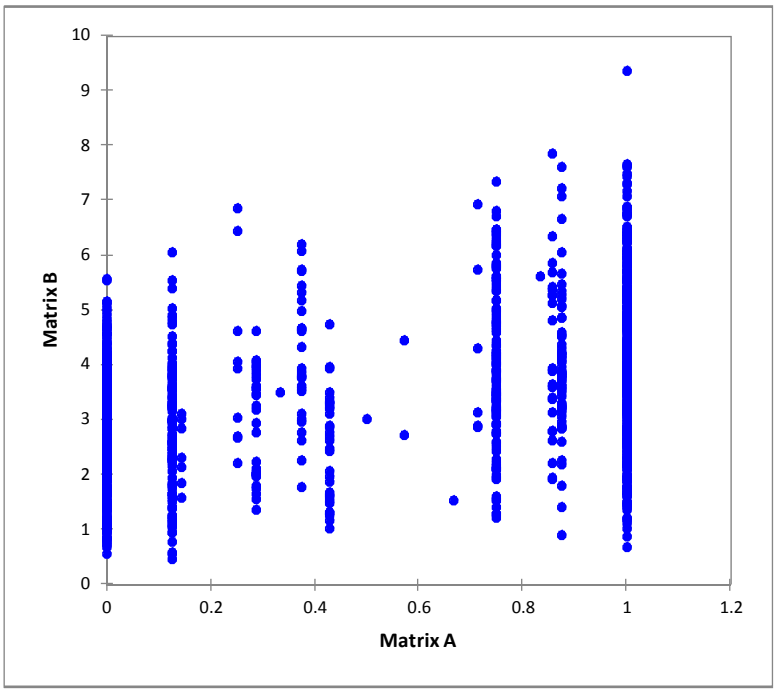


Figure 47. Correlation between proximity matrix (Euclidian distances) of adjusted morphological parameters and allele based genetic distances matrix

Table 37. Correlation between proximity matrix (Euclidian distances) of adjusted morphological parameters and allele based genetic distances matrix

r(AB)	**0.409**
p-value (Two-tailed)	< 0.0001
alpha	0.01

6. Conclusions

Morphological descriptors recommended by FAO and approved by Bioversity International are able to effectively discriminate *Opuntia spp*. accessions and to segregate forage-oriented varieties from fruit-oriented ones. The number of morphological descriptors may be restricted to 7 traits which with no reduction in segregating population accuracy which is even improved. All used SSR microsatellites are either informative or highly informative. These markers allow efficient segregation of cactus genetic resources and lead to grouping of accessions similar to phenotypic discrimination. The positive relationship between phenotypic and molecular characterization is significant suggesting that SSR markers may complement or substitute morphological characterization for cactus forages. A priority for future research is to develop additional SSR primers specific to the cactus crop.

REFERENCES

ARBA, M. 2006. 'Dellahia' a cactus pear cultivar from the Mediterranean Coast of Northern Morocco. . In: Eds. C. Mondragon-Jacobo. Proc. Vth International Congress on Cactus pear and cochineal. **Acta Hort.** 728: 37-41

ARNHOLDT-SCHMITT, B., GIRAO, L.C., LLAMOCA-ZARATE, R.M., CAMPOS, F.A.P. 2001. Genome characterization of *Opuntia ficus-indica*: a simple and efficient micromethod. **JPACD** 57-65

ARRUDA, G.P. AND WARUMBY, J.F. 1999. Inytroduccion y utilizacion de las cactaceae Nopalea cochellinifera (L) y *Opuntia ficus indica* en el Brasil. In: Aguirre R.R. and J.A. Reyes, A (eds.) Memoria del VIII Congreso nacional y VI Internacional sobre el conocimiento y approvechamiento del Nopal. San Luis Potosi. Mexico

BACHMANN, K., BLATTER, F., FISHER, D., FRIESEN, N., FRITSCH, R., KLAAS, M., MES, T. AND POLLER, S. 2001. Molecular markers in Allium: range of application, reliability and taxonomic applications. Proceedings of the International Symposium on Molecular Markers. **Acta Hort.** 546: 159-163

BARBERA, G. 1995. History, economic and agro-ecological importance. In Barbera G., Inglese P., Pimienta-Barrios E. (eds., Agroecology, cultivation and uses of cactus pear. **FAO Plant Production and Protection Paper**, Rome, Italy. pp I-II

BÁRCENAS, ROLANDO T.; YESSON, CHRIS & HAWKINS, JULIE A.; 2011. "Molecular systematics of the Cactaceae", ***Cladistics*** 27 (5): 470–489, doi:10.1111/j.1096-0031.2011.00350)

BARTHLOTT, W. and D.R. HUNT., 1993. Cactaceae. Pp. 161–196 in The families and genera of vascular plants, eds. K. Kubitzki, J. G. Rohwer, and V. Bittrich. Berlin: Springer-Verlag.

BENDHIFI, M., BARAKET, G., ZOURGUI, L., SOUID, S., SALHI-HANNACHI, A. 2013. Assessment of genetic diversity of Tunisian Barbary fig (*Opuntia ficus-indica*) cultivars by RAPD markers and morphological traites. **Scientia Horticulturae** 158: 1-7

BOTSTEIN, D, WHITE, KL, SKOLNICK, M and DAVIS, RW, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. **Am. J. Hum. Genet.** 32 : 314-331.

CAETANO-ANOLLES, G., BASSAM, GRESSHOFF, P.M., 1991. DNA amplification fingerprinting using short arbitrary oligonucleotide primers. **Bio/Technology** (New York) 9, 553-557.

CARUSO, M., CURRO, S., LAS CASAS, G., LA MALFA, S. and GENTILE, A. 2010. Microsatellite markers help to assess genetic diversity among *Opuntia ficus indica* cultivated genotypes and their relation with related species. **Plant Syst. Evol.** 290: 85-97. DOI 10.1007/s00606-010-0351-9

CASAS, A., and G. BARBERA, 2002. Mesoamerican domestication and diffusion. In P. S. Nobel [ed.], Cacti: biology and uses, 143–162. **University of California, Berkeley, California**, USA.

CERVANTES HERRERA, J., REYES AGÜERO, J.A., GALLEGOS VASQUEZ, C., FERNANDEZ MONTES, R. MONDRAGON-JACOBO, C., MARTINEZ, J.C., and LUNA VASSQUEZ, J. 2006. Mexican cultivars of Opuntia ficus-indica (L.) Mill.

With economic importance. In: Eds. C. Mondragon-Jacobo. Proc. Vth International Congress on Cactus pear and cochineal. **Acta Hort**. 728: 29-35.

CHAPMAN, B., C. MONDRAGON-JACOBO , R.A. BUNCH AND H. PATERSON, 2002. Breeding and Biotechnology. In: Nobel, P.S. (Ed.) **Cacti: Biology and Uses**, pp 255-271. University of California Press, California, USA.

CHATTI, K., SALHI-HANNACGI, A., MARS, M., MARRAKCHI, M., TRIFI, M. 2003. Genetic diversity and phylogenic relationships in Tunisian fig (*Ficus carica* L.) cultivars mediated by RAPD. **Biologia** 2: 1-4

CHESSA, I., NIEDDU, G. and MULAS, M. 1995. Variability of *Opuntia ficus-indica* in a Mediterranean area: definition of a descriptor list and germplasm characterization. In: Proceedings of the Second International Congress on Cactus Pear and Cochineal.

CHESSA, I., NIEDDU G., 1997. Descriptors for Cactus pear (*Opuntia spp*.). **FAO CACTUSNET Newsletter**, Special issue, pp. 39

CHESSA, I., NIEDDU, G., SERRA, P., INGLESE, P., and LA MANTIA, T.. 1997. Isozyme characterization of *Opuntia* species and varieties from Italian germplasm. **Acta Hort**. 438 : 45-56

CHESSA, I., ERRE P., BARBATO M., OCHOA J., NIEDDU G., 2013. Polymorphic microsatellite DNA markers in Opuntia spp. collections. **Acta Hort.** num. 995: 35-42

CRP DRYLAND SYSTEMS, 2015. http://drylandsystems.cgiar.org/content/worlds-dry-areas

DE AMORIM, P.L, MARTUSCELLO, J.A., ARAUJO FILHO DE, J.T., CUNHA DA, D.N.F.V., JANK, L. 2015. Morphological and productive characterization of froage cactus varieties. **Revista Caatinga**, Mossoro, v. 28, n. 3, p.230-238, jul.-set., 2015.

DE LA CRUZ, M., RAMIREZ, F. and HERNANDEZ, H. 1997. DNA isolation and amplification from Cacti. **Plant Mol Biol Rep** 15:319-325.

DE LYRA, M.C.C.P., SILVA, DA SILVA, M.L.R.B., MEGULHÃO, A.C.E.S., MONDRAGON-JACOBO, C., MERTINEZ-ROMERO, E. 2015. Molecular studies of forage prickly-pear cactus from the semiarid of Pernambuco State – Brazil. **Journal of Applied Biology and Biotechnology** Vol. 3 (02), pp. 001-005. http://www.jabonline.in

DONKIN, R. A.,1977. Spanish red: An ethnographical study of cochineal and the *Opuntia* cactus. **Transactions of the American Philosophical Society**. 67:1–84.

DOS SANTOS, D. C. ; ALBUQUERQUE, S. G. de, 2001. *Opuntia* as fodder in the semi-arid northeast of Brazil. In: Cactus (Opuntia spp.) as Forage. Mondragon-Jacobo and Perez-Gonzalez Ed., **FAO Plant production and protection papers N°169**: 161p. Opuntia as forage. 37-50,

DOYLE, J. J. and EGAN, A. N. 2010. Dating the origins of polyploidy events. **New Phytologist**, 186: 73–85. doi: 10.1111/j.1469-8137.2009.03118.x

DUBEUX, Jr. J.C.B., DOS SANTOS, M.V.F., DE MELLO, A.C.L., VIEIRA DE CUNHA, M., de A. FERREIRA, M., DOS SANTOS, D.C., DE A. LIRA, M. and DA C. SILVA, M. 2015. Forage potential of Cacti on drylands. In: P Inglese, G. Liguori A Nefzaoui (eds.) proceedings of the Eighth International Congress on Cactus Pear and Cochineal. **Acta Hort**. 1067: 181-186

ERRE, P., CHESSA, I., NIEDDU, G. and JONES, P.G. 2009. Diversity and spatial distribution of *Opuntia* spp. in the Mediterranean Basin. **J. Arid Environ**. 73: 1058-422

ERRE, P., NIEDDU, G. and CHESSA, I. 2010. Identification of microsatellite loci in *Opuntia* spp. And their characterization in cultivars and species. **Acta Hort**. 918: 327-332

ERRE , P. and CHESSA, I. 2013. Discriminant analysis of morphological descriptors to differentiate the opuntia genotypes. **Acta Hort**. (ISHS) 995:43-50

FELKER, P., RODRIGUEZ, S.C., CASOLIBA, R.M., FILIPPINI, R., MEDINA, D. and ZAPATA, R. 2005. Comparison of *Opuntia ficus indica* varieties of Mexian and Argentina origin for fruit yield and quality in Argentina. **J. Arid Environ**. 60: 405-422

FLORES-FLORES, V. and TEKELENBURG, A. 1999. Dacti (Dactylopius coccus Costa) dye production. In : G Barbera, P. Inglese and E Pimienta-Barrios (eds) Agroecology, cultivation and uses of cactus pear. **FAO Plant Production and Protection Paper** 132: 167-184

FORD-LLOYD, B., 1996. Measuring genetic variation using molecular markers. International Plant Genetic Resources Institute (IPGRI). Version 1.2. 66 pp. https://www.unirc.it/documentazione/materiale_didattico/140_2012_316_15694.pdf

GARCIA-ZAMBRANO, E.A., SALINAS, G., VAZQUEZ, R., CARDENAS, E. and GUTIERREZ, A. 2006. Clasificacion y estimacion de la diversidad genetica de Nopal Opuntia spp. En base a descriptors fenotipicos y marcados genetico moleculares. **International Journal of Experimental Botany**, 75: 125-135

GARCÍA-ZAMBRANO, E.A., ZAVALA-GARCIA, F., GUTIERREZ-DIEZ, A., OJEDA-ZACARIAS, M.C. and CERDA-HURTADO, I. 2009. Estimation of genetic diversity of *Opuntia* spp. using molecular markers AFLP. **International Journal of Experimental Botany**, 78:117-120

GIBSON, A.C and P.S. NOBEL, 1986. The Cactus Primer. **Harvard University Press**, Cambridge, Massachusetts.

GRIFFITH, M. P., 2004. The origins of an important cactus crop, *Opuntia ficus-indica* (Cactaceae): new molecular evidence. **American Journal of Botany** 91: 1915-1921.

GRIFFITH, M. PATRICK and PORTER, J. MARK; 2009. Phylogeny of Opuntioideae (Cactaceae). **International Journal of Plant Science** 170 (1): 107-116. Doi: 10.1086/593048

GUPTA, P. K., VARSHNEY, R. K., SHARMA, P. C. and RAMESH, B. 1999. Molecular markers and their applications in wheat breeding. **Plant Breeding**. Volume 118, Issue 5, pages 369–390, doi: 10.1046/j.1439-0523.1999.00401.x

HELSEN, P., VERDYCK, P., TYE, A. and VAN DONGEN, S. 2009. Low levels of genetic differentiation between Opuntia echios varieties on Santa Cruz (Galapagos). **Plant Syst. Evol.** 279: 1-10

HOOGENDIJK M. and WILLIAMS D. E., 2001. "Characterizing the Genetic Diversity of Home Garden Crops: Some Examples from the Americas," In: J. W. Watson and P. D. Eyzaguirre, Eds., Home Gardens and in situ Conservations of Plant Genetic Resources in Farming Systems, **Proceeding of the Second International Home Gardens Workshop**, Witzenhausen, 17-19 July 2001, pp. 34-40.

INGLESE P., BASILE F., M. SCHIRRA., 2002. Cactus pear fruit production. In "P.S. Nobel (eds) Cacti. **California University Press**, 163-183.

JIN L AND CHAKRABORTY R, 1993. Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data**. Mol Biol Evol** 11: 120–127.

KIESLING, R. 1998. Origen, domesticacion y distribucion de Opuntia ficus-indica. **JPACD** 3: 50-59

KLUG, W.S. and CUMMINGS, M.R. 2002. Concepts of genetics, 6th Edition pp. 713-733. **Prentice Hall International**, New Jersey, USA

LABRA, M. GRASSI, F., BARDINI, M., IMAZIO, S. GUIGGI, A ;, CITTERIO, S. BANFI, E. and SGORBATI, S. 2003. Relationships in *Opuntia* Mill. Genus (Cactaceae) detected by molecular marker. **Plant Sci**. 165: 1129-1136

LE HOUÉROU, H.N. 2002. The role of Opuntia Cacti in the Agricultural Development of Mediterranean Arid Zones. In A Nefzaoui and P inglese (eds) Proceedings of the IVth International Congress on Cactus Pear and Cochineal. **Acta Hort**. 581: 21-46

LUNA-PAEZ, A., VALADEZ-MOCTEZUMA, E., BARRIENTOS-PRIEGO, A.F. and GALLEGOS-VAZQUEZ, C. 2007. Characterization of *Opuntia* spp. By means of seed with RAPD and ISSR markers and its possible use for differentiation. **JPACD** 9: 43-59

LYNCH, M., MILLIGAN, B.G., 1994. Analysis of population genetic structure with RAPD markers. **Mol. Ecol**. 3, 91-99.

MANTEL, N., 1967. The detection of disease clustering and a generalized regression approach. **Cancer Res.** 27, 209–220.

MASHOPE B, 2007. Characterization of cactus pear germplasm in South Africa. Thesis Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa.

MERGULHÃO, A.C.E.S., RITO, K.F., COREDEIRO, D.D.S, DA SILVA, M.L.R.B., DE LYRA, M.D.C.C.P, DA SILVA, M.V., 2012. Marcadores moleculares para deteção de variabilidade genética em variedades de palma forrageire. Pesq. Agropec. Pernamb., Recife, v. 17, n. unico, p. 78-82.

MOHAMMADI, S.A. and PRASANNA B.M. 2003. Review and interpretation of Analysis of Genetic Diversity in Crop Plants—Salient Statistical Tools and Considerations. **Crop Sci.** 43:1235–1248 (2003)

MONDRAGON-JACOBO, C. and BORDELON, B.B. 1996. Cactus pear (*Opuntia* spp. Cactaceae) breeding for fruit production. **JPACD** 1: 19-35

MONDRAGON-JACOBO, C. 1999. Preliminary genetic studies on cactus pear (*Opuntia* ssp. Cactaceaae) germplasm from Central Mexico. PhD thesis. **Purdue University**, West Lafayette, Indiana.

MONDRAGON-JACOBO, C. 2001. Cactus pear breeding and domestication. **Plant Breed Rev**. 20: 135-166

MONDRAGON-JACOBO, C.; PEREZ-GONZALEZ, S., 2001. Cactus (*Opuntia spp*.) as forage. **FAO Plant production and protection papers** Vol 169. FAO

MONDRAGON-JACOBO, C. and BORDELON, B.B. 2002. Apomixis in crosses of Mexican pear cactus, preliminary molecular identification. **Rev. Fitotec. Mex**. 25: 247-252

MONDRAGÓN-JACOBO, C. 2003. Caracterizacion molecular mediante RAPDs de una coleccion de nopal de (*Opuntia* spp. Cataceae) del centro de Mexico, como base del mejoramiento genetico. **Revista Chapingo** 9: 97-114

MONDRAGON-JACOBO C., CHESSA I., 2013. A global perspective on genetic resources of cactus pear; and asset for the future sustainability of semi-arid lands. **Acta Horticulturae** num. 995: 19-26.

NAZARENO, M. 2013. New insights about medicinal uses and health-beneficial properties of cactus products. In Nefzaoui A Inglese P Liguori G (eds) Proceedings VII International Congress on Cactus Pear and Cochineal. **Acta Horticulturae** 995: 237-250

NEDER, D.G., DA COSTA, F.R., EDVAN, R.L. and SOUTO FILHO, L.T., 2013. Correlations and path analysis of morphological and yield traits of cactus accessions. Crop Breeding and Applied Biotechnology, 13: 203-207.

NEFZAOUI, A. and BEN SALEM, H. 2002. Forage, fodder and animal nutrition. In Nobel P.S. (eds.) **Cacti Biology and Uses**. California University Press, USA, pp 199-210

NEFZAOUI A.; 2009. Cactus: A crop to meet the challenges of climate change in dry areas. **Annals of Arid Zones**, 48: 1-18.

NEFZAOUI, A., KETATA, H. and EL MOURID, M. 2012. Agricultural Technological and Institutional Innovations for Enhanced Adaptation to Environmental Change in North Africa. In: Eds. Stephen S. Young and Dr. Steven E. Silvern **"International Perspectives on Global Environmental Change"**. ISBN 978-953-307-815-1

NEFZAOUI, A., LOUHAICHI, M., and BEN SALEM, H.; 2014. Cactus as a Tool to Mitigate Drought and to Combat Desertification. **Journal of Arid Land Studies**; 24-1, 121 - 124 (2014)

NEI M, 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NIEDDU, G., CHESSA, I. 1997. Distribution of phenotypic characters within a seedling population from Opuntia ficus-indica (cv. Gialla). **Acta Hort**. 438: 37-43

NIEDDU, G., CHESSA, I., and BARBERIS, A., 2006. Characterization of seedlings obtained from open pollinated 'Gialla' cactus pear (Opuntia-ficus indica). Acta. Hort. 438: 37-43

NOBEL, P.S. 1988. Environmental biology of agaves and cacti. Cambridge Univ. Press, New York

NOBEL, P.S. 2009. **Cacti Biology and Uses**. California University Press, USA, 280 pp.

PAIXÃO, S.L., 2012. Avaliação morfologica de clones e progênies de palma forrageira. http://200.17.137.108/tde_busca/arquivo.php?codArquivo=1792

PEÑA-VALDIVIA, C.B., LUNA-CAVAZOS, M., CARRANZA-SABAS, J.A., REYES-AGÜRO, J.A., AND FLORES, A., 2008. Morphological characterization of Opuntia spp.: A multivariate analysis. J.PACD, pp. 1-21

PESSOA, A.S. 1967. Cultura da Palma Forrageira. SUDENE - Divisão de Documentação 98p. **Agricultura**, 5. Recife, Brazil.

PIMIENTA-BARRIOS, E. 1994. Prickly pear (*Opuntia* spp.): a valuable fruit crop for the semi-arid lands of Mexico. **J. Arid Environ**. 28: 1-12

PIMIENTA-BARRIOS, E. and MUÑOZ-URÍAS, A. 1995. Domestication of opuntias and cultivated varieties. p.58-63, in: G. Barbera, P. Inglese and E. Pimienta-Barrios (eds) Agro-ecology, cultivation and uses of cactus pear. **FAO Plant Production and Protection Paper**, 132.

REBMAN, J. and D. PINKAVA. 2001. *Cylindropuntia delgadilloana*, a new cholla (Cactaceae) from Baja California, Mexico. **Journ. Ariz.-Nev. Acad. Sciences**. 33: 154-156.

REYES-AGUERO, J.A., AGUIRRE, R.J.R., VALIENTE-BANUET, A. 2006. Reproductive biology of *Opuntia*: a review. **J. Arid Environ**. 64: 549-585

REYNOLDS, S.G. and ARIAS, E., 2001. Introduction. In: C. Mondragón-Jacobo and S. Pérez-González, pp.1-4. Cactus (*Opuntia spp*.) as forage. **FAO Plant production and protection paper** 169. 146 pp.

SAENZ HERNANDEZ, C. CORRALES-GARCIA, J., AQUINO-PÉREZ, G. 2002. Nopalitos, mucilage, fiber and cochineal. In Cacti Biology and Uses (P.S. Nobel, ed.). **University of California Press**, Berkeley and Los Angeles, California, Pp 211-234

SAGHAI-MAROOF MA, SOLIMAN K, JORGENSEN RA, ALLARD RW, 1984. Ribosomal DNA spacer length polymorphism in barley: Men- using microsatellite markers. **Theor Appl Genet.** 105: 699–707.

SCHEINVAR, L. 1995. Taxonomy of utilized Opuntias. In: Barbera G., Inglese P., Pimienta-Barrios E (eds) Agroecology, cultivation and uses of cactus pear. **FAO Plant Production and Protection Paper** 132, FAO Rome, pp 20-27

SNOUSSI TRIFA, H., LABRA, M., and BEN SALEM, H. 2009. Molecular characterization of three Tunisian collections of cactus. **Acta Hort**. 811: 287-291

SOKAL RR and MICHENER CD, 1958. A statistical method for evaluating systematic relationships. University of Kansas Science Bulletin 38, pages 1409–1438.

SOUTO-ALVES, T., VANUSA DA SILVA, M. ALVES DE ALMEIDA, C.M., Oliveira Jordao do Amaral, D., Cordeiro dos Santos, D. , Farias, I., Tenorio Sabino Donato, V.M. and Da Costa, A.F. 2009. Genetic diversity in cactus clones using ISSR markers. **Acta Hort**. 811: 55-58

TAMURA, K, STECHER, G, PETERSON, D, FILIPSKI, A, and KUMAR, S. 2013. **Molecular Biology and Evolution,** 30: 2725-2729. doi: 10.1093/molbev/mst197

TEGEGNE, F., 2001. Nutritional value of Opuntia ficus-indica as a ruminant feed in Ethiopia. In Cactus (Opuntia spp.) as Forage. **FAO Plant Production and Protection Paper** 169. Pp. 91-99

TEL-ZUR, N. ABBO, S., MYSLABODSKI, D. and MIZRAHI, Y. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selecnicereus* (Cactaceae). **Plant molecular biology reporter** 17: 249-254

UDUPA, SM, WEIGAND, F, SAXENA, MC, KAHL, G, 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. **Theor. Applied Genet** 97: 299-307.

UZUN, I. 1997. Fruit and cladode isozymes in cactus pear. **Acta Hort**. 438: 53-56

VOS P., R., HOGAR, M., BLEEKER, M., REIJANS, T., VAN DE LEE, M., HORNES, A., FREITERS, J., POT, J., PELEMAN, M., KUIPER and M. ZABEAU. 1995. AFLP: A new technique for DNA fingerprinting. **Nucleic Acids Research** 23: 4407-4414.

WANG, X., FELKER, P., BURROW, M.D. and PATERSON, A.H.. 1998. Comparison of RAPD marker patterns to morphological and physiological data in the classification of *Opuntia* accessions. **JPACD** 3: 3-14

WILLIAMS, J.G.K., KUBELIC, A.R., LIVACK, K.J., RAFALSKI, J.A., TINGEY, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucl. Acide Res.** 18 (22): 6531-6535

WILLIAMS, J.G.K., RAFALSKI, J.A., TINGEY, S.V. 1992. Genetic analysis using RAPD markers. **Methods in Enzymology** 218: 704-740

ZOGHLAMI, N., CHRITA, I., BOUAMAMA, B., GARGOURI, M., ZEMNI, H. GHORBEL, A. and MLIKI, A. 2007. Molecular based assessment of genetic diversity within barbari fig (*Opuntia ficus indica* (L.) in Tunisia. **Sci. Hort**. 113: 134-141

Annex 1. List of accessions and phenotypic data

Table 4. List of accessions selected from the Mediterranean/Morocco collection at Agadir (Morocco) used for morphological and molecular characterization

Variety	Abreviation	Origin	Code number
Bianca Bonacardo	B_Bona	Italy	M01
Bianca Macomer	B_Maco	Italy	M02
Nudosa	Nudosa	Mexico	M03
Bianca Roccapalumba	B_Roc	Sicily, Italy	M04
Rossa Roccapalumba	R_Roc	Sicily, Italy	M05
Rossa San Cono	R_SCon	Sicily, Italy	M06
Rojalisa	Roja	Mexico	M07
Gialla Roccapalumba	G_Roc	Sicily, Italy	M08
Trunzara Rossa San Cono	TR_SCon	Sicily, Italy	M09
Bianca San Cono	B_SCon	Sicily, Italy	M10
Algerian	Alger	South Africa	M11
Seedless Roccapalumba	SL_Roc	Sicily, Italy	M12
Morado	Mora	South Africa	M13
Gialla San Cono	G_SCon	Sicily, Italy	M14
Seedless Santa Margherita	SL_SMar	Ethiopia	M15
Israele Monastra	I_Mona	Israel	M16
Local ecotype 1, Morocco	L_Mor1	Mel zhar Morocco	M17
Local ecotype 2, Morocco	L_Mor2	Tétouan Morocco	M18
Local ecotype 3, Morocco	L_Mor3	Eljadida 1 Morocco	M19
Local ecotype 4, Morocco	L_Mor4	Eljadida 2 Morocco	M20

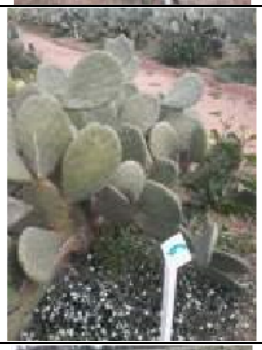
Table 15. List of IPA accessions (North East Brazil) used for morphological characterization

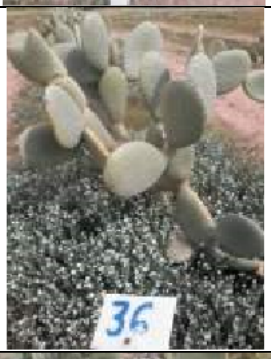
code	Accession name	code	Accession name	code	Accession name
B1	Sanrizil IV	B54	IPA 98-T30F4	B107	IPA 98-T37F6
B2	Chile Fruit/1118	B55	IPA 98-T38F4	B108	IPA 98-T27F6
B3	Sanrizil III	B56	IPA 98-T31F3	B109	IPA 98-T33F3
B4	Additional cv. 1281	B57	IPA 98-T18F3	B110	IPA 98-T34F6
B5	Skiner court	B58	IPA 98-T20F4	B111	IPA 98-T27F5
B6	Fusicaulis	B60	IPA 98-T8F2	B112	IPA 98-T35F5
B7	Sanrizil II	B61	IPA 98-T33F2	B113	IPA 98-T20F5
B8	Additional cv. 1279	B62	IPA 98-T39F2	B114	IPA 98-T18F5
B10	Direkteur	B63	IPA 98-T5F2	B115	IPA 98-T45F5
B11	México Unkonwn/1296	B64	IPA 98-T7F2	B116	IPA 98-T43F5
B12	Villa nueva	B65	IPA 98-T12F2	B117	IPA 98-T49F5
B13	Jalpa	B66	IPA 98-T27F4	B118	IPA 98-T33F5
B14	Liso forrageiro	B67	IPA 98-T32F1	B119	IPA 98-T41F5
B15	Blanco San Pedro	B68	IPA 98-T29F1	B120	IPA 98-T34F5
B16	Nopalea M. Aleman	B69	IPA 98-T26F1	B121	IPA 98-T37F5
B17	Politlán	B70	IPA 98-T24F1	B122	IPA 98-T29F5
B18	Oaxaca	B71	IPA 98-T21F1	B123	IPA 98-T39F5
B19	Copena V1	B72	IPA 98-T14F1	B124	IPA 98-T35F4
B20	Atlixco	B73	IPA 98-T9F1	B125	IPA 98-T25F5
B21	Moradilla	B74	IPA 98-T7F1	B126	IPA 98-T9F7
B22	Copena F1	B75	IPA 98-T4F1	B127	IPA 98-T7F7
B23	Liso M. Aleman	B76	IPA 98-T5F2	B128	IPA 98-T6F7
B24	Tobarito	B77	IPA 98-T8F1	B129	IPA 98-T5F7
B25	Blanco Michoacán	B78	IPA 98-T12F1	B130	IPA 98-T23F6
B26	IPA 90-73	B79	IPA 98-T19F1	B131	IPA 98-T19F6
B27	1294 – México Vegetable	B80	IPA 98-T22F1	B132	IPA 98-T12F6
B28	Marmillon Fodder/1327	B81	IPA 98-T25F1	B133	IPA 98-T43F6
B29	Algeria Fodder/1267	B82	IPA 98-T27F1	B134	IPA 98-T42F6
B30	Marmillon Fodder/1311	B83	IPA 98-T30F1	B135	IPA 98-T41F6
B31	Redonda	B84	IPA 98-T11F1	B136	IPA 98-T11F6
B32	Additional cv. 1258	B85	IPA 98-T6F3	B137	IPA 98-T7F6
B33	IPA 90-92	B86	IPA 98-T27F3	B138	IPA 98-T6F6
B34	IPA 90-18	B87	IPA 98-T13F3	B139	IPA 98-T53F4
B35	Algerian	B88	IPA 98-T22F3	B140	IPA 98-T39F6
B36	Gigante	B89	IPA 98-T19F3	B141	IPA 98-T45F6
B37	México Fodder/1278	B90	IPA 98-T7F3	B142	IPA 98-T24F6
B38	IPA 90-155	B91	IPA 98-T38F3	B143	IPA 98-T33F8
B39	IPA 94-Clone-20	B92	IPA 98-T25F4	B144	IPA 98-T31F8
B40	IPA 90-75	B93	IPA 98-T9F4	B145	IPA 98-T52F8
B41	IPA 90-106	B94	IPA 98-T7F4	B146	IPA 98-T40F8
B42	IPA 90-111	B95	IPA 98-T51F3	B147	IPA 98-T36F8
B43	IPA 94-Doce Miúda	B96	IPA 98-T37F3	B148	IPA 98-T27F7
B44	Chile Fruit/1317	B97	IPA 98-T26F3	B149	IPA 98-T22F7
B45	IPA 90-156	B98	IPA 98-T23F5	B150	1318
B46	Liso Forrajeiro (s)	B99	IPA 98-T6F5	B151	Marmillon Fodder/1327
B47	Penca Alargado	B100	Oreja de Elefante (Mexicana)	B152	IPA 98-T23F11
B48	Oreja de Elefante (Mexicana)	B101	Additional cv. 1258	B153	IPA 98-T20F11
B49	Amarillo Milpa Alta (s)	B102	IPA 98-T49F7	B154	IPA 98-T19F11
B50	Blanco San Pedro	B103	IPA 98-T35F7	B155	IPA 98-T12F11
B51	Chile Fruit/1317	B104	IPA 98-T30F6	B156	IPA 98-T10F11
B52	IPA 98-T30F5	B105	IPA 98-T33F6	B157	IPA 98-T34F10
B53	IPA 98-T28F4	B106	IPA 98-T40F4	B158	IPA 98-T31F10

Table 15 (ctd.). List of IPA accessions (North East Brazil) used for morphological characterization

code	Accession name	code	Accession name	code	Accession name
B159	IPA 98-T32F10	B200	Chile Fruit/1316	B249	IPA 98-T27F2
B160	IPA 98-T31F10	B201	IPA Sertânia	B250	Chilean/1313
B161	IPA 98-T35F10	B202	IPA 98-T34F11	B252	IPA 98-T24F7
B162	IPA 98-T24F10	B204	IPA 98-T28F11	B254	IPA 98-T4F7
B163	IPA 98-T30F10	B205	IPA 98-T14F11	B255	IPA 98-T3F7
B164	IPA 98-T19F10	B206	IPA 98-T10F11	B256	IPA 98-T22F6
B165	IPA 98-T4F11	B207	IPA 98-T15F11	B258	IPA 98-T15F6
B166	IPA 98-T9F11	B208	IPA 98-T20F11	B259	IPA 98-T55F6
B167	IPA 98-T10F11	B209	IPA 98-T22F11	B260	IPA 98-T26F6
B168	IPA 98-T11F9	B210	IPA 98-T13F10	B261	IPA 98-T50F6
B169	IPA 98-T12F9	B211	IPA 98-T31F10	B262	IPA 98-T61F10
B170	IPA 98-T18F8	B212	IPA 98-T12F10	B264	IPA 98-T27F8
B171	IPA 98-T15F8	B214	IPA 98-T40F4	B265	IPA 98-T25F8
B172	IPA 98-T32F8	B215	IPA 98-T41F4	B266	IPA 98-T35F8
B173	IPA 98-T33F8	B216	IPA 98-T13F4	B267	IPA 98-T24F8
B174	IPA 98-T35F8	B217	IPA 98-T32F4	B268	IPA 98-T28F8
B175	IPA 98-T40F8	B218	IPA 98-T15F4	B269	IPA 98-T30F8
B176	IPA 98-T17F7	B219	IPA 98-T30F3	B270	IPA 98-T14F9
B177	IPA 98-T21F7	B220	IPA 98-T41F3	B271	IPA 98-T21F9
B178	IPA 98-T18F7	B221	IPA 98-T53F3	B272	IPA 98-T27F9
B179	IPA 98-T16F7	B222	IPA 98-T8F3	B273	IPA 98-T28F9
B180	IPA 98-T10F6	B223	IPA 98-T7F5	B274	IPA 98-T33F9
B181	IPA 98-T13F6	B224	IPA 98-T31F5	B276	IPA 98-T61F11
B182	IPA 98-T39F6	B225	IPA 98-T50F5	B278	IPA 98-T26F11
B183	IPA 98-T42F6	B228	IPA 98-T11F5	B279	IPA 98-T42F11
B184	IPA 98-T11F6	B230	IPA 98-T50F3	B280	IPA 98-T46F11
B185	IPA 98-T9F6	B231	IPA 98-T24F3	B281	IPA 98-T41F11
B186	IPA 98-T12F6	B232	IPA 98-T55F3	B282	IPA 98-T23F11
B187	IPA 98-T10F6	B233	IPA 98-T17F4	B283	IPA 98-T21F11
B188	IPA 98-T18F6	B234	IPA 98-T58F4	B284	IPA 98-T16F11
B189	IPA 98-T15F4	B235	IPA 98-T17F3	B285	IPA 98-T7F11
B190	IPA 98-T25F6	B236	IPA 98-T16F3	B286	IPA 98-T2F10
B191	IPA 98-T34F6	B237	IPA 98-T3F5	B287	IPA 98-T4F11
B192	IPA 98-T33F6	B238	IPA 98-T35F2	B288	IPA 98-T43F8
B193	IPA 98-T30F8	B239	IPA 98-T23F2	B289	IPA 98-T51F10
B194	IPA 98-T38F8	B240	IPA 98-T29F2	B290	IPA 98-T37F10
B195	IPA 98-T37F8	B241	IPA 98-T4F2	B291	IPA 98-T42F10
B196	IPA 98-T39F8	B242	IPA 98-T13F2	B293	IPA 98-T28F10
B197	IPA 98-T57F8	B243	IPA 98-T3F2	B297	IPA 98-T7F10
B198	IPA 98-T33F7	B246	IPA 98-T2F2	B299	IPA 98-T56F9
B199	IPA 98-T31F7	B248	IPA 98-T17F2	B300	Algeria Fodder/1267

Pictures 1. Selected Opuntia ficus indica Mill accessions from Mediterranean/Morocco (INRA Agadir) used for phenotypic characterization

Accession	Abreviation	Origin	Plant photo	Cladode photo
BIANCA BONACARDO	B_Bona	Italy		
BIANCA MACOMER	B_Maco	Italy		
NUDOSA	Nudosa	Mexico		
Gialla ROCCAPALUMBA	B_Roc	Sicily, Italy		
Rossa ROCCAPALUMBA	R_Roc	Sicily, Italy		

Rossa SAN CONO	R_SCon	Sicily, Italy		
ROJALISA	Roja	Mexico		
Gialla ROCCAPALUMBA	G_Roc	Sicily, Italy		
TRUNZARA RED SAN CONO	TR_SCon	Sicily, Italy		
WHITE SAN CONO	B_SCon	Sicily, Italy		

ALGERIAN	Alger	South Africa		
SEEDLESS ROCCAPALUMBA	SL_Roc	Sicily, Italy		
MORADO	Mora	South Africa		
YELLOW SAN CONO	G_SCon	Sicily, Italy		
SEEDLESS SANTA MARGHERITA	SL_SMar	Ethiopia		

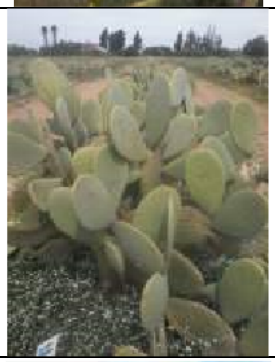
ISRAELE MONASTRA	I_Mona	Israel		
Local ecotype Morocco 1	L_Mor1	Mel zhar Morocco		
Local ecotype Morocco 2/ LAHMAMYA	L_Mor2	Tétouan Morocco		
Local ecotype Morocco 3 (Rgion Eljadida)	L_Mor3	Eljadida 1 Morocco		
Local ecotype Morocco 4 (Region Eljadida)	L_Mor4	Eljadida 2 Morocco		

Table 16. Phenotypic traits of the IPA accessions

Accession	P_Height	P_Width	Cl_length	Cl_width	Cladsh Ix	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B1	1.07	1.88	40	22	1.82	20	90	0	0	0	0
B2	1.07	1.23	44	22	2.00	20	120	0	0	0	3
B3	1.68	2.07	41	22	1.86	20	122	0	0	0	0
B4	1.15	1.07	51	19	2.68	21	140	0	0	0	0
B5	1.2	1.26	45	16	2.81	16	134	0	0	0	0
B6	1.21	0.92	35	12	2.92	25	132	0	0	0	5
B7	1.19	1.29	41	20	2.05	12	114	0	0	0	0
B8	0.51	0.7	28	17	1.65	10	116	0	0	0	0
B10	0.74	0.7	41	10	4.10	12	154	0	0	0	0
B11	1.67	1.34	45	18	2.50	20	148	3	10	1	3
B12	1.42	1.79	39	27	1.44	10	168	3	15	1	0
B13	1.54	1.5	44	27	1.63	18	114	0	0	0	0
B14	1.12	0.69	32	20	1.60	22	116	3	7	1	7
B15	1.45	1.92	27	16	1.69	15	60	0	0	0	3
B16	0.98	1.38	24	13	1.85	12	32	0	0	0	0
B17	1.49	1.83	29	22	1.32	20	110	3	5	1	3
B18	1.46	1.57	31	22	1.41	15	120	3	16	2	0
B19	0.98	1.37	32	19	1.68	16	140	0	0	0	0
B20	0.86	0.79	25	16	1.56	12	160	3	10	1	0
B21	0.84	1.03	35	22	1.59	20	118	0	0	0	0
B22	1.7	1.66	59	17	3.47	20	132	0	0	0	3
B23	0.81	2.08	34	26	1.31	30	130	5	20	4	3
B24	1.46	1.96	51	24	2.13	21	124	0	0	0	0
B25	1.58	2.05	32	17	1.88	16	48	0	0	0	0
B26	1.4	1.74	34	18	1.89	20	122	0	0	0	0
B27	1.44	1.27	43	24	1.79	18	100	0	0	0	0
B28	1.52	1.48	36	23	1.57	19	114	0	0	0	0
B29	0.92	1.78	46	31	1.48	22	66	3	26	1	3
B30	1.12	0.83	48	17	2.82	18	130	0	0	0	0
B31	1.02	1.06	33	27	1.22	26	128	0	0	0	0
B32	1.26	1.35	50	18	2.78	31	128	0	0	0	7
B33	1.5	1.52	41	25	1.64	26	126	0	0	0	0
B34	1.6	1.7	36	20	1.80	21	138	0	0	0	0
B35	2.06	1.85	45	14	3.21	21	84	0	0	0	0
B36	1.08	1.4	39	20	1.95	24	94	3	10	1	0
B37	1.6	2.11	44	19	2.32	13	124	0	0	0	0
B38	1.48	1.44	42	21	2.00	20	120	0	0	0	0
B39	1.02	1.03	39	19	2.05	23	120	0	0	0	0
B40	0.95	1.17	31	17	1.82	24	106	0	0	0	0
B41	1.32	1.94	50	23	2.17	20	100	0	0	0	0
B42	1.21	1.94	37	22	1.68	21	116	0	0	0	0
B43	1.26	1.24	24	12	2.00	16	38	0	0	0	0
B44	0.78	1.29	31	15	2.07	18	84	0	0	0	0
B45	1.3	1.37	35	17	2.06	17	124	0	0	0	0
B46	0.91	1.25	36	22	1.64	24	86	3	16	1	7
B47	0.7	0.78	21	13	1.62	25	80	5	10	1	7
B48	1.16	1.95	51	34	1.50	10	36	0	0	0	3
B49	0.95	1.66	36	22	1.64	19	162	3	11	1	3
B50	0.88	0.76	25	22	1.14	20	92	3	15	1	3
B51	0.92	1.68	38	19	2.00	28	140	0	0	0	3
B52	0.94	1.45	40	16	2.50	24	126	0	0	0	0
B53	1.02	1.45	35	20	1.75	20	118	0	0	0	0

Accession	P_Height	P_Width	Cl_length	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B54	1.14	1.4	37	20	1.85	21	106	0	0	0	0
B55	1.09	1.58	39	21	1.86	20	100	0	0	0	0
B56	1.35	1.58	45	22	2.05	20	132	0	0	0	0
B57	1.22	1.78	34	17	2.00	22	148	0	0	0	0
B58	1.48	1.79	26	11	2.36	12	36	0	0	0	0
B60	1.16	1.74	41	22	1.86	18	114	0	0	0	0
B61	1.21	1.55	35	20	1.75	19	120	0	0	0	0
B62	1.29	1.73	49	25	1.96	20	128	3	10	1	0
B63	1.26	1.6	41	23	1.78	22	112	0	0	0	0
B64	1.22	1.45	39	22	1.77	23	120	0	0	0	0
B65	1.09	1.78	35	19	1.84	22	122	0	0	0	0
B66	1.45	1.95	36	22	1.64	17	124	0	0	0	0
B67	1.4	1.65	37	19	1.95	19	100	0	0	0	0
B68	1.16	1.7	35	20	1.75	20	100	0	0	0	0
B69	1.17	1.32	36	19	1.89	19	130	0	0	0	0
B70	1.4	1.69	49	23	2.13	23	92	0	0	0	0
B71	1.39	1.63	40	22	1.82	18	122	0	0	0	0
B72	0.79	1.4	19	24	0.79	22	124	0	0	0	0
B73	0.98	1.93	37	17	2.18	27	128	3	9	1	0
B74	1.08	1.5	40	18	2.22	14	106	0	0	0	0
B75	1.07	1.38	28	24	1.17	27	108	3	14	1	0
B76	1.55	1.29	41	24	1.71	20	104	0	0	0	0
B77	1.21	1.45	40	20	2.00	22	142	0	0	0	0
B78	1	1.41	48	26	1.85	22	132	3	7	1	0
B79	1.05	1.18	36	19	1.89	22	110	0	0	0	0
B80	1.4	1.09	38	21	1.81	26	118	0	0	0	0
B81	1.4	1.93	47	22	2.14	20	118	0	0	0	0
B82	1.41	1.71	41	19	2.16	25	128	0	0	0	0
B83	0.97	1.65	36	21	1.71	21	160	0	0	0	0
B84	1.49	1.41	47	21	2.24	24	142	0	0	0	0
B85	1.44	1.7	41	22	1.86	22	112	0	0	0	0
B86	1.31	1.44	41	23	1.78	26	120	0	0	0	0
B87	1.22	1.34	33	19	1.74	21	128	0	0	0	0
B88	0.81	1.93	26	17	1.53	19	136	0	0	0	0
B89	1.03	1.48	37	17	2.18	24	120	0	0	0	0
B90	1.18	1.6	35	19	1.84	15	106	0	0	0	0
B91	1.19	1.5	35	18	1.94	21	134	0	0	0	0
B92	1.13	1.48	31	18	1.72	26	106	0	0	0	0
B93	0.97	1.55	33	19	1.74	23	122	0	0	0	0
B94	1.04	1.45	38	20	1.90	20	134	0	0	0	0
B95	1.45	1.55	35	23	1.52	23	156	0	0	0	0
B96	1.28	1.47	35	17	2.06	26	136	0	0	0	0
B97	1.55	1.63	34	25	1.36	20	122	0	0	0	0
B98	1.27	1.3	34	20	1.70	24	134	0	0	0	0
B99	1.18	1.46	37	19	1.95	29	128	0	0	0	0
B100	1.04	1.85	26	24	1.08	21	44	0	0	0	5
B101	1.6	1.89	53	21	2.52	22	128	0	0	0	5
B102	1.28	1.73	31	18	1.72	24	126	0	0	0	0
B103	1.33	1.74	36	18	2.00	23	124	0	0	0	0
B104	1.09	1.55	35	21	1.67	21	110	0	0	0	0
B105	1.18	1.62	34	17	2.00	22	130	0	0	0	0
B106	1.35	1.46	34	22	1.55	25	124	0	0	0	0
B107	1.47	1.85	36	19	1.89	18	156	0	0	0	0
B108	1.11	1.33	48	26	1.85	20	118	0	0	0	0

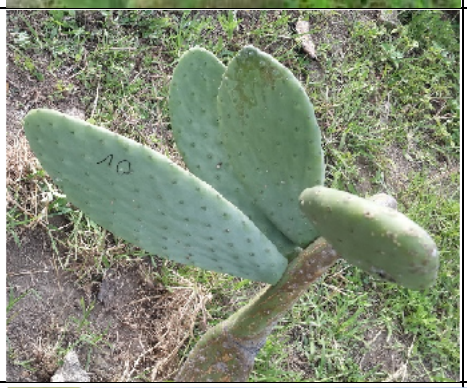
Accession	P_Height	P_Width	Cl_length	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B109	0.86	1.09	41	21	1.95	20	138	3	16	1	0
B110	1.15	1.18	30	15	2.00	22	110	3	15	1	0
B111	1.41	1.73	40	24	1.67	23	134	0	0	0	0
B112	0.91	1.4	32	18	1.78	24	136	0	0	0	0
B113	1.11	1.38	37	20	1.85	24	110	0	0	0	0
B114	1.28	1.49	28	18	1.56	20	120	0	0	0	0
B115	1.01	1.42	38	23	1.65	19	132	0	0	0	0
B116	1.16	1.28	37	21	1.76	21	128	0	0	0	0
B117	1.51	1.4	36	23	1.57	21	134	0	0	0	0
B118	1.12	1.21	34	21	1.62	25	130	0	0	0	0
B119	1.11	1.35	29	20	1.45	19	130	0	0	0	0
B120	1.14	1.32	39	23	1.70	24	138	3	10	1	0
B121	1.51	1.54	38	20	1.90	17	138	0	0	0	0
B122	1.22	1.36	31	19	1.63	27	148	0	0	0	0
B123	1.28	1.4	33	21	1.57	19	106	0	0	0	0
B124	1.22	1.31	24	19	1.26	19	136	0	0	0	0
B125	1.07	1.51	44	19	2.32	17	148	0	0	0	0
B126	1	1.51	32	18	1.78	18	128	0	0	0	0
B127	1.09	1.34	35	20	1.75	21	140	3	12	1	0
B128	0.86	1.2	29	18	1.61	17	136	3	10	1	0
B129	1.13	1.98	30	21	1.43	16	156	0	0	0	0
B130	1.04	1	32	21	1.52	21	128	0	0	0	0
B131	1.13	1.37	31	21	1.48	17	130	0	0	0	0
B132	1.11	1.13	37	20	1.85	17	134	0	0	0	3
B133	1.35	1.13	41	22	1.86	20	102	0	0	0	3
B134	1.57	1.75	39	21	1.86	18	132	0	0	0	5
B135	1.41	1.62	40	21	1.90	19	110	0	0	0	0
B136	1.6	1.26	42	22	1.91	20	140	0	0	0	0
B137	1.07	1.53	38	22	1.73	18	138	0	0	0	0
B138	1.08	1.14	35	20	1.75	21	138	3	6	1	0
B139	1.23	1.24	38	22	1.73	22	142	0	0	0	0
B140	1.59	1.36	43	25	1.72	21	146	3	14	1	3
B141	1.16	1.29	36	22	1.64	17	132	0	0	0	7
B142	1.37	1.88	44	25	1.76	19	134	3	9	1	3
B143	1.1	1.71	43	23	1.87	21	128	3	10	1	3
B144	1.25	1.7	42	24	1.75	24	130	3	6	1	3
B145	1.17	1.21	45	25	1.80	20	138	3	7	1	3
B146	1.2	1.56	36	22	1.64	21	118	0	0	0	3
B147	1.37	1.25	39	22	1.77	14	150	3	7	1	3
B148	1.17	1	39	22	1.77	23	130	0	0	0	0
B149	0.84	1.14	30	19	1.58	20	110	3	13	1	3
B150	0.87	1.56	38	19	2.00	22	108	3	5	1	0
B151	1.14	2.32	46	26	1.77	22	130	3	5	1	0
B152	1.11	1.39	40	22	1.82	26	122	3	5	1	0
B153	1.27	1.62	39	22	1.77	24	132	3	7	1	0
B154	1.15	1.63	42	25	1.68	25	122	3	10	1	5
B155	1.42	1.78	29	20	1.45	27	114	0	0	0	3
B156	1.55	1.71	37	25	1.48	25	116	3	15	1	3
B157	1.42	1.96	35	20	1.75	22	116	0	0	0	0
B158	1.44	0.96	37	24	1.54	25	90	3	10	1	0
B159	1.1	1.56	35	22	1.59	29	112	0	0	0	5
B160	1.15	1.51	41	22	1.86	20	120	3	10	1	3
B161	1.1	1.29	39	21	1.86	24	110	0	0	0	3
B162	1.01	1.37	42	26	1.62	22	108	0	0	0	0

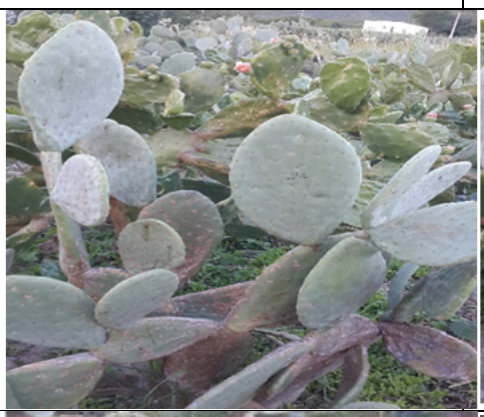
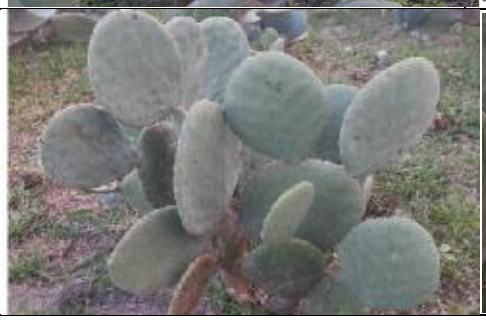
Accession	P_Height	P_Width	Cl_length	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B163	1.4	1.37	38	23	1.65	24	110	0	0	0	0
B164	1.33	1.28	36	18	2.00	25	126	3	11	1	5
B165	1.06	1.33	38	22	1.73	20	124	0	0	0	0
B166	1.17	1.4	43	23	1.87	30	120	3	5	1	5
B167	1.35	1.54	33	20	1.65	21	112	3	10	1	5
B168	1.06	1.13	30	19	1.58	25	116	3	14	1	3
B169	1.36	1.7	40	27	1.48	26	120	0	0	0	3
B170	1.19	1.54	32	21	1.52	26	134	3	10	1	0
B171	0.99	1.2	36	26	1.38	30	130	3	8	1	3
B172	1.22	1.75	37	21	1.76	26	110	3	5	1	0
B173	1.14	1.68	38	22	1.73	21	132	3	6	1	0
B174	1.06	1.35	43	24	1.79	22	120	0	0	0	7
B175	1.19	1.42	43	24	1.79	21	102	3	13	1	7
B176	1.4	1.45	33	20	1.65	19	126	0	0	0	0
B177	1.12	1.37	40	24	1.67	21	114	3	10	1	3
B178	1.09	1.51	35	23	1.52	27	120	3	9	1	3
B179	1.3	2	37	20	1.85	26	122	3	13	1	0
B180	1.15	2.05	39	24	1.63	20	116	3	5	1	3
B181	0.96	1.27	37	23	1.61	21	116	3	12	1	0
B182	1.05	1.52	42	20	2.10	21	132	3	11	1	0
B183	1.05	1.44	35	20	1.75	22	128	0	0	0	7
B184	1.16	1, 33	37	20	1.85	27	120	3	6	1	0
B185	1.08	1.38	33	19	1.74	24	126	3	9	2	3
B186	1.21	0.9	33	21	1.57	23	122	3	10	1	3
B187	1.08	1.46	35	23	1.52	26	108	3	10	1	3
B188	0.96	1.14	31	16	1.94	18	124	3	11	1	3
B189	1.17	1.28	39	22	1.77	27	116	0	0	0	0
B190	1.45	1.19	41	23	1.78	23	112	0	0	0	0
B191	1.06	0.97	41	22	1.86	20	114	0	0	0	7
B192	0.81	1.36	41	22	1.86	21	128	3	13	1	7
B193	0.94	0.9	32	19	1.68	21	94	0	0	0	0
B194	1.11	1.36	39	23	1.70	21	104	0	0	0	7
B195	1.25	1.32	34	25	1.36	28	122	0	0	0	0
B196	1.3	1.63	40	20	2.00	26	138	3	11	1	0
B197	1.16	1.49	35	22	1.59	28	136	3	11	1	0
B198	1.16	1.71	39	20	1.95	27	128	0	0	0	0
B199	1.1	1.4	39	22	1.77	21	140	0	0	0	0
B200	1.27	1.4	33	19	1.74	25	106	0	0	0	0
B201	1.1	1.43	30	21	1.43	28	88	0	0	0	0
B202	0.81	0.9	38	21	1.81	21	102	3	11	1	0
B204	0.75	1.32	35	19	1.84	25	128	0	0	0	7
B205	0.71	1.02	28	18	1.56	20	102	0	0	0	0
B206	0.73	1.02	34	18	1.89	23	84	0	0	0	0
B207	0.81	1.04	23	15	1.53	20	124	0	0	0	0
B208	0.87	0.99	31	18	1.72	22	126	0	0	0	0
B209	0.97	1.68	28	25	1.12	26	116	0	0	0	0
B210	0.87	1.2	31	25	1.24	26	134	3	5	1	0
B211	0.79	1.25	35	20	1.75	26	100	0	0	0	0
B212	0.67	1.24	33	18	1.83	21	106	0	0	0	0
B214	0.77	1.19	32	19	1.68	22	94	3	10	1	0
B215	0.88	1	32	23	1.39	21	130	0	0	0	0
B216	0.61	0.81	33	27	1.22	25	88	3	9	1	0
B217	0.96	1.12	42	23	1.83	27	96	0	0	0	5
B218	0.94	1.13	33	21	1.57	24	118	3	5	1	0

Accession	P_Height	P_Width	Cl_length	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B219	0.9	1.47	29	17	1.71	23	110	0	0	0	0
B220	1.14	1.6	40	21	1.90	23	132	0	0	0	0
B221	0.7	1.44	41	19	2.16	21	110	0	0	0	0
B222	0.75	1.03	36	22	1.64	22	118	0	0	0	0
B223	0.66	1.08	38	20	1.90	23	90	3	9	1	0
B224	0.67	1.02	33	16	2.06	21	92	3	7	1	0
B225	0.91	1	36	21	1.71	24	116	3	5	2	0
B228	0.84	0.97	31	18	1.72	24	120	3	10	1	0
B230	0.87	1.1	33	19	1.74	22	96	0	0	0	0
B231	0.95	1.29	41	23	1.78	23	118	3	9	1	0
B232	0.57	1.15	25	23	1.09	27	120	3	13	1	0
B233	0.71	1.51	32	19	1.68	24	128	0	0	0	0
B234	0.82	0.93	33	22	1.50	26	106	0	0	0	0
B235	0.9	1.49	34	21	1.62	22	120	0	0	0	0
B236	0.9	1.52	35	23	1.52	23	118	0	0	0	0
B237	1.56	1.26	35	21	1.67	30	112	0	0	0	0
B238	0.77	0.93	28	16	1.75	27	102	0	0	0	0
B239	0.82	1.44	40	24	1.67	21	134	3	5	1	0
B240	0.9	1.47	35	23	1.52	26	90	3	6	1	0
B241	0.95	1.24	26	16	1.63	24	104	0	0	0	0
B242	0.64	0.8	30	20	1.50	21	92	3	5	1	0
B243	0.85	1.11	35	18	1.94	22	116	0	0	0	0
B246	0.71	1.09	25	14	1.79	19	90	0	0	0	0
B248	0.92	1.29	35	15	2.33	22	66	0	0	0	0
B249	0.95	1.66	38	22	1.73	27	108	0	0	0	0
B250	1.06	1.43	36	16	2.25	24	138	0	0	0	0
B252	0.96	0.98	36	23	1.57	24	110	0	0	0	7
B254	0.85	1.48	36	21	1.71	23	108	3	13	1	0
B255	0.86	1.32	39	19	2.05	23	126	3	8	1	0
B256	0.7	1.07	30	20	1.50	25	132	3	7	1	0
B258	0.7	1.07	31	20	1.55	21	126	0	0	0	0
B259	0.42	0.52	27	16	1.69	23	106	3	11	1	0
B260	0.94	1.31	32	20	1.60	20	124	0	0	0	0
B261	0.99	1.3	32	17	1.88	25	102	0	0	0	0
B262	0.58	0.78	39	22	1.77	24	98	0	0	0	0
B264	0.7	0.67	33	19	1.74	29	110	3	6	1	0
B265	1.06	1.41	35	21	1.67	21	134	0	0	0	0
B266	0.86	1.17	39	24	1.63	17	130	0	0	0	0
B267	0.85	1.17	39	25	1.56	24	106	0	0	0	0
B268	0.66	1.1	31	18	1.72	24	126	0	0	0	0
B269	0.74	1.07	33	19	1.74	24	116	0	0	0	0
B270	0.54	0.77	32	19	1.68	24	108	3	15	1	0
B271	1.07	1.62	40	25	1.60	27	130	0	0	0	0
B272	1	1.18	37	23	1.61	27	124	0	0	0	0
B273	0.81	0.81	32	21	1.52	20	116	0	0	0	0
B274	0.85	1.04	33	19	1.74	30	130	3	12	1	0
B276	0.89	1.15	33	21	1.57	21	110	0	0	0	0
B278	0.96	1.16	32	20	1.60	23	30	0	0	0	0
B279	1.09	1.9	37	16	2.31	30	122	3	5	1	0
B280	0.76	0.78	38	22	1.73	21	126	3	11	1	0
B281	1.19	1.33	41	22	1.86	26	138	0	0	0	0
B282	0.52	1.12	31	22	1.41	30	116	3	13	1	0
B283	0.82	0.75	33	19	1.74	20	108	3	11	1	0
B284	1	1.53	38	21	1.81	22	116	3	13	1	0

Accession	P_Height	P_Width	Cl_length	Cl_width	CladshIx	Cl_thickness	N_areole	N_spines	Spine_size	NSpine_areole	N_glochides
B285	0.93	1.11	22	20	1.10	26	112	0	0	0	0
B286	0.95	1.95	35	18	1.94	24	140	0	0	0	0
B287	0.66	0.85	27	23	1.17	22	100	0	0	0	0
B288	0.92	1.14	33	18	1.83	21	148	0	0	0	0
B289	0.92	1.17	31	19	1.63	25	114	0	0	0	0
B290	0.92	1.46	31	18	1.72	23	116	0	0	0	0
B291	0.96	1.83	37	25	1.48	27	140	0	0	0	0
B293	0.64	1.11	38	22	1.73	22	126	3	13	1	0
B297	1.04	1.51	28	16	1.75	24	116	3	10	1	0
B299	0.85	0.7	32	19	1.68	25	108	3	10	1	0
B300	0.66	1.7	37	28	1.32	26	70	3	20	1	5

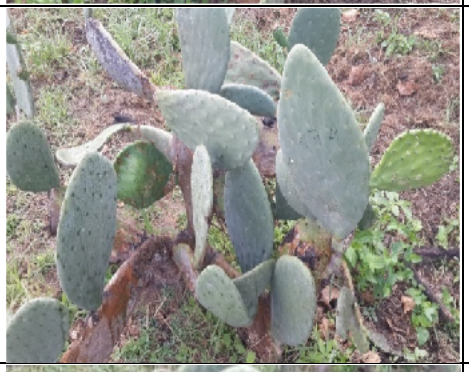
Pictures 2. Cactus accessions selected from IPA Arcoverde (Brazil) Collection to study morphological and molecular characterization

Acession code/ Entry number	IPA number	Name	Plant	Cladode
5/28	200193	Skiner court		
8/29	200179	Additional cv. 1279		
10/41	200191	Direkteur		
11/30	200183	México Unkonwn/1296		

19/F2	200001	Copena V1		
22/F1	200001	Copena F1		
24/F6	200006	Tobarito		
31/64	100002	Redonda		

34/-	100407	IPA 90-18		
37/20	200177	México Fodder/1278		
42/-	100412	IPA 90-111		
43/-	100404	IPA 94-Doce Miúda		

46/F-17	200017	Liso Forrajeiro (s)		
47/F-34	200034	Penca Alargado		
70/ Progeny PD/PL	100434	IPA 98-T24F1		
108/ Progeny PD/PL	100470	IPA 98-T27F6		
122/ Progeny PD/PL	100484	IPA 98-T29F5		

123/ Progeny PD/PL	100485	IPA 98-T39F5		
145/ Progeny PD/PL	100507	IPA 98-T52F8		
154/ Progeny PD/PL	100514	IPA 98-T19F11		
174/ Progeny PD/PL	100534	IPA 98-T35F8		
202/ Progeny PD/PL	100560	IPA 98-T34F11		

223/ Progeny PD/PL	100581	IPA 98-T7F5		
235/ Progeny PD/PL	100593	IPA 98-T17F3		
238/ Progeny PD/PL	100596	IPA 98-T35F2		
262/ Progeny PD/PL	100617	IPA 98-T61F10		

269/ Progeny PD/PL	100624	IPA 98-T30F8		
272/ Progeny PD/PL	100627	IPA 98-T27F9		
276/ Progeny PD/PL	100631	IPA 98-T61F11		
279/ Progeny PD/PL	100634	IPA 98-T42F11		

Table 23. Phenotypic traits of the combined batch of accessions from IPA and Mediterranean/Morocco collections

Accessions	Hp (cm)	Wp (cm)	Cl_Len (cm)	Cl_wid (cm)	Cl_thi (mm)	L/W index	Na	NSpine_areole	Lepl (mm)
B01	120	126	45	16	16	2.813	134	0	0
B02	51	70	28	17	10	1.647	116	0	0
B03	74	70	41	10	12	4.100	154	0	0
B04	167	134	45	18	10	2.500	148	1	10
B05	98	137	32	19	16	1.684	140	0	0
B06	170	166	59	17	20	3.471	132	0	0
B07	146	196	51	24	21	2.125	124	0	0
B08	102	106	33	27	26	1.222	128	0	0
B09	160	170	36	20	21	1.800	138	0	0
B10	160	211	44	19	13	2.316	124	0	0
B11	121	194	37	22	21	1.682	116	0	0
B12	126	124	24	12	16	2.000	38	0	0
B13	91	125	36	22	24	1.636	86	1	16
B14	7	78	21	13	25	1.615	80	1	10
B15	140	169	49	23	23	2.130	92	0	0
B16	111	133	48	26	20	1.846	118	0	0
B17	122	136	31	19	27	1.632	148	0	0
B18	128	140	33	21	19	1.571	106	0	0
B19	117	121	45	25	20	1.800	138	1	7
B20	115	163	42	25	25	1.680	122	1	10
B21	106	135	43	24	22	1.792	120	0	0
B22	81	90	38	21	21	1.810	102	1	11
B23	66	108	38	20	23	1.900	90	1	9
B24	90	149	34	21	22	1.619	120	0	0
B25	77	93	28	16	27	1.750	102	0	0
B26	58	78	39	22	24	1.773	98	0	0
B27	74	107	33	19	24	1.737	116	0	0
B28	100	118	37	23	27	1.609	124	0	0
B29	89	115	33	21	21	1.571	110	0	0
B30	109	190	37	16	30	2.313	122	1	5
M01	117	136	42	20	18.6	2.100	108	1.6	10
M02	159	234	44.4	20	19.8	2.220	106	1.8	11
M03	136	192	42.6	24	18.2	1.775	138	2	11.6
M04	124	178	39.8	21	20.4	1.895	139	1.6	11.6
M05	125	171	35.2	18.8	14.2	1.872	112	1.2	9
M06	122	178	39.2	20.8	14.8	1.885	120	1.5	9.8
M07	100	142	33.8	20.2	13.4	1.673	109	1.4	7.8
M08	116	169	34.4	19.2	14	1.792	114	1	7.3
M09	111	176	34.8	21	24.2	1.657	119	1	8
M10	108	197	35	19.6	16	1.786	103	1	10.2
M11	115	148	32.2	19.6	18.4	1.643	129	1.38	8.70

Accessions	Hp (cm)	Wp (cm)	Cl_Len (cm)	Cl_wid (cm)	Cl_thi (mm)	L/W index	Na	NSpine_areole	Lepl (mm)
M12	125	205	33.25	16.25	16	2.046	93	1	6.7
M13	143.75	187.5	36.5	17.75	18.75	2.056	112	1.67	10
M14	130	208.75	30.5	16.5	14.5	1.848	97	1	8
M15	113.33	181.67	34	18.67	15	1.821	126	1	9
M16	90	148.33	32.33	13.33	20.33	2.425	104	3	4
M17	108.33	152.67	32.67	18	12.67	1.815	99	1	6.5
M18	111.67	136.67	31	18	16.67	1.722	99	1	9
M19	108.33	175	35.67	19	18.67	1.877	106	1	6.7
M20	180	110	45	16	21.67	2.813	136	1.38	8.70

Annex 2 : Preparation of reagents for molecular characterization

- Solution of Tris-HCL of 1 M and pH = 8

To prepare 100mL of solution, 12.114 g of Tris base powder (Amresco) was dissolved in an Erlenmeyer containing distilled water. The pH solution was adjusted to 8 by adding HCL. The total volume is adjusted to 100 mL by adding distilled water.

- Concentration of 5 M NaCl solution

To prepare 100 mL of solution, 29.22 g of NaCl powder (Merck) were dissolved in an Erlenmeyer containing distilled water of equal volume to 100 mL.

- EDTA solution of 0.5 M concentration and pH 8

To prepare 100 mL of solution, 18.6 g of EDTA powder (Amresco) were dissolved in an Erlenmeyer containing distilled water. The pH solution was adjusted to 8 by adding NaOH and the volume was brought to 100 mL by adding distilled water.

- Solution Chloroform / Isoamyl alcohol

To prepare 100mL of solution, 96 ml of chloroform (Scharlau) are mixed in a beaker with 4 mL of isoamyl alcohol or 3-methylbutanol (Sigma).

- Solution 70% Ethanol

To prepare 100mL of solution, 70 mL of absolute ethanol or denatured ethanol Anhydrous (Amresco) are mixed in a beaker with 30 mL of distilled water.

- Solution 5X TBE

To prepare 1 liter of 1X TBE solution, 27.5 g of boric acid (Amresco) and 54 g Tris base (Sigma) were dissolved in a large beaker containing 20 ml of EDTA (0.5 M, pH = 8) and distilled water. The pH was adjusted to 8 by the addition of boric acid and distilled water was added for a second time to reach a final volume of 1 liter.

- TBE 1X solution

To prepare 75 mL of 1X TBE solution, 15 mL of TBE 1X are mixed to 60 mL of distilled water.

- Agarose blue solution

To prepare 10 ml of this solution, 20 mg of bromophenol blue were dissolved in a beaker containing 0.5 mL of Tris (1M, pH = 7.5), 0.1 ml of EDTA (0.5 M, pH = 8) and 5 ml of glycerol. Finally, distilled water was added to reach 10 mL.

- Blue solution of Acrylamide

To prepare 25 ml of blue acrylamide, 12.5 g of Xylene cyanole and 0.0125 g of bromophenol blue were dissolved in a volume of 23.75 mL formamide and 1.25 ml of sterile distilled water.

- Solution 40% Acrylamide

To prepare 100 mL of this solution, 38.66 g of acrylamide (Promega) and 1.34 g of bis-acrylamide or N, N'-methylenebisacrylamide (Sigma) were dissolved in a beaker containing 100 mL of distilled water.

APS 10 %:

1 g of ammonium persulfate is dissolved in 10 ml of distilled water.

Loading buffer (25 ml)

Formamide: 23,75 ml
Bromophenol blue: 12,5 mg
Xylene cyanol: 12,5 mg
Sterile distilled water: 1,25 ml

CTAB Buffer:

To prepare 2 x CTAB buffer, a final volume of 50 ml was needed.
The extraction buffer contained 5 ml of 1 M **Tris-HCL** (pH=8), 14 ml of 5 M **NaCl**, 2 ml of 0.5M **EDTA** (ph=8) and 1 g of **CTAB** powder. Then, the volume was completed with distilled water. Finally, 0.1 g of Sodium sulfite and 0.25 g of PVP were added to the 2 X CTAB solution.

- Tris maintain the pH, as It interacts with lipopolysacharids presente on the outer membrane which helps to permeabilize the membrane.
- NaCl helps the DNA separation, making It less hydrophile.
- EDTA is a chelating agent that binds mg2+ ions, It protects DNA from endonucleases.
- Sodium sulfite in the extraction buffer is used for DNA isolation from olives, faba bean, etc...and older leaves.
- PVP inhibit the polyphenol oxydase activity responsible for the coloration

Annex 3. Methodology for molecular characterization

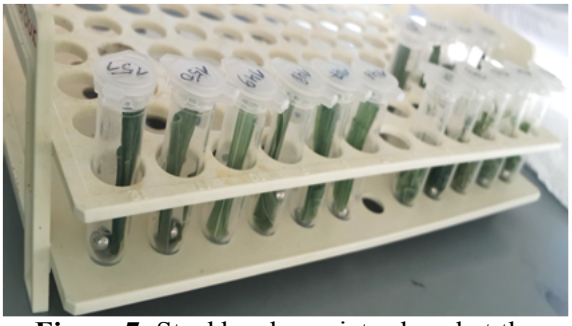


Figure 7. Steel beads are introduced at the bottom of each tube containing lyophilized material

Figure 8. Mechanical grinder

Figure 9. Proteins separating from DNA

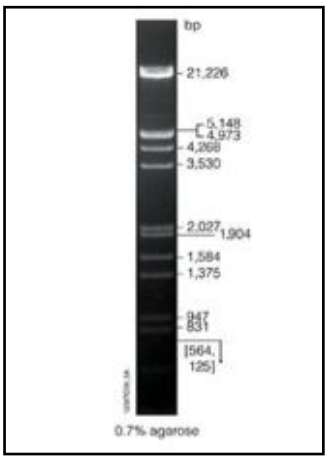


Figure 10. DNA size marker MIII

Lambda DNA/EcoRI+ HindIII Cat. # G1731 (Promega)

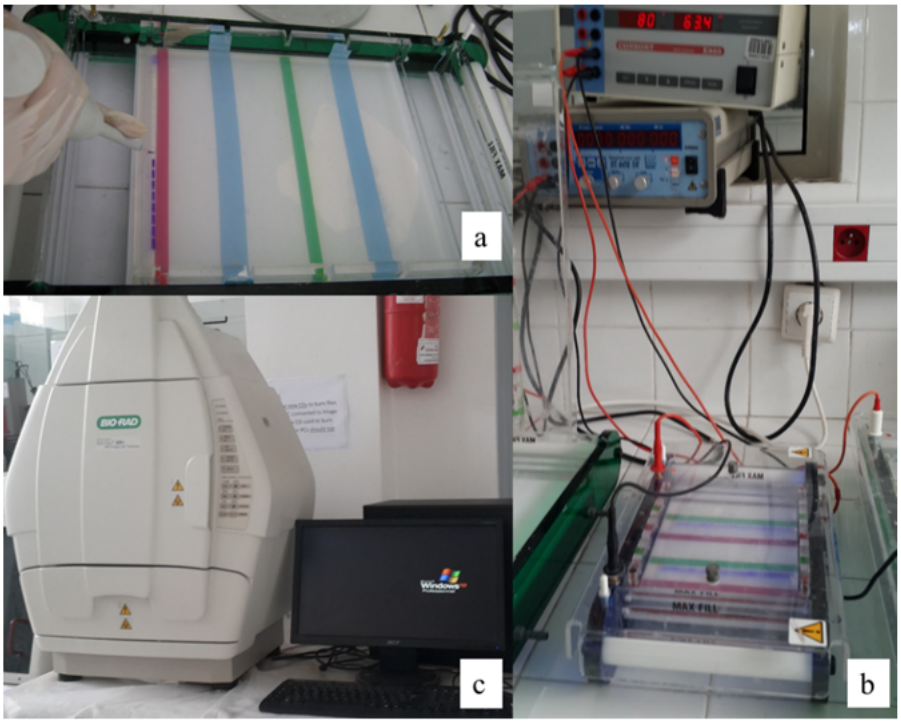


Figure 11. Deposit phase, running and visualization of 1% agarose gel

Figure 12. Taq polymerase (Roche)

Figure 13. On the left, the master mix is being centrifuged and on the right 9 µL of the mix are deposited in each PCR tube

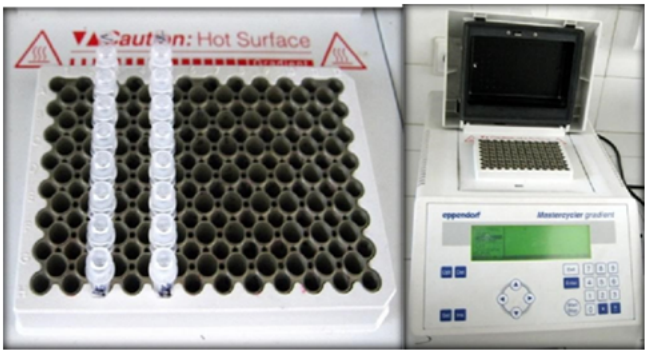


Figure 14 Transfer of PCR tubes in the thermocycler

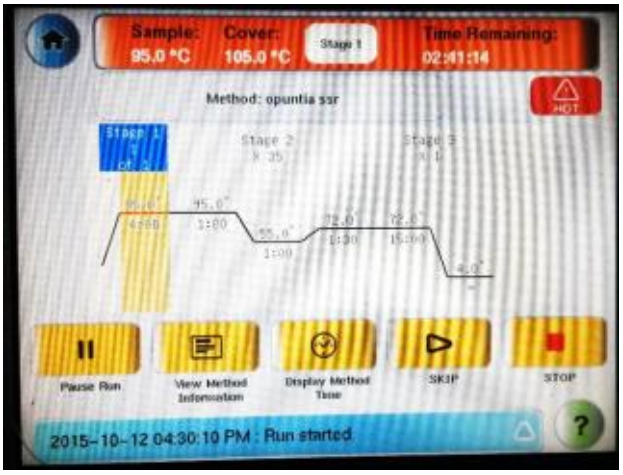


Figure 15. PCR program for *Opuntia*

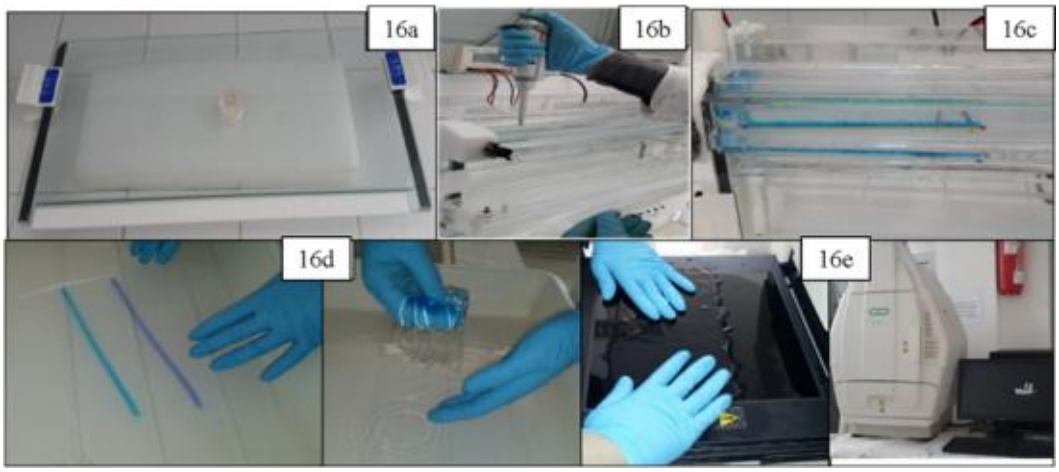


Figure 16. Polymerization, deposit, migration, staining and visualization of 6 % native Acrylamide gel.

Figure 17. 100bp size marker

Annex 4. Amplification profiles

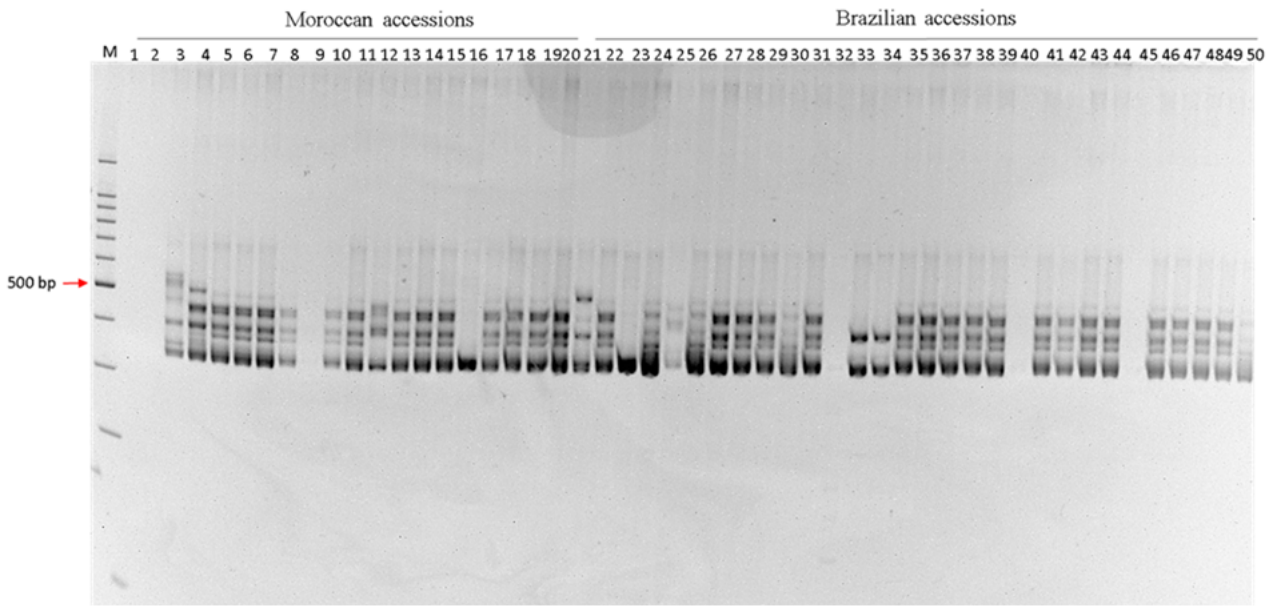


Figure 36. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 3 on 6% native polyacrylamide gel. Lane M, size marker of 100 bp.

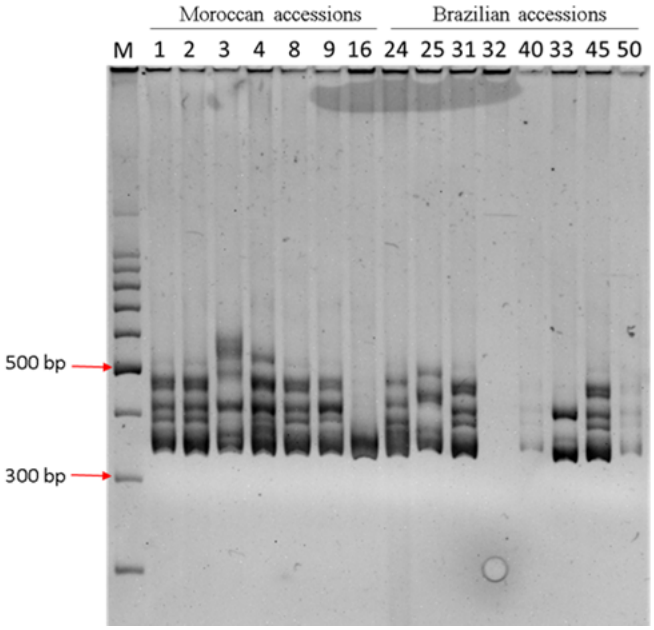


Figure 37. Amplification profile of 15 genotypes of *Opuntia* Mill. by the microsatellite primer Opuntia 3 on 6% native polyacrylamide gel; Lane M, size marker of 100 bp ; 1, 2, 9, 32, 40 and 45 were the missing bands in the previous profile with the same primer (figure below); Lanes 3, 4, 8, 16, 24, 25, 31, 33 and 50 are representatives of each different pattern.

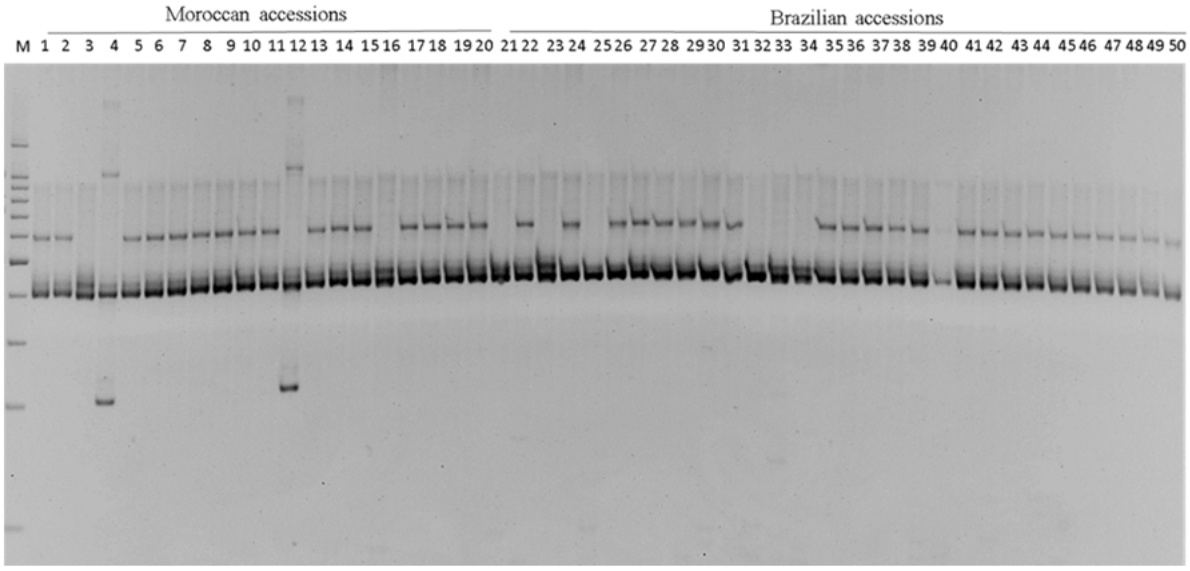


Figure 38. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 5 on 6% native polyacrylamide gel. Lane M, size marker of 100 bp.

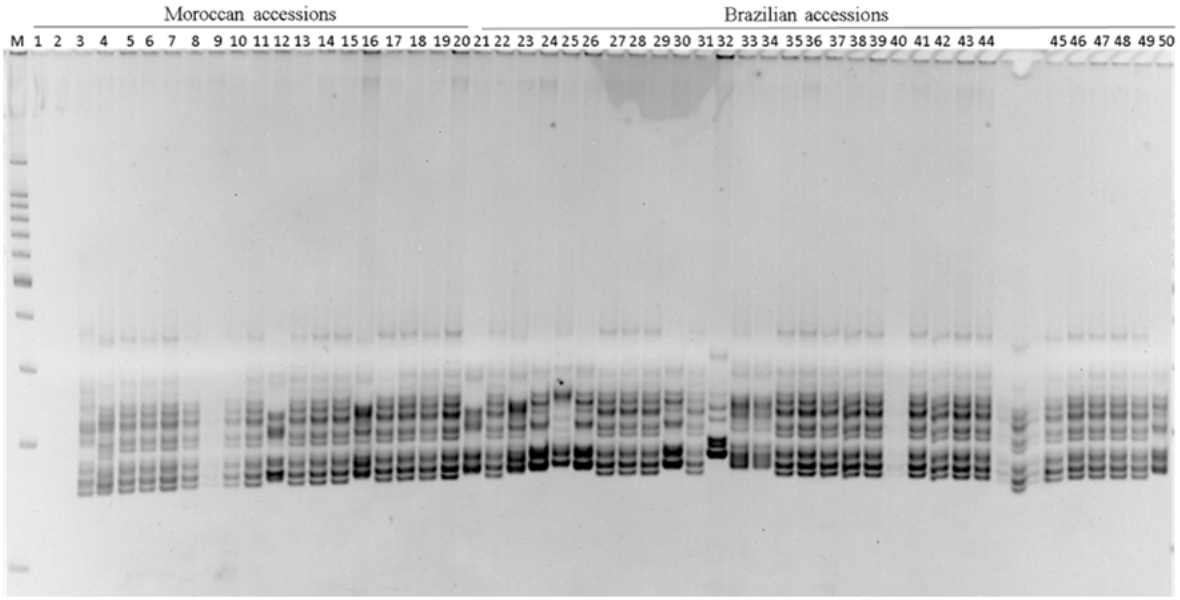


Figure 39. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 9 on 6% native polyacrylamide gel. Lane M, size marker of 100 bp.

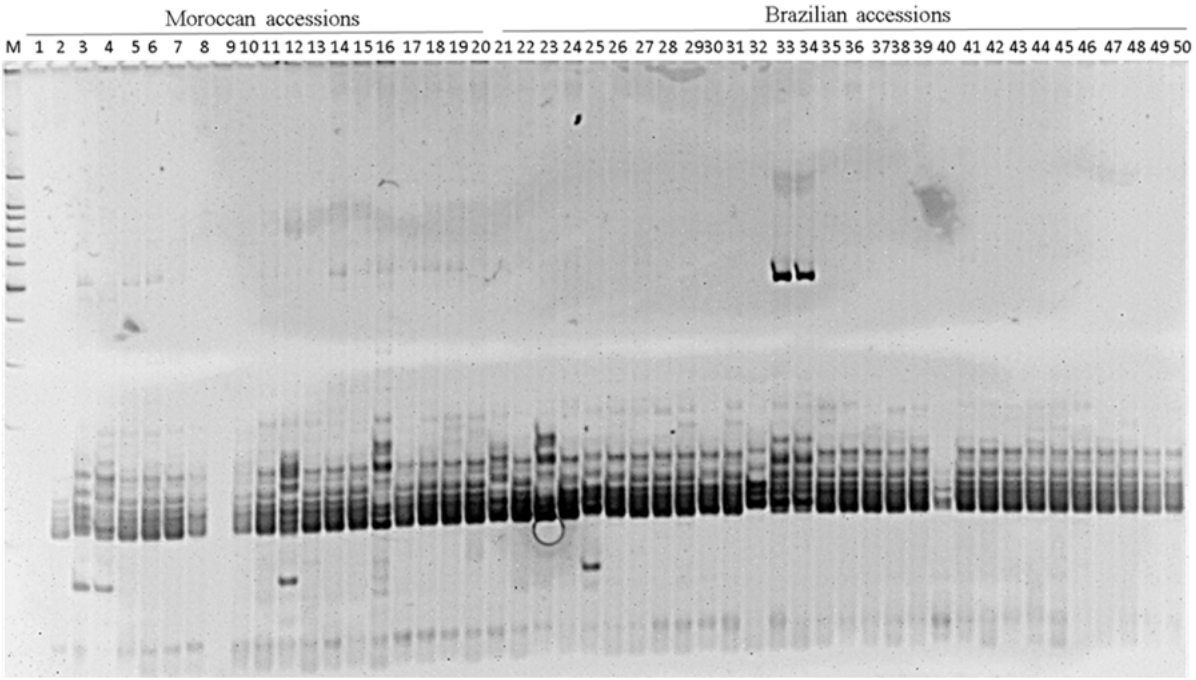


Figure 40. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 11 on 6% native polyacrylamide gel. Lane M, size marker of 100 bp.

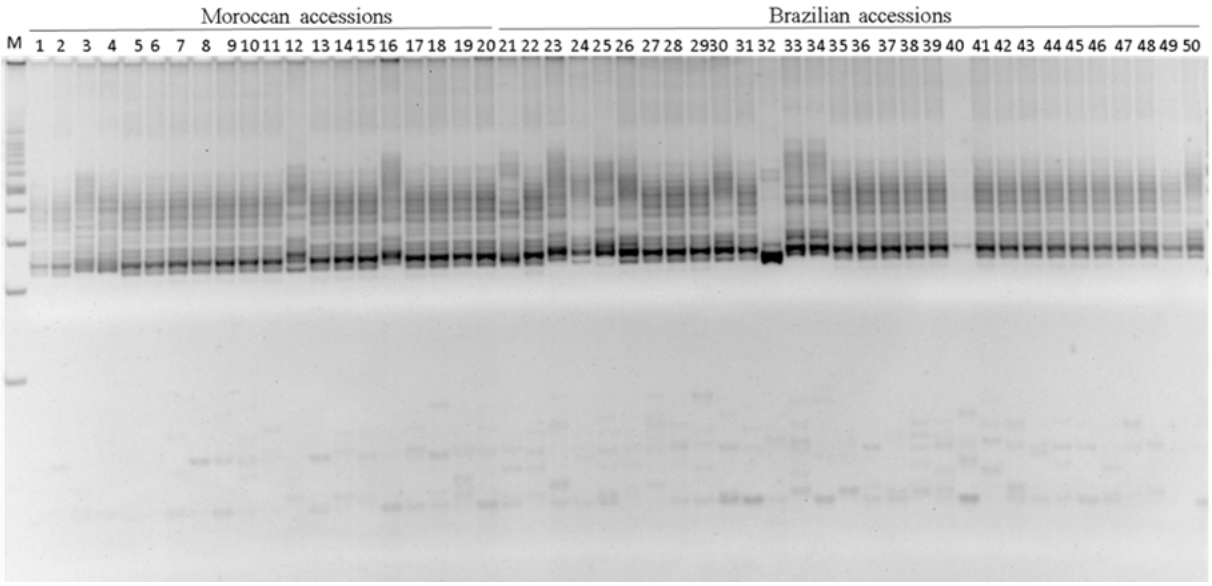


Figure 41. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 12 on 6% native polyacrylamide gel. Lane M, size marker of 100 bp.

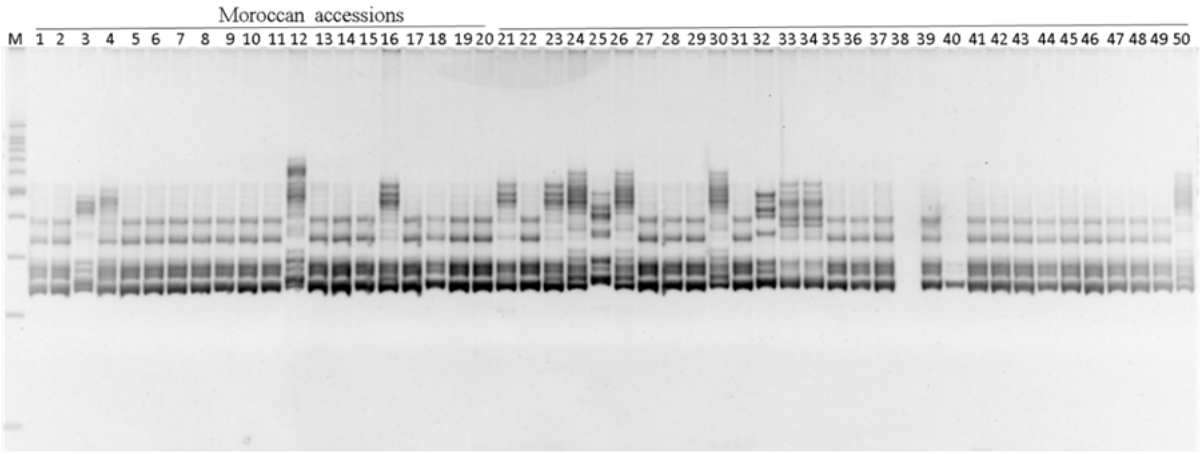


Figure 42. Amplification profile of 50 cactus genotypes by the microsatellite primer Opuntia 13 on 6% native polyacrylamide gel.Lane M, size marker of 100 bp.

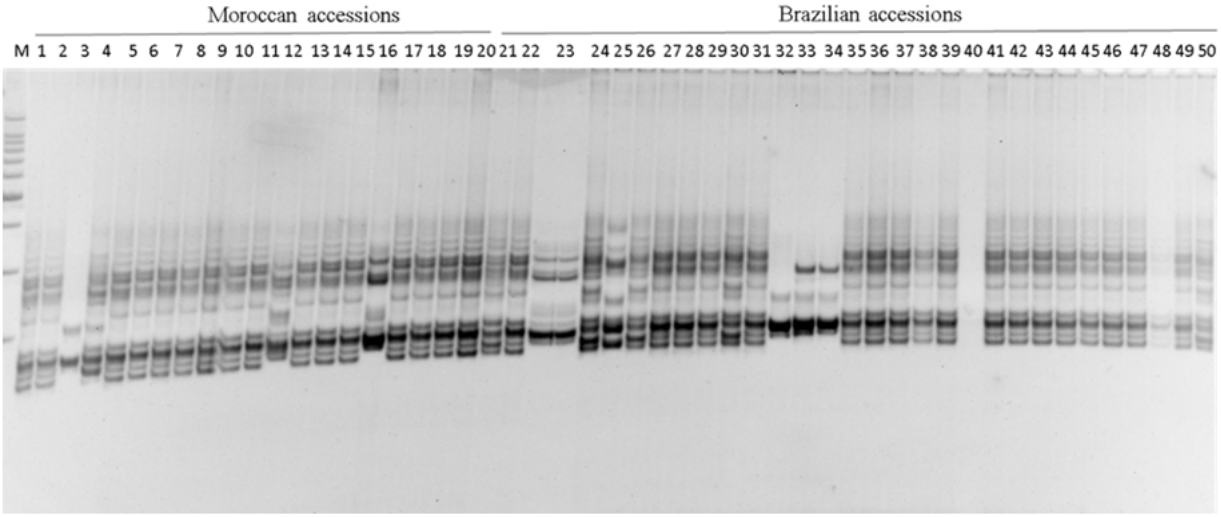


Figure 43. Amplification profile of 50 cactus genotypes by the microsatellite primer Ops9 on 6% native polyacrylamide gel.Lane M, size marker of 100 bp.

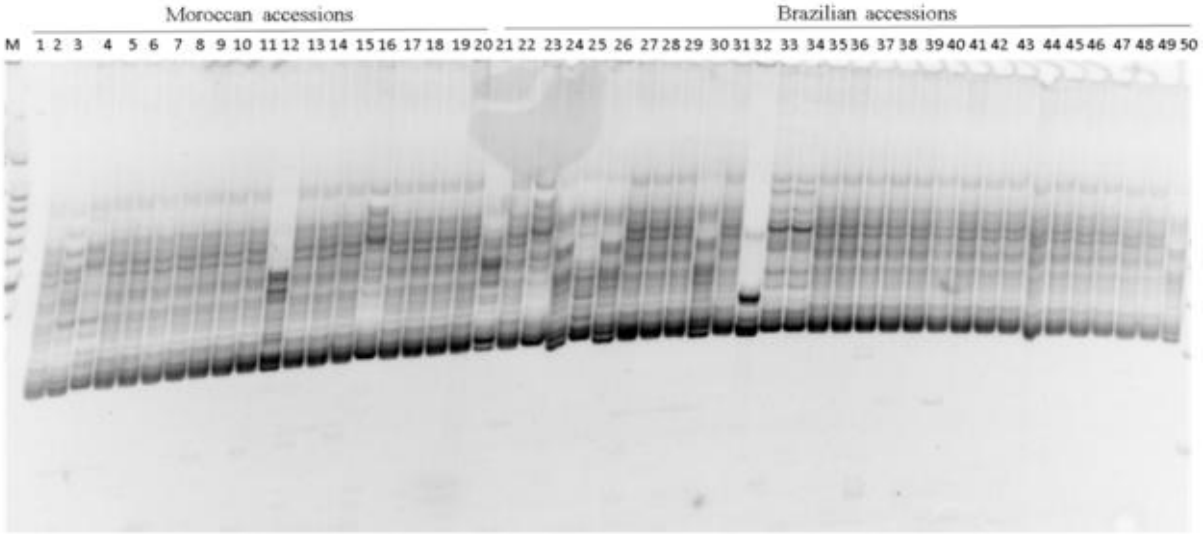


Figure 44. Amplification profile of 50 cactus genotypes by the microsatellite primer Ops24 on 6% native polyacrylamide gel.Lane M, size marker of 100 bp.