

**GENETIC DIVERGENCE AMONG MELON
(*Cucumis melo* L.) GROUPS FOR GROWTH,
YIELD AND QUALITY TRAITS**

Thesis

**Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of**

**MASTER OF SCIENCE
in
HORTICULTURE (VEGETABLE SCIENCE)
(Minor Subject: Plant Breeding and Genetics)**

By

**Dildar Singh
(L-2014-A-152-M)**

**Department of Vegetable Science
College of Agriculture
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LUDHIANA-141004**

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CERTIFICATE – I

This is to certify that the thesis entitled, “**Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits**” submitted for the degree of **Master of Science**, in the subject of **Vegetable Science** (Minor subject: **Plant Breeding and Genetics**) to the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Dildar Singh (L-2014-A-152-M)** under my supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. Sat Pal Sharma)

Major Advisor

Assistant Vegetable Botanist
Department of Vegetable Science
Punjab Agricultural University
Ludhiana – 141 004 (India).

CERTIFICATE – II

This is to certify that the thesis entitled, “**Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits**” submitted by **Dildar Singh (L-2014-A-152-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science**, in the subject of **Vegetable Science** (Minor subject: **Plant Breeding and Genetics**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

(Dr. Sat Pal Sharma)
Major Advisor

(Dr. A.D. Munshi)
External Examiner
Principal Scientist
Division of Vegetable Science
IARI, New Delhi

(Dr. A.S. Dhatt)
Head of the Department

(Dr. Neelam Grewal)
Dean, Post-Graduate Studies

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Place:

Date:

Dildar Singh

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Name of the student and Admission No. : Dildar Singh
(L-2014-A-152-M)

Major Subject : Vegetable Science

Minor Subject : Plant Breeding and Genetics

Name and Designation of Major Advisor : Dr. Sat Pal Sharma
Assistant Vegetable Botanist

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ABSTRACT

The present investigation entitled, "Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits" was conducted at Department of Vegetable Science and School of Agricultural Biotechnology, PAU, Ludhiana during the spring-summer seasons of 2015 and 2016. Seventy melon accessions belonging to five horticultural groups, *cantalupensis*, *reticulatus*, *inodorus*, *momordica* and *callosus* collected from diverse geographical locations such as, India, USA, Israel and Afghanistan were characterized for thirty-three morphological traits based on IPGRI descriptors. The genetic diversity based on quantitative morphological characters of genotypes was assessed by principle component analysis. The biplot of first two components grouped all the genotypes into four major clusters irrespective of their geographical region. Accessions belonging to *momordica* and *callosus* groups were grouped in Cluster I and Cluster II, respectively. The accessions having large fruits, particularly from *inodorus* group were clustered in cluster III. While, the remaining genotypes, mostly from *reticulatus* and *cantalupensis* groups were clustered in cluster IV. Genetic variability of muskmelon genotypes was determined by using 30 SSR markers. All of the 30 SSR markers amplified. A total of 67 alleles were detected by 24 polymorphic SSR loci and maximum 4 alleles were observed with an average of 2.8 alleles per primer pair. The markers CMAG59, CMTCN38, CMMS3-1 and EMS178 were highly informative as these revealed maximum number of alleles (4), PIC values (0.550, 0.600, 0.442) and genetic diversity (0.56, 0.61, 0.45, 0.54), respectively. Seven SSR markers revealed specific/unique alleles and identified twenty-three genotypes. Across the genotypes, maximum number of alleles (58) was detected in IC-274034 and the percentage of polymorphic marker was maximum (86.96) in PAUS-19. In present study, average observed heterozygosity (53%) within a melon germplasm was higher as compared to the average expected heterozygosity (48%). The dendrogram illustrating genetic relationship, grouped the accessions belonging to *reticulatus* group in cluster-I and accessions belonging to *cantalupensis*, *callosus*, *momordica* and *inodorus* were grouped in cluster-II and cluster-III. The maximum genetic similarity coefficient was 0.83 in cluster-I. Similarly, it ranged from 0.88 to 0.91 in cluster-II and III. Intermixing of accessions of five horticultural groups within clusters indicates that classification of muskmelon groups on morphological does not necessarily correspond to the genetic relationship shown by molecular analysis. Hence, out crossing behavior may be the cause of genetic introgression in melons.

Keywords: Cantaloupe, heterozygosity, melon, morphological characterization, molecular markers

Signature of Major Advisor

Signature of the Student

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ਮੌਜੂਦਾ ਅਧਿਐਨ “ਵਿਕਾਸ, ਝਾੜ ਅਤੇ ਗੁਣਵਤਾ ਗੁਣਾਂ ਦੇ ਲਿਹਾਜ਼ ਨਾਲ ਖਰਬੂਜੇ (ਕੁਕੁਮਿਸ ਮੀਲੋ ਐਲ.) ਸ਼੍ਰੇਣੀਆਂ ਵਿੱਚ ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਭਿੰਨਤਾ” ਸਿਰਲੇਖ ਅਧੀਨ ਪੀ.ਏ.ਯੂ. ਲੁਧਿਆਣਾ ਦੇ ਸਬਜ਼ੀ ਵਿਗਿਆਨ ਵਿਭਾਗ ਅਤੇ ਸਕੂਲ ਆਫ ਐਗਰੀਕਲਚਰਲ ਬਾਇਓਟੈਕਨੋਲੋਜੀ ਵਿਖੇ ਸੰਨ 2015 ਅਤੇ 2016 ਦੀ ਬਸੰਤ-ਗਰਮੀ ਰੁੱਤੇ ਕੀਤਾ ਗਿਆ। ਵੱਖੋ-ਵੱਖਰੇ ਭੂਗੋਲਿਕ ਖੇਤਰਾਂ ਜਿਵੇਂ ਕਿ ਭਾਰਤ, ਯੂ.ਐਸ.ਏ., ਇਜ਼ਰਾਈਲ ਅਤੇ ਅਫਗਾਨਿਸਤਾਨ ਤੋਂ ਪੰਜ ਬਾਗਬਾਨੀ ਸ਼੍ਰੇਣੀ ਕੈਂਟਾਲੂਪੈਨਸਿਸ, ਰੈਟੀਕੁਲੇਟਿਸ, ਰੈਟੀਕੁਲੇਟਿਸ, ਇਨੋਡੋਰਸ, ਮਮੋਰਡੀਕਾ ਅਤੇ ਕੇਲੋਸਿਸ ਨਾਲ ਸਬੰਧਤ ਖਰਬੂਜੇ ਦੇ 70 ਅਸੈਸ਼ਨ ਲਏ ਗਏ ਅਤੇ IPGRI ਵਰਨਣਕਰਤਾਵਾਂ ਦੇ ਅਧਾਰ ਤੇ 33 ਆਕ੍ਰਿਤਕ ਗੁਣਾਂ ਲਈ ਇਹਨਾਂ ਦਾ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਸਿਧਾਂਤਕ ਮੌਲਿਕ ਮੁਲਾਂਕਣ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਜੀਨੋਟਾਈਪਾਂ ਦੇ ਗੁਣਾਤਮਕ ਆਕ੍ਰਿਤਕ ਗੁਣਾਂ ਦੇ ਅਧਾਰ ਤੇ ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਭਿੰਨਤਾ ਦਾ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਪਹਿਲੇ ਦੋ ਅੰਸ਼ਾਂ ਦੇ ਬਾਈਪਲਾਟ ਨੇ ਇਹਨਾਂ ਜੀਨੋਟਾਈਪਾਂ ਨੂੰ ਚਾਰ ਸ਼੍ਰੇਣੀਆਂ ਵਿੱਚ ਸ਼੍ਰੇਣੀਗਤ ਕੀਤਾ ਅਤੇ ਇਸ ਪ੍ਰਕਿਰਿਆ ਉਪਰ ਜੀਨੋਟਾਈਪਾਂ ਦੇ ਭੂਗੋਲਿਕ ਖੇਤਰਾਂ ਦਾ ਕੋਈ ਵੀ ਪ੍ਰਭਾਵ ਨਹੀਂ ਵੇਖਿਆ ਗਿਆ। ਮਮੋਰਡੀਕਾ ਅਤੇ ਕੇਲੋਸਿਸ ਸ਼੍ਰੇਣੀਆਂ ਨਾਲ ਸਬੰਧਤ ਅਸੈਸ਼ਨਾਂ ਨੂੰ ਕ੍ਰਮਵਾਰ ਸ਼੍ਰੇਣੀ I ਅਤੇ ਸ਼੍ਰੇਣੀ II ਵਿੱਚ ਸ਼੍ਰੇਣੀਗਤ ਕੀਤਾ ਗਿਆ। ਵੱਡੇ ਫਲਾਂ ਵਾਲੇ ਖਾਸ ਤੌਰ ਤੇ ਇਨੋਡੋਰਸ ਸ਼੍ਰੇਣੀ ਨਾਲ ਸਬੰਧਤ ਅਸੈਸ਼ਨਾਂ ਨੂੰ ਸ਼੍ਰੇਣੀ III ਵਿੱਚ ਰੱਖਿਆ ਗਿਆ। ਜਦੋਂਕਿ ਬਾਕੀ ਬਚਦੇ ਜੀਨੋਟਾਈਪਾਂ, ਜਿਹਨਾਂ ਵਿੱਚੋਂ ਜ਼ਿਆਦਾਤਰ ਰੈਟੀਕੁਲੇਟਿਸ ਅਤੇ ਕੈਂਟਾਲੂਪੈਨਸਿਸ ਸ਼੍ਰੇਣੀਆਂ ਨਾਲ ਸਬੰਧਤ ਸਨ, ਨੂੰ ਸ਼੍ਰੇਣੀ IV ਵਿੱਚ ਰੱਖਿਆ ਗਿਆ। 30 ਐਸ.ਐਸ.ਆਰ. ਮਾਰਕਰਾਂ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਖਰਬੂਜੇ ਦੇ ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਭਿੰਨਤਾ ਦਾ ਪਤਾ ਲਗਾਇਆ ਗਿਆ। ਸਾਰੇ ਦੇ ਸਾਰੇ 30 ਐਸ.ਐਸ.ਆਰ. ਮਾਰਕਰਾਂ ਨੂੰ ਐਂਪਲੀਫਾਈ ਕੀਤਾ ਗਿਆ। 24 ਬਹੁਰੂਪਕ ਐਸ.ਐਸ.ਆਰ. ਲੋਕਾਇ ਦੁਆਰਾ ਕੁੱਲ 67 ਐਲੀਲਸ ਦਾ ਪਤਾ ਚੱਲਿਆ ਅਤੇ ਪ੍ਰਤੀ ਪ੍ਰਾਈਮਰ ਜੋੜੇ ਔਸਤਨ 2.8 ਐਲੀਲਸ ਨਾਲ ਵੱਧ ਤੋਂ ਵੱਧ 4 ਐਲੀਲ ਦਾ ਪਤਾ ਚੱਲਿਆ। CMAG59, CMTCN38, CMMS3-1 ਅਤੇ EMS178 ਮਾਰਕਰ ਸਭ ਤੋਂ ਵਧੇਰੇ ਅਸਰਦਾਰ ਸਨ ਕਿਉਂਕਿ ਇਹਨਾਂ ਨੇ ਸਭ ਤੋਂ ਵਧੇਰੇ ਐਲੀਲਸ (4), ਪੀ.ਆਈ.ਸੀ. ਮਿਕਦਾਰਾਂ (0.550, 0.600 ਅਤੇ 0.442) ਅਤੇ ਅਨੁਵਾਂਸ਼ਿਕੀ ਵਿਭਿੰਨਤਾ (0.56, 0.61, 0.45, 0.54) ਵਿਖਾਈ। ਸਤ ਐਸ.ਐਸ.ਆਰ. ਮਾਰਕਰਾਂ ਨੇ ਖਾਸ/ਵਿਲੱਖਣ ਐਲੀਲਸ ਵਿਖਾਏ ਅਤੇ 25 ਜੀਨੋਟਾਈਪਾਂ ਦੀ ਪਹਿਚਾਣ ਕੀਤੀ। ਜੀਨੋਟਾਈਪਾਂ ਵਿੱਚੋਂ, IC-274034 ਜੀਨੋਟਾਈਪ ਵਿੱਚ ਸਭ ਤੋਂ ਵਧੇਰੇ ਐਲੀਲਸ (58) ਦਾ ਪਤਾ ਚੱਲਿਆ ਅਤੇ PAUS-19 ਵਿੱਚ ਬਹੁਰੂਪਕ ਮਾਰਕਰ ਦੀ ਪ੍ਰਤੀਸ਼ਤਤਾ ਸਭ ਤੋਂ ਵਧੇਰੇ (86.96%) ਪਾਈ ਗਈ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ, ਖਰਬੂਜੇ ਦੇ ਜ਼ਰਮਪਲਾਜ਼ਮ ਵਿੱਚ ਦਰਜ ਕੀਤੀ ਗਈ ਔਸਤਨ ਹੈਟੇਰੋਜੀਗੋਸਿਟੀ (53%), ਅਪੇਕਸਤ ਹੈਟੇਰੋਜੀਗੋਸਿਟੀ (48%) ਤੋਂ ਵਧੇਰੇ ਸੀ। ਅਨੁਵਾਂਸ਼ਿਕੀ ਸਬੰਧ ਦਰਸਾਉਣ ਵਾਲੇ ਡੈਂਡੋਗ੍ਰਾਮ ਨੇ ਰੈਟੀਕੁਲੇਟਿਸ ਨਾਲ ਸਬੰਧਤ ਅਸੈਸ਼ਨਾਂ ਨੂੰ ਸ਼੍ਰੇਣੀ-I ਅਤੇ ਕੈਂਟਾਲੂਪੈਨਸਿਸ, callosus, ਮਮੋਰਡੀਕਾ ਅਤੇ ਇਨੋਡੋਰਸ ਨਾਲ ਸਬੰਧਤ ਅਸੈਸ਼ਨਾਂ ਨੂੰ ਸ਼੍ਰੇਣੀ-II ਅਤੇ ਸ਼੍ਰੇਣੀ-III ਵਿੱਚ ਸ਼੍ਰੇਣੀਗਤ ਕੀਤਾ। ਸ਼੍ਰੇਣੀ-I ਵਿੱਚ ਅਨੁਵਾਂਸ਼ਿਕੀ ਸਮਾਨਤਾ ਗੁਣਾਂਕ ਸਭ ਤੋਂ ਵਧੇਰੇ (0.83) ਸੀ। ਇਸੇ ਤਰ੍ਹਾਂ, ਸ਼੍ਰੇਣੀ-II ਅਤੇ ਸ਼੍ਰੇਣੀ-III ਵਿੱਚ ਅਨੁਵਾਂਸ਼ਿਕੀ ਸਮਾਨਤਾ ਗੁਣਾਂਕ 0.88 ਤੋਂ 0.91 ਤੱਕ ਸੀ। ਸਮੂਹਾਂ ਵਿੱਚ ਪੰਜ ਬਾਗਬਾਨੀ ਸ਼੍ਰੇਣੀਆਂ ਦੇ ਅਸੈਸ਼ਨਾਂ ਦੀ ਇੰਟਰਮਿਕਸਿੰਗ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਹ ਜ਼ਰੂਰੀ ਨਹੀਂ ਹੈ ਕਿ ਆਕ੍ਰਿਤਕ ਗੁਣਾਂ ਦੇ ਲਿਹਾਜ਼ ਨਾਲ ਖਰਬੂਜੇ ਦੇ ਸਮੂਹਾਂ ਦਾ ਵਰਗੀਕਰਣ, ਆਂਣਵਿਕ ਮੁਲਾਂਕਣ ਦੁਆਰਾ ਦਰਸਾਏ ਗਏ ਅਨੁਵਾਂਸ਼ਿਕੀ ਸਬੰਧਾਂ ਦੀ ਪੁਸ਼ਟੀ ਕਰੇ। ਇਸ ਲਈ, ਆਉਟ-ਕ੍ਰਾਸਿੰਗ ਵਤੀਰਾ ਖਰਬੂਜੇ ਵਿੱਚ ਅਨੁਵਾਂਸ਼ਿਕੀ ਅਨੁਕ੍ਰਮਣ ਦਾ ਕਾਰਨ ਹੋ ਸਕਦਾ ਹੈ।

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CHAPTER I

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is a diploid ($2n=24$) morphologically diverse cucurbitaceous crop of commercial importance relished for its sweet taste. Taxonomic evidence available indicates that *Cucumis melo* L. is an extremely polymorphic species possessing wide range growth habits, flowering behavior and fruit characters (Robinson and Decker-Walters 1997). The high level of morphological variation has led to intra-specific classification up to 16 horticultural groups within this species (Pitrat *et al* 2000). Thus, there is large intra-specific phenotypic diversity available in muskmelon which is expected to translate to genotypic diversity at genomic DNA level.

The world production of melon was estimated at 29.5 million tons from an area of 1.2 million ha with an average yield of 24.9 tons ha⁻¹ (Anonymous 2014). The leading muskmelon producing countries of world are China, Iran, Turkey, Egypt, India, U.S.A and Spain. In India, during 2015 melons were produced on an area of 47 thousand ha with production of 878 thousand MT with average productivity of 20 tons ha⁻¹ (Anonymous 2015). Melon is extensively cultivated in hot and dry areas of Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar and Karnataka. In Punjab, muskmelon was cultivated on an area of 4.9 thousand ha with production of 86.1 thousand tons with average yield of 17.4 tons ha⁻¹ (Anonymous 2015). Thus, muskmelon is an important commercial crop in the region which calls for precise characterization of locally adapted germplasm for the development high yielding cultivars/ hybrids.

Although the center of origin of muskmelon is not yet clear, but on the basis of availability of several species of wild *Cucumis*, Africa is considered to be the region of domestication of muskmelon (Pitrat 2008; Whitaker and Davis 1962). However, recent taxonomic and molecular data support an Asian origin (Schaefer *et al* 2009). The wild progenitor of melon has been located in India and its closest relative (*Cucumis picrocarpus* F. von Mueller) has been reported in Australia (Sebastian *et al* 2010). The primary center of cultivated melon diversity is located in South-west and Central Asia, including Syria, Turkey, Afghanistan, Iran, Central and North India and Transcaucasia, Tajikistan, Uzbekistan and Turkmenistan, and secondary centers of muskmelon diversity are found in the Korea, China and Iberian Peninsula (Esquinas-Alcazar and Gulick 1983). Melon species are distributed around the world especially in Africa, tropical America and South-east Asia.

Foremost, Linne described the species of *C. melo* L. in 1753 (Stepansky *et al* 1999). Later, melons were characterized on basis of their morphological traits such as leaf shape, vine growth, flower types, habitat and types of fruit (Stepansky *et al* 1999). This led to classification of melon species into ten different varieties which became basis of all ensuing studies (Naudin 1859). Further, Munger and Robinson (1991) clarified Naudin's taxonomic

classification by dividing *C. melo* into 7 groups which included trinomial names *C. melo* var. *inodorus* Naud. (Honeydew or casaba, winter melons), *C. melo* var. *cantalupensis* Naud. (muskmelon or cantaloupe), *C. melo* var. *momordica* (Snap melon or phoot), *C. melo* var. *chito* (Mango melon) and *dudaim* Naud. (Queen's pocket melon), *C. melo* var. *agrestis* Naud. (Wild melon), *C. melo* var. *conomon* Mak. (Chinese white cucumber, pickling melon) and *C. melo* var. *flexuosus* Naud. (Snake melon) (Stepansky *et al* 1999).

Among the seven groups of melon, *cantalupensis*, *reticulatus* and *inodorus* have great economic importance, substantially due to their pulp savour (McCreight *et al* 1993) besides occurrence of enormous fruit morphological variations that allow for sub-grouping of these two into distinct market classes, such as: galia, cantaloupe, charentais (*cantalupensis*) and piel de sapo, yellow canary and honeydew (*inodorus*). Wild melon and *momordica* genotypes are a good reservoir of resistance genes for various biotic and abiotic stresses (Fergany *et al* 2011; Dhillon *et al* 2007 and Garcia *et al* 1998).

Before the evolution of molecular techniques, visual characteristics were used to differentiate between various muskmelon cultivar groups/species. Unfortunately, morphological characteristics such as leaf, flower, and fruit traits are influenced by the environment and growth stage (Bernet *et al* 2003). But, phenotypic characteristics are integral to assess genetic resources which are required for initial diversity studies and also to distinguish traditional genotypes (Konopka and Hanson 1985). Although, molecular markers have vantage over all other kinds, where these exhibit genetic distinction on a more circumstantial level without any environment impact and provide quick results (Garcia *et al* 2004 and Goncalves *et al* 2008).

Molecular markers are fragments of the chromosomes which do not compulsory encode any traits and are not factitious by environment but these are inherited in a Mendelian fashion. The most commonly used techniques in genetic diversity studies are, diversity array technology (Kilian *et al* 2005), single nucleotide polymorphisms (Chen and Sullivan 2003), amplified fragment length polymorphism (Vos *et al* 1995), inter-simple sequence repeats (Zietkiewicz *et al* 1994), randomly amplified polymorphic DNA (Williams *et al* 1990) or arbitrarily primed PCR (Welsh and McClelland 1990), simple sequence repeat markers (Tautz 1989), restriction fragment length polymorphism (RFLP) (Botstein *et al* 1980) and other high throughput platforms.

The main goal of present study was to estimate the authentic extent of molecular variation in muskmelon germplasm and to arbitrate whether the apparent diversity in the botanical and horticultural traits is reflected at DNA level. Genetic diversity at molecular level was assessed with help of simple sequence repeat markers. Simple sequence repeats (SSR) are tandem repeated units of one to six nucleotides which are also known as microsatellites (Reddy *et al* 2002). Simple sequence repeat markers have many vantages over

all other molecular markers as these allow the identification of numerous alleles at a individual locus. Abundance and distribution throughout the genome, a high degree of polymorphism and specificity, co-dominant nature, a relatively large number of alleles per loci, PCR based, high reproducibility within and between labs, and good transportability across species within the same genus are the desirable characteristics of SSR markers (Jones *et al* 1997). Several studies have been published utilizing SSR markers in muskmelon over genetic linkage mapping (Park *et al* 2009 and Gonzalo *et al* 2005) and genetic the diversity study (Danin-Poleg *et al* 2001; Staub *et al* 2004; Fergany *et al* 2011 and Ning *et al* 2014).

Genetic diversity is essential for any breeding program. Not only it is necessary for the incorporation of improved horticultural characteristics such as sweetness, flavour and increased yield, but it is also needed for the integration of disease and pest resistance. Given the economic importance of muskmelon, several diversity studies have been performed on this crop within India (Fergany *et al* 2011; Dhillon *et al* 2007 and Malik *et al* 2014) and throughout the world (Laun *et al* 2008; Staub *et al* 2004; Staub *et al* 2000 and Stepansky *et al* 1999). However, majority of these explorations only look at diversity of local germplasm from a few horticultural groups of the species, *Cucumis melo* L.

Keeping above points in mind, this research was done to study the significance of genetic divergence through seventy melon accessions of various horticultural groups, viz. *cantalupensis*, *reticulatus*, *inodorus*, *momordica* and *callosus* which were obtained from muskmelon germplasm repository, Department of Vegetable Science, PAU, Ludhiana. Diversity assessment of these melon accessions which were collected from various agro-ecological regions of the world like Afghanistan, USA, Israel and India was done with thirty-three morphological descriptors and 30 simple sequence repeat markers. The overall aim of the study was to elucidate genetic diversity of the melon germplasm for future utility for crop improvement and for conservation with following specific objectives:-

- i) To characterize muskmelon genotypes for growth, yield and quality under field conditions.
- ii) Assessment of genetic diversity by using SSR markers.
- iii) To investigate the genetic relatedness among melon genotypes collected from various geographic locations.

CHAPTER II

REVIEW OF LITERATURE

Genetic diversity is a repository for novel and improved traits and genes which will be instrumental for breeders to select desirable parents of hybrid for new cultivar production besides maintenance of natural population polymorphism. As the performance of hybrids seems to be related to the genetic divergence of parental lines, information on the genetic similarity between genotypes may ease the prophecy of crosses which will produce F₁ with a higher interpretation (Sekhon and Gupta 1995; Luan *et al* 2010). However, an intensive inbreeding practice also reduces the genetic variability and is responsible for the ineffective progression of the new combinations (Ramanatha and Hodgkin 2002). Therefore, there is an ardent need to determine the genetic diversity of the crop accessions and perform phytochemical mining of a germplasm repository using appropriate markers so as to identify the potential accessions to be utilized for future breeding programs. The literature relevant to the present investigation has been reviewed under the following heads:

2.1 Morphological characterization of muskmelon accessions

2.1.1 Vegetative, yield and its component traits

2.1.2 Fruit quality traits

2.2 Molecular characterization of muskmelon accessions

2.1 Morphological characterization of muskmelon accessions

Analysis of plant genetic diversity by using accurate morphological evaluation of regional collections is useful for the germplasm curators as it helps to explain cultivars by geographical regions and which will provide solid historical reference data for the future genetic investigations aimed at seizure genetic erosion, exploration potential and site preservation precedence.

2.1.1 Vegetative, yield and its component traits

Melons exhibit tremendous variations in their leaf morphology, vine length, branching pattern, and growth habits. The fruit characters viz., fruit size, shape, fruit cavity, skin, rind and flesh colour, and presence or absence of netting and sutures also vary among different cultivars. Variation among the closest of muskmelon provides chance to ameliorate gene pool of muskmelon with unique alleles which eventually could improve quality, productivity and adaptation or lessen the risk of genetic vulnerability (Stepansky *et al* 1999).

Malik *et al* (2014) reported genetic characterization of ninety-six genotypes of melon from three groups in two subspecies (*C. melo* subsp. *agrestis* Momordica group, and *C. melo* subsp. *melo* Cantalupensis group and *Reticulatus* group). Significant differences were reported among the eighty-eight Indian landraces and eight USA *reticulatus* group reference cultivars for plant and fruit traits. Number of primary branches per plant in Indian germplasm

ranged from 2.4 to 6.8, while in the USA germplasm the range was 2.2–3.1 and *momordica* group had highest number of primary branches. Similarly, in Indian landraces the days from sowing to marketable fruit maturity ranged from 78.5 to 89.4, while in USA reference cultivars the range was from 82.7 to 86.1 days. The *cantalupensis* Group was significantly earlier than the two other Indian groups and the USA reference cultivars. Fruit weight of USA cultivars ranged from 0.94 to 1.1 kg. While in *momordica* group it ranged from 0.70 to 1.41 and 0.81 to 0.87 kg and *cantalupensis* that ranged from 0.46 to 1.47 kg. Number of fruit per plant did not significantly differ among the Indian accessions; however these were higher than the USA reference cultivars. Number of fruits per plant ranged from 1.6 to 3.0 and 2.0 to 2.5 among the *reticulatus* and *momordica* accessions, respectively. Fruits per plant among Indian *reticulatus* landraces ranged from 1.6 to 3.0 as compared to 1.5 to 2.0 in USA genotypes. Rind thickness among Indian accessions ranged from 1.5 to 3.1 mm and 3.2 to 3.7 mm for USA cultivars which can be positively correlated with storage life. Also, USA cultivars had more shelf life than *reticulatus*, *cantalupensis* and *momordica* group, respectively.

Reddy *et al* (2013) evaluated thirty five genotypes and observed that phenotypic coefficient of variation was highest for fruit yield (28.3%) followed by fruit cavity length (28.2%) and rind thickness (26.4%), while lowest for days to appearance of first pistillate flower (7.8%) followed by days to last fruit harvest (8.9%) and days to first fruit harvest (9.6%). Similarly, genotypic coefficient of variation was highest for fruit cavity length (26.7%) followed by average fruit weight (22.8%) and rind thickness (22.1%), while lowest for days to last fruit harvest (3.9%) followed by days to first fruit harvest (3.9%) and days to appearance of first pistillate flower (5.8%). It signifies that there is greater potential for favorable advance in selection in these attributes.

Koli *et al* (2013) assessed the phenotypic diversity among thirty three landraces of *C. melo* and these were clustered into three clusters based on principle component analysis. Accessions in I cluster, showed large variation in number of branches on 60th day (12 to 20), vine length (101 cm to 220 cm), length of tendril (24 cm to 51 cm), shape of fruit (ovate to oblate), length of fruit (15 cm to 43 cm), number of nodes on 60th day (25 to 43), width of fruit (7 cm to 12 cm) and these collections were basically from the group *acidulous*. Genotypes having longer leaf length and vine length, light green to yellow fruit color, large fruit length, soft rind, high fruit weight, light orange flesh color, intermediate and grainy flesh, famished shelf life (1 month), with light brown seeds, short growth duration (within 90 days) were grouped in the II cluster, comprised of group *momordica*. III Cluster having landraces which had almost similar characters were of group *momordica*. There existed single unclustered landrace “CmKc-9” having higher number of nodes on 60th day, longer vein length, small size of leaf, heavy fruit weight, high fruit breadth and with the medium growth durations (less than 120 days), which belongs to *acidulous* group.

In a correlation study, Malik *et al* (2012) reported that no. of fruits per vine exhibits a significantly negative correlation with the seed cavity length, fruit weight, length of fruit, thickness of rind, and the ascorbic acid content, while fruit weight showed positive correlation with the length of fruit, length of seed cavity and also with dry matter content. Positive and significant correlation of days from sowing to marketable maturity was reported with days from sowing to last fruit harvest and also with fruit weight. Similarly, node at which 1st hermaphrodite flower appear displayed a significantly positive correlation with the fruit weight and with titrable acidity.

Manohar *et al* (2012) studied phenotypic divergence in 44 melon accessions, from two important snapmelon varieties, *Cucumis melo* var. *momordica* and *Cucumis melo* var. *acidulus*. In scatter diagram, *acidulus* and *momordica* accessions grouped into different clusters. The maximum and minimum vine length and the nodes per plant were same in both the genotypes; except in one accession, CMC 108 (*momordica* group) in which the highest vine length was 291.1 cm. Mean number of branches in group *acidulus* (6.63 ± 1.65) was higher as compared to *momordica* group (5.90 ± 2.15). Length of fruit varied widely, from 12.1- 40.7 cm and 12.1- 26.1 cm in *acidulus* and *momordica*, respectively. The mean fruit weight (g) in *acidulus* group (1109.36 ± 362.72) was more as compared to *momordica* group (883.40 ± 5.08). The fruits of *acidulus* group were oblate in the shape, but of group *momordica* were elongated with exception in genotypes CMC 28 and CMC 104 which in these were oblate in shape. Flesh of *momordica* fruits was cream to orange in colour, soft, but that of *acidulus* group was crispy and with off white color. However, rind of the *momordica* fruits was soft and cracks at the maturity stage and had a less shelf-life, while that of *acidulus* accessions were hard and had longer shelf-life.

Nasrabadi *et al* (2012) examined the characteristics of eleven Iranian melon cultivars for morphological traits and results showed greater diversity. Correlation analysis the between traits reported a significantly positive relation between length of fruit and with content of sugar. Similarly, fruit weight was positively correlated with diameter of fruit, length of fruit, thickness of flesh and skin and with the sugar content. Negative correlation was reported between length of fruit with diameter of fruit, thickness of flesh and skin. The minimum and maximum length of fruit was 22.6 and 48.1 cm in “Zin Abade” and “Jafarabadi” cultivars, respectively. Similarly, minimum and maximum diameters of fruit were reported to be 15.2 and 19.6 cm in “Jabari” and “Zemestani” cultivars, respectively, but in another experiment, ranges were reported from 10 to 34.3 cm for length of fruit and 8.8 to 16.3 for diameter of fruit (Naroui *et al* 2010). Maximum fruit weight was recorded 4.9 kg in “Jafarabadi” and minimum 2.30 kg in cultivar “Abade”.

Roy *et al* (2012) reported greater diversity among 37 wild melon accessions. Mean weight of fruit was ranged from 5.3-155.8 g. There existed no association between numbers of fruit /vine and the fruit weight, but two genotypes WM 62 and WM 4 showed highest total fruit weight (155.8 g and 103.5 g) and highest fruits number per vine 34.9 and 34.4, respectively. Plant growth habit was intermediate (60%) and prostrate (40%). Dense stem pubescence, intermediate and low observed in 41%, 21%, and 38% of genotypes, respectively. Similarly, dense leaf pubescence intermediate and low found in 46, 19 and 35 % of the accessions, respectively. No correlation was existed between leaf pubescence and stem; about 50% of the genotypes with low stem pubescence also had the low leaf pubescence. Long (150 cm), medium (100 to150 cm) and short (less than 100 cm) vines were found in the 13, 38 and 49% of the cultivars, respectively. Primary branches per plant ranged from 2.3 to 4.5. In whole germplasm, four fruit shapes were reported. Fruit of half of the genotypes produced the oblate type fruits, others produce ovate (8 genotypes), elliptical (10 genotypes), or round (2 genotypes) fruits.

Fergany *et al* (2011) reported greater variation was present in melon landraces of southern India. Number of primary branches per vine varied from 2 to 7.4, days to marketable maturity varied from 50.6 to 77.2, fruit number per vine ranged from 2.8 to 9, fruit weight varied from 0.26 to 1.77 kg and average yield/ plant ranged from 0.87 and 5.33 kg /vine. Majority of genotypes belonged either to oblate (40%) or elongated (42%) category; pyriform and elliptical types were presented by 6 and 10 % of the genotypes, respectively. The majority of genotypes (60%) had yellow primary skin color, while other genotypes were orange (14%), light green in color (18%) or 4% had green color. Similarly, majority of the genotypes had green color (68%) or dark green color (14%) secondary skin color whereas; single genotype (AM 39) was orange in color. At maturity, fruit cracking was found in 62% of the landraces and these genotypes had striped (27%) and speckled (72%) skin patterns.

Szamosi *et al* (2010) compared morphological characteristics of fifty-eight Hungarian and Turkish *Cucumis melo* L. accessions for seventeen quantitative characters (3 seedlings, 3 leaves, 2 flowers, 9 fruit). Among, Hungarian accessions average fruit weight (g) ranged from 734.0 to 1333.7, fruit length varied from 7.9 to 29.8, fruit diameter ranged from 8.5 to 24.5, TSS varied from 5.5 to 15.5, length of seed cavity varied from 4.7 to 18.7. While, in Turkish melon accessions average fruit weight ranged from 653.50 to 1017.70, fruit length varied from 5.5 to 26.7, fruit diameter ranged from 4.2 to 16.7, TSS ranged from 2.7 to 12.7 and length of seed cavity varied from 4 to 12.7. The results indicated that both Hungarian and Turkish germplasm resources present a wide range of diversity for morphological traits.

Tomar *et al* (2008) reported that genetic diversity of fifty melon genotypes, which were clustered into seven groups based on the genetic diversity. Cluster III exhibited the highest mean value for fruit weight (0.91), flesh thickness (2.50), number of node at which 1st

female flower appeared (6.7), moisture percent (91.90) and length of fruit (18.85). While, II cluster, exhibited highest total soluble sugars content (6.77) and TSS content (11.50) and cluster VI having fruit yield/ plant (3.28) and number of fruits/ plant (5.41). However, clusters V, IV and VII exhibited percent acidity (0.21), higher fruit girth (43.4) and days to 1st picking (81.67), respectively. Further results indicated that maximum number of similar genotypes (24) were appeared in I cluster. Twelve and eight genotypes were composed of clusters II and V, respectively. While, III, IV and VI, VII clusters were composed of 2 accessions and a individual genotype, respectively.

Dhillon *et al* (2007) reported large variation in 27 snapmelon accessions collected from Punjab, Haryana and Rajasthan, India. Number of primary branches varied from 2.9 to 11.8, number of fruits per plant 1 to 3.5, average fruit weight from 0.24 to 1.4 kg within accessions. Greater variability was present in fruit shapes.

2.1.2 Fruit quality traits

Sweetness, flavour, texture and the level of phytonutrients such as β -carotene, vitamin C and folic acid in flesh tissue are the determinants of fruit quality in melons (Yamaguchi *et al* 1977 and Lester 2008). Orange-fleshed muskmelons are known for their unique flavour and high sugar levels (Yamaguchi *et al* 1977). Being low in calories, muskmelons are an excellent sources of vitamins, many minerals and antioxidants (Lester 2008). With the increased awareness about the benefits of healthful foods, melons have acquired a reputation as excellent source of health promoting phytonutrients though the consumer preference is still largely determined by sweetness, aroma and texture (Lester 2006).

a) Sweetness

Sweetness is the most important determinant of melon fruit quality. The total soluble solids (TSS) content is a reliable indicator of quality and is routinely used by breeders to screen germplasm for sweetness. Soluble sugars account for more than 97% of the total soluble solids in maturing muskmelon fruits, with sucrose accounting for nearly 50% of all sugars (Bianco and Pratt 1977). As per USDA standards, high-quality muskmelon fruit should contain 9 to 11% of total soluble solids content (Rubatzky and Yamaguchi 1997).

Nasrabadi *et al* (2012) in a correlation analysis reported a significant positive relation of sugar content with fruit length and fruit weight. On the basis of quantitative data, accessions were divided into 6 groups. The clusters VI, IV and III had maximum flesh thickness and III cluster had the highest sugar content than the other clusters.

Obando *et al* (2009) characterized organic acid and sugar profiles in melon by using NILs which were derived from the Piel de Sapo (PS), a Spanish cultivar and Shongwan Charmi (PI 161375) which is an exotic Korean genotype. Data used to map sixty quantitative trait loci (QTLs): seven for the total sugar content, eight for sucrose equivalents, five for

glucose-to-fructose ratio, twenty for organic acids and eighteen for the sugars. Quantitative trait loci which were associated with sugar profile, twenty seven defined the content of sugar: nine for sucrose, six for glucose, eight for fructose and four for sucrose equivalents. Similarly, within thirty-two mapped for sensory traits, twenty-seven were associated with low scores in sweetness (8 QTLs), taste (9 QTLs) or global quality appreciation (9 QTLs); 2 with increased sweetness or fruit sourness and 3 with increased fruit bitterness. The QTLs defined herein may assist curators to understand overall organoleptic balance (umami taste, sweetness and sourness) in muskmelon fruit, particularly those which located within linkage groups III, V, VI and VIII to XI.

Tomar *et al* (2008) in a path analysis study reported that the TSS exhibits +ve direct effect on fruit yield/ plant. Thus, TSS along with number of fruits/ plant may be given more percentage for effective selection to improve fruit yield in melon. TSS followed by total soluble solids and fruit yield/ plant contributed the most towards divergence.

Obando *et al* (2008) examined a set of 27 melon NILs to study the genetic control of a large number of muskmelon quality traits, flavor texture, including morphological and external appearance. For all the traits, heritability was significant, being >0.5 for the whole area of the longitudinal section of the fruit, skin lightness color, flesh proportion, flesh-extractable juice and hue angle coordinate of flesh colour. Near isogenic lines classified with help of principal-component analysis. The 1st principal component (22% variation) was affected by the morphological traits, the 2nd component (10% variation) was influenced by internal and external morphology patterns and the colour and 3rd component (9% variation) was controlled by flavour traits. Averages of the 5.6 quantitative trait loci /trait were identified (range, between 1 and 12 QTL; 134 quantitative trait loci in total).

Ram *et al* (2002) suggested exclusive improvement of TSS without any adverse effect on other traits, as TSS did not show any significant correlation with any other plant or fruit character in muskmelon. In quality evaluation of melon cultivars, Pardossi *et al* (2000) observed that flavour was positively correlated with pH and soluble solids content which confirm consumer preference for fruits with a high pH and high sugar content. Similarly, Reddy *et al* (1990) examined eleven muskmelon varieties comprising both desert and non - desert types for TSS variation. They reported that medium TSS varieties had very high variability for TSS content.

b) pH

Koubala *et al* (2016) reported pH of the various fruit fractions varied from 4.27 to 5.29. But, according to Bianchi *et al* (2016) the pH of melon significant varies from 5.2 to 6.5 depending on the cultivar used. Parveen *et al* (2012) also noted similar variations in pH (5.38

to 5.58) with *Ravi* melon variety. However, Beaulieu *et al* (2007) reported a slightly higher pH value (6.79) with *cantaloupe* variety.

c) β -carotene content

β -carotene (84.7%), ζ -carotene (6.8%), α -carotene (1.2 %) and lutein (1.0 %) are the main carotenoids in muskmelon. Orange fleshed muskmelons are a good source of carotenoids (Fraps 1947). Henane *et al* (2013) reported significant differences among muskmelon varieties for carotenoid content. Carotenoid content varied from 1.15 in *cv.* Maazoul to 12.82 mg/ kg fruit weight in *cv.* Galaoui. Highest content of carotenoid was recorded in *cv.* Galaoui. In fact, total carotenoid content obtained in ‘Galaoui’ was more than 12-fold higher than those obtained in ‘Maazoul’. This study demonstrated that total carotenoid and total phenolic contents were widely influenced by accessions emphasizing the need for evaluation of melon biodiversity to improve their nutritional value.

Crosby *et al* (2007) also reported that carotenoid content changed with the flesh colour and ranged from 0 in white-fleshed to 40 $\mu\text{g g}^{-1}$ in dark orange-fleshed genotypes. Cultivars ‘TAM Uvalde’ and ‘Mission’ possessed more than 36 $\mu\text{g g}^{-1}$ carotenoids. Further, Watanabe *et al* (1991) reported that β -carotene content varied from 9.2 to 18.0 $\mu\text{g g}^{-1}$ depending upon the varieties, while green-fleshed honeydew melons have low carotenoid concentrations.

d) Ascorbic acid content

Koubala *et al* (2016) studied the developmental stage effect on physicochemical characters in muskmelon fruits. Results indicated that mesocarp weight ratio (44 to 42%) declined meanwhile that of endocarp (41 to 44%) raised with the influence of growth. The proteins content of peels decreased from 1.7 to 0.4 mg/ g during maturation of fruit, while that of endocarp and mesocarp increased from 1.4 to 4.3 and 0.14 to 0.48 mg/ g respectively. At 1st stage of development, the peels were 2 times richer in vitamin C content (14.9 $\mu\text{g per g}$) than other fruit fractions (4.17 to 8.38). However, from 2nd to 5th stage (full maturation), the endocarp and mesocarp appeared to be richer. Dry matter content of melon peels (8.18 to 11.49%) increased throughout fruit maturation.

Burger *et al* (2004) evaluated 350 melon accessions for ascorbic acid content of their flesh as sucrose is a primary determinant of taste and ascorbic acid (Vitamin C). They identified few accessions having consistently high ascorbic acid content. Sharma and Lal (2004) analyzed ten varieties of muskmelon for variation in physicochemical constituents and found that vitamin C content ranged from 8.3 to 23.1 mg/100 g of fresh weight.

In a correlation study, Kaur *et al* (1997) concluded that vitamin C was positively associated with total sugar but negatively with free reducing sugar, fruit weight and flesh

thickness. Thus, selection for total sugars will also be correlated with high vitamin C. These reports indicate wide variation for ascorbic acid in melon genotypes which emphasizes the need to evaluate the muskmelon biodiversity in order to improve its nutritional value.

2.2 Molecular characterization of muskmelon accessions

In molecular diversity studies different techniques are used for assessment of variability like diversity array technology (DarT) (Kilian *et al* 2005), single nucleotide polymorphisms (SNPs) (Chen and Sullivan 2003), amplified fragment length polymorphism (AFLP) (Vos *et al* 1995), inter-simple sequence repeats (ISSR) (Zietkiewicz *et al* 1994), randomly amplified polymorphic DNA (RAPD) (Williams *et al* 1990) or arbitrarily primed PCR (AP-PCR) (Welsh and McClelland 1990), simple sequence repeats (SSR) (Tautz 1989), restriction fragment length polymorphism (RFLP) (Botstein *et al* 1980) and other high throughput platforms.

a) Amplified fragment length polymorphism (AFLP) marker study

AFLP markers are based on genomic DNA fingerprinting technique, which are utilized for the detection of DNA polymorphism. This is a polymerase chain reaction (PCR) based technique, which has been widely used for the genetic diversity determination and phylogenetic relationship between closely related individuals. Amplified fragment length polymorphism (AFLP) marker are leading in nature and do not require preceding knowledge of the genomic composition (Vos *et al* 1995).

Danesh *et al* (2015) studied the genetic diversity of six Iranian muskmelon cultigens by Amplified fragment length polymorphism (AFLP) markers. Fifteen individuals from each of the five well known and popular Iranian local melon (*Cucumis melo* L.) cultivars were examined. Ten primer pairs used on ninety individuals producing 318 polymorphic fragments, with an average of 31.8 fragments per primer combination. The percent polymorphism rate varied from 80 to 100%. Genetic similarities among genotypes were calculated by Dice's Similarity Index and dendrogram constructed based on the Un-weighted Pair Group Method with Arithmetic Average (UPGMA). The genetic distance measured with help of AFLPs varied from 0.29 to 0.63, with an average of 47. Five Iranian muskmelon accessions and "Ananasi" genotype were considered as 2 separate groups on the basis of cluster analysis. Results indicated that wide genetic diversity was present among Iranian muskmelon genotypes. The high expected genetic diversity and high number of alleles detected by the AFLP marker reported that the Iranian muskmelon genotypes had disparate characteristics and were an important genetic diversity pool.

b) Randomly amplified polymorphic DNA (RAPD) marker studies

First PCR based molecular marker technique was randomly amplified polymorphic DNA which is simplest one also (Williams *et al* 1990). Approximately 10 bases, short

polymerase chain reaction primers are randomly and imperiously selected for the amplification of random DNA segments throughout the genome. This technique was used widely used in studying genetic diversity between the plant species.

Yi *et al* (2009) evaluated genetic diversity with 27 RAPD markers of Myanmar muskmelon germplasm and morphological characteristics using forty-one of muskmelon accessions, out of which thirty-six genotypes were small-seeded type. The genetic diversity was 0.239, higher than for group *conomon* from East Asia and equivalent to Indian melon populations. Melon accessions were classified into six major clusters. Cluster IV which was largest among the groups was comprised specially *conomon* group which was closely related to cluster V consisted of *agrestis* group. Most of accessions of *cantalupensis* group were clustered in II or VII clusters and were consisted of *conomon* and *agrestis* groups. The relationship to muskmelon genotypes from adjoining countries was also accessed. Twenty-four genotypes of clusters IV and V were mostly clustered together with the small-seeded type muskmelon from India, but fourteen genotypes of clusters VI and VII were mostly grouped together with large-seeded type muskmelon from India. Results indicate that large genetic variability of Indian muskmelon is preserved in the Myanmar.

Luan *et al* (2008) have examined the melon accessions from India and Africa, Crete/Greece, Japan, Europe, U.S.A., Spain, and 68 Chinese cultigens by using seventeen randomly amplified polymorphic DNA primers (thirty-two mapped loci). The average similarity between 2 pairs of genotypes investigated as estimated by randomly amplified polymorphic DNA variation was 0.47 ± 0.14 . Within-group genetic resemblance varied between 0.08 (non netted thick-skinned type) and 0.94 (thin-skinned type). Percentage of polymorphism in Chinese accessions: non-netted thin skinned, non-netted thick skinned, netted thick skinned and non-netted thin skin vegetable was 81.3 %, 87.5%, 78.1, 56.3%, respectively. Among reference accessions of Africa 84.4 % percentage of polymorphism was reported by Mliki *et al* (2001). Similarly, Crete 65.6 % Staub *et al* (2004), Europe and U.S.A. 87.5% Staub *et al* (2000), India 65.6% McCreight *et al* (2004), Japan 81.3 % Nakata *et al* (2005), Spain 71.9% Lopez-Sese *et al* (2003), Turkey 89.9% Sensoy *et al* (2007), respectively. Genotypes were partitioned into two main branches consisting of group *cantalupensis* and *inodorus* reference accessions and Chinese accessions with help of cluster analysis. A second cluster analysis partitioned African, China, and Indian accessions into single major group, and accessions from Europe, U.S.A. and Japan into another.

Dhillon *et al* (2007) studied diversity among 36 snap melon landraces, collected from 2 agro-ecological regions of India (9 agro-climatic sub-regions), assayed using RAPD and SSR primers, morphological and fruit traits, 2 yield related traits, pest and disease resistance

and the biochemical composition (titrable acidity, Total soluble solid content and ascorbic acid). RAPD based grouping analysis revealed that Indian snap melon rich in genetic variation. The percentage of polymorphic bands shown by RAPD markers, ranged from 80 to 100, with a mean of 96.6. Accessions of var. *agrestis* and var. *momordica* clustered together and there was a separate cluster of the accessions of var. *reticulatus*.

Sensoy *et al* (2007) determined genetic relationships among 56 melon (*Cucumis melo* L.) genotypes by comparing molecular and phenotypic traits with those of twenty-three foreign and local muskmelon accessions to examine taxonomic relationships and genetic variability of Turkish muskmelon genotypes. To determine genetic similarity among the muskmelon individuals by dendrograms or two and three dimensional scaling was used. Sixty-one phenotypic characters and 109 polymorphic RAPD markers obtained from 33 primers were used in the present study. High correlations ($r \geq 0.97$) among the four resulting matrices was used for characterization at molecular level. The correlations among molecular and phenotypic Euclidean, Simple matching Nei analyses and Jaccard were $r = 0.41$, $r = -0.40$, $r = -0.43$ and $r = -0.40$, respectively. Molecular and phenotypic analysis reported that non-sweet muskmelon types were different from sweet types and the diversity of Turkish muskmelon accessions was higher than those of sweet foreign genotypes examined, as turkish accessions (89.9%), foreign accessions (94.5%), non-sweet foreign accessions (74.3%), sweet foreign accessions (72.5%), foreign *cantalupensis* group (55.1%), foreign *inodorus* group (56.0 %) have polymorphism, respectively. It was also reported that sweet Turkish muskmelon accessions belonging to *inodorus* groups and *cantalupensis* group were highly variable and could be intermated or crossed with other non-sweet type muskmelons.

Nakata *et al* (2005) studied genetic diversity of sixty-seven muskmelon (*C. melo* L.) genotypes from five Japanese seed companies with twenty-five random amplified polymorphic DNA (RAPD) primers (56 bands) and 9 simple sequence repeat (SSR) (36 alleles) markers. These genotypes belonged to the 3 horticultural groups: *cantalupensis*, *inodorus* and *conomon*. They reported that Japanese market class (*conomon*) had highest molecular polymorphism than Oriental class [79 (RAPD) to 89% (SSR)]. Charentais market classes and Japanese House (*cantalupensis*) were also had moderate polymorphism [56 (random amplified polymorphic DNA) to 67% (Simple sequence repeat)]. Earl's type market class genotypes (*cantalupensis*) possess relatively less polymorphism [11 (simple sequence repeat) to 38% (random amplified polymorphic DNA)]. Hence, Oriental market class and Japanese House genotypes were relatively rich in the genetic variation, followed closely by Galia and Charentais types. Genetic introgression of genes from Charentais and Galia germplasm can be done to increase the genetic diversity of these market classes.

Staub *et al* (2004) assessed diversity among seventeen melon landraces and inbred lines of Group *cantalupensis*, *inodorus* and *flexuosus* germplasm from Greece using twenty-four RAPD primers, 11 morphological traits of fruit, two yield-related characteristics and resistance to powdery mildew. They reported that *flexuosus* and *inodorus* accessions from Greece possessed genetic affinities with Western Asian (Group *conomon*) and Mediterranean (Group *Inodorus*) market classes, respectively. Comparative analysis of Greek germplasm and an admix of former represented reference genotypes spanning major market classes employing 19 RAPD primers, indicates that all but one of the accessions from Greece showed genetic affinities among themselves and with 23 reference accessions of various origin and market class. Thus, enhancement of USA, Asian and European muskmelon market classes can be done by Greek muskmelon landraces.

Lopez-Sese *et al* (2003) assessed genetic relationships of 125 Spanish muskmelon (*Cucumis melo* L.) genotypes from a Spanish germplasm collection by using thirty-four RAPD markers bands and 72 reference genotypes. The reference genotypes consisted of a broad range African, Crete, Japanese, and USA and Europe of different horticultural groups like *flexuosus*, *chito*, *cantalupensis*, *inodorus* and *conomon*,) and also from different muskmelon market classes like US European and Western Shipper types Charentais, Ogen, Galia, Casaba and Honeydew). Spanish muskmelon genotypes (*inodorus* group, largely Casaba,) were distinctive from reference accessions and other *inodorus* group muskmelons of various origins. Level of polymorphism (88.2%) was observed among the muskmelon originated from Spain and Rochet genotype from Andalusia region and lowest among Crete accessions 67.6%. It was concluded that Spanish muskmelon genotypes can be used for broadening genetic base of foreign and local Casaba germplasm and to enhance genetic variability of US and European commercial muskmelon germplasm and also to outline collection arrangement for the acquisition of further spanish accessions.

c) Simple sequence repeats (SSR) marker studies

Simple sequence repeats (SSRs) are also known as microsatellites and are small, tandemly repeated segments of DNA (Chiba *et al* 2003; Danin-Poleg *et al* 2000; Ritschel *et al* 2004). SSR have a high level of transferability to related species and for that reason, these markers are significantly valuable (Varshney *et al* 2005). SSR markers are preferred for some of their favorable traits. For example, they are hyper-variable and co-dominant in nature. Moreover, they are very reproducible and highly informative. They are also locus-specific markers (Danin-Poleg *et al* 2001). Although they have some advantages, their development is time consuming for plant species and requires DNA sequence information (Wang *et al* 2004).

The SSR markers are very useful for varietal/line identification (multiplexing of primers necessary), framework/region specific mapping, create genetic maps, F1 identification, comparative mapping, breeding, diversity studies, novel allele detections,

marker-assisted selection, high-resolution mapping, seed testing, map-based gene cloning and also they provide detailed information about genetic structure and gene flow (FAO/IAEA 2002, Wang *et al* 2004).

Henane *et al* (2015) analyzed the genetic divergence of Tunisian muskmelon genotypes (*Cucumis melo* L.) and Fakous (*cucumis melo* var. *flexuosus*). Twelve SSR primer pairs were used for five Tunisian varieties and Fakous (*Cucumis melo* var. *flexuosus*). Out of total twelve primer pairs, 11 simple sequence repeat were polymorphic and reproducible. The number of alleles/ locus ranged from 2 and 3 alleles, with the average of 2.54 alleles per marker. They used Darwin 5 software to study genetic relationship among melon varieties and fakous. Dissimilarity coefficient varied from 0.090 to 0.818, with average value of 0.454, which showed that varieties of muskmelon and fakous constituted an intergral pool of genetic diversity.

Malik *et al* (2014) studied genetic diversity of eighty-eight landraces melon (*Cucumis melo* L.) and eight USA reference cultivar using 30 SSR markers. They reported mean heterozygosity of 0.44. Sixty-seven alleles were found across the 88 Indian accessions and reference cultivars. An average number of alleles/ SSR locus was 2.2. Mean Polymorphism Information Content (PIC) value, a reflection of allele diversity and frequency was 0.57. Sixteen (23.8 %) alleles were present within *momordica* accessions, and twelve (17.9 %) alleles were present in *cantalupensis* and *reticulatus* accessions of Indian origin. Twenty-five (37.3 %) alleles were present in USA reference cultivars were not observed in any of 88 genotypes of the Indo-Gangetic plains of India. The 88 Indian melons clustered into six groups in the NJ tree based on variability of 30 SSR loci. The 16 Reticulatus accessions and the eight USA reference cultivars formed a distinct group designated B. The 60 Cantalupensis accessions clustered into the other four groups designated A, C, D and E and 12 Momordica accessions formed a distinct group designated F.

Ning *et al* (2014) analyzed genetic variability of sixty-four muskmelon accessions including forty-three “Xinjiang Hami” muskmelon cultivars by thirty-six simple sequence repeat markers, which yielded 145 alleles. The PIC values of SSR markers varied from 0.09 to 0.83 with an average of 0.45. Muskmelon genotypes were clustered into two major groups (thin-skinned and thick muskmelons) based on the SSR markers. Also, sub-cluster analysis partitioned different botanical groups, even separating similar agronomic trait groups (Xinjiang landraces var. *ameri* and var. *inodorus*) on the basis of SSR markers. Marker analysis showed that four primers (CMCTT144, CMBR150, CMBR12 and CMBR84) produced the different sized polymorphic bands between these 2 botanical groups. Those 4 primers might be closely related to muskmelon fruit maturing time. However, a low level of genetic variability was observed in “Xinjiang” muskmelon genotypes. Genetic distances

signified relatively narrower genetic base, but explicit taxonomic status of Xinjiang genotypes compared with foreign reference cultivars.

Roy *et al* (2012) reported large genetic variation within the wild melon germplasm. Diversity among forty-three wild melon genotypes assessed for morphological traits of fruit morphological traits and plant habit, two yield-related traits by using 16 simple sequence repeat markers. Also, nineteen accessions included in the study as reference genotypes. A total of 165 alleles were reported across the muskmelon genotypes. Mean number of alleles / simple sequence repeat locus was 10.3. The average polymorphic information content value, a reflection of allele variability and frequency was 0.692. Average observed heterozygosity for the wild melon genotypes was 0.51 compared with 0.17 for the reference accessions. Forty-seven alleles (34.5%) present exclusively in the wild melon genotypes; the reference accession had thirty-three (20%) specific alleles.

Kacar *et al* (2012) examined 81 melon genotypes and 15 reference accessions for genetic diversity by using 20 SSR markers. A total of 123 alleles generated among 96 genotypes with polymorphism of 97.5%. Number of alleles identified by individual primer set varied from 2 to 12, with mean of 6.15. Average number of alleles was higher in the study than those of previous studies. Similarly, Tzitzikas *et al* (2009) used simple sequence repeat markers to explore genetic variability and population structure of Cypriot and Greek melon cultivars. All the SSR primers were polymorphic in nature with the total number of 81 alleles, having average of 4.7 alleles/ locus. In another study, total 232 (SSR) alleles were identified with an average of 10.3 alleles / simple sequence repeat were acquired for Indian snapmelons (*C. melo* var. *momordica*) (Dhillon *et al* 2007). However, Kong *et al* (2007) studied genetic diversity *C. melo* with EST-SSR markers and reported that number of alleles varied from 2 to 5 with mean of 2.9 alleles/ locus. Similarly, simple sequence repeat polymorphism rate (97.5%) also higher than that reported other literatures [66.7%, Lopez-Sese *et al* (2002); 86%, Danin-Poleg *et al* (2001) and 71%, Katzir *et al* (1996)]. Large number of genotypes used and the detection of a higher number of alleles may be the reason for obtaining high (SSR) polymorphism values, Monforte *et al* (2003).

Fergany *et al* (2011) presented genetic characterization of muskmelon accessions from south India. Assessment of genetic variability among fifty muskmelon genotypes collected from different agro ecological regions of the southern India was done by variation at seventeen SSR loci, traits of fruit and plant habit, 2 yield-related traits, disease and pest resistance, biochemical compositions (carotenoids, ascorbic acid and titrable acidity). Differences among accessions were observed in plant and fruit traits. Large genetic variation in muskmelon germplasm was done by SSR analysis. Across the full set of muskmelon genotypes, total 114 alleles were observed. Mean number of alleles/ marker was 6.8. The average polymorphic information content value, which is an reflection of allele diversity and

frequency among was 0.544. The average observed heterozygosity for collected accessions was 0.23 whereas it was 0.13 for reference populations. Thirty-one alleles (24.2%) were present sole in collected genotypes and the reference individual had identical proportion of specific alleles.

Yildiz *et al* (2011) determined genetic variability of sixty-three muskmelon (*Cucumis melo* L.) accessions which were obtained from different locations of Turkey by comparing their molecular RAPD, ISSR, and SRAP primers with nineteen foreign muskmelon genotypes to study genetic variability and taxonomic relationships of Turkish muskmelon cultivars. To investigate genetic similarity within the muskmelon accessions with two and three dimensional scaling or dendrogram, total 162 polymorphic primers (75, 18 and 69 were obtained from RAPD, SRAP and ISSR markers, respectively). Average similarity coefficient between any 2 pairs of genotypes examined as estimated by molecular variation (0.7 ± 0.4). The genetic similarities varied between 0.96 and 0.46 within the group. Closely related cultivars or accessions obtained from same locations were clustered within same groups. The accessions of Southeastern Anatolian region were distinct from *cantalupensis* (sweet) group and *inodorus* group accessions. This concluded that Turkey is secondary center for genetic variability of muskmelon germplasm. The genetic variability within Turkish accessions ($I = 0.42$ and $H = 0.28$) was little less than accessions of the world ($I = 0.45$ and $H = 0.30$). On the other hand, percent polymorphism within Turkish muskmelon accessions (90.7%) was higher than accessions of the world (87.6%).

Escribano *et al* (2008) studied genetic variability of 5 winter muskmelon genotypes and 4 reference genotypes including single genotype from snake melon, using the allelic variation at 19 SSR loci. Seventy-two polymorphic bands scored which provided for adequate discrimination among the accessions examined. Cluster analysis (UPGMA) resulted in a dendrogram with two major clades. Moreover, high level of heterogeneity observed within the accessions indicates that the melons examined possess broad genetic diversity.

Sheng *et al* (2007) assessed genetic diversity among 46 Chinese thin-skinned melon cultivars (*Cucumis melo* L.) by using 50 simple sequence repeat (SSR) markers. Thirty of 48 primers amplified polymorphisms and 179 bands were produced. The number of polymorphic marker bands detected within each accession was 1-8, where most of the SSR markers had an expected size of 150 bp or less. The gene diversity among these lines generally lower than that reported by Staub *et al* (1999).

Diversity analysis using SSR markers of 36 snap melon landraces by Dhillon *et al* (2007) showed the mean heterozygosity (0.42) for the Indian snapmelon genotypes, while it was 0.09 in reference genotypes.

Table 2.1: Summarization of molecular diversity studies in muskmelon

Markers	Genotypes	Conclusions	Scientist
10 AFLP	7 Cultigens	Wide genetic diversity present in Iranian melon cultigens	Danesh <i>et al</i> 2015
27 RAPD	41 melon accessions	Genetic diversity of Indian melon is conserved in Myanmar.	Yi <i>et al</i> 2009
17RAPD	68 melon cultivars	Chinese genotypes are great source of genetic variability for plant improvement.	Luan <i>et al</i> 2008
RAPD and 18 SSR	36 snapmelon landraces	Genotypes of var. <i>momordica</i> and var. <i>agrestis</i> grouped together and a distinct cluster was observed for the genotypes of var. <i>reticulatus</i> .	Dhillon <i>et al</i> 2007
33 RAPD	56 melon genotypes	Turkish muskmelon accessions belonging to <i>cantalupensis</i> and <i>inodorus</i> groups were highly diverse and can be crossed or intermated with other non-sweet type of muskmelons.	Sensoy <i>et al</i> 2007
24 RAPD	17 melon landraces	Greek muskmelon accessions were unique which can be used for the enhancement of Asian, US, and European muskmelon market classes.	Staub <i>et al</i> 2004
34 RAPD	125 spanish melon accessions	To broaden t genetic base of foreign and local casaba germplasm, spanish muskmelon genotypes could be used.	Lopez-sese <i>et al</i> 2003
12 SSR	5 turkish varieties and Fakous (<i>Cucumis melo</i> var. <i>flexous</i>)	Turkish varieties and Fakous constitute an important pool of diversity	Henane <i>et al</i> 2015
30 SSR	96 melon accessions	Great genetic diversity among melon accessions was reported	Malik <i>et al</i> 2014
36 SSR	64 melon genotypes	Relatively narrow genetic base	Ning <i>et al</i> 2014
16 SSR	43 wild melon accessions	Great genetic variation was observed within wild muskmelon accessions	Roy <i>et al</i> 2012
20 SSR	81 melon and 15 reference populations	Large genetic diversity was observed in melon genotypes.	Kacer <i>et al</i> 2012
17SSR	50 melon landraces	High level of genetic variability observed in melon landraces	Fergany <i>et al</i> 2011
69 (ISSR), 18 (SRAP) and 75 (RAPD)	63 melon genotypes	Percentage of polymorphic loci among Turkish melon genotypes (90.7%) was higher than that of the world accessions (87.6%).	Yildiz <i>et al</i> 2011
19 SSR	5 winter melon landraces and 4 reference populations	High level of heterogeneity indicated broad genetic diversity present in melon landraces	Escribano <i>et al</i> 2008
50SSR	46 cultivars	Low genetic diversity present Chinese thin skinned cultivars	Sheng <i>et al</i> 2007
25 RAPD	67 melon cultivar	Genetic introgression of genes from the Galia and Charentais germplasm can be done to increase genetic diversity	Nataka <i>et al</i> 2005

CHAPTER III

MATERIAL AND METHODS

In this study, diversity among melon accessions was characterized for twenty-seven morphological characters, six fruit quality traits and at molecular level using thirty simple sequence repeat (SSR) markers. The experiments were conducted at Department of Vegetable Science and School of Agricultural Biotechnology, PAU, Ludhiana (30.9°N 75.85°E and 244 m above sea level), during the spring-summer seasons of 2015 and 2016. Details of each method are presented below:

3.1 Experiment 1: Genetic diversity for different morphological traits

3.1.1 Experiment material

In this study seventy melon accessions collected from diverse geographical locations such as, USA, India, Israel and Afghanistan consisting of various horticultural melon groups, viz. *cantalupensis*, *reticulatus*, *inodorus*, *momordica* and *callosus* were used for diversity analysis.

3.1.2 Experimental procedure

Nursery of melon genotypes sown on 25th February in 2015 and on 10th February in 2016 in 100 gauge thicknesses polythene bags of 15×10 cm size punched at base and filled with soil and well rotten farm yard manure in equal proportion. Seedlings were transplanted in the field at a spacing of 3.0 m × 0.45 m on 20th and 25th March in 2015 and 2016, respectively. In 2015, the selected genotypes were hand emasculated and self-pollinated for multiplying the seeds. The emasculation of hermaphrodite flowers was done in the evening during 5.00-7.00 p.m., bagging with parchment paper bags and pollinating it with pollen from the bagged male flower of same plant in the next morning at 6.00-8.00 a.m. After pollination, the flowers were covered with parchment paper bags and the bags were removed after setting of fruits. The fruits were harvested at full-slip stage and were cut-opened to collect seeds. The seeds were kept overnight in polythene bags at room temperature and were washed under tap water in mesh sieves. The washed seeds were dried in partial shade for two days and were stored in paper bags at room temperature.

In 2016, eight plants of each accession were transplanted in the field with 3 m row to row spacing and 0.45 m plant to plant spacing in a randomized complete block design (RCBD) with two replications. Four plants from each accession were randomly selected for recording data. The observations on various quantitative and qualitative parameters viz., days to last fruit harvest, days to 1st fruit harvest, days to 1st female flower appearance, number of fruits per vine, fruit weight (g), yield per plant (kg/plant), length of fruit (cm), fruit breadth (cm), flesh thickness (cm), rind thickness (mm), seed cavity size (length × breadth (cm²)), vine length (cm), firmness (lb/ inch²), fruit shape index, seed cavity length (cm), seed cavity breadth (cm), seed cavity index, flesh color (creamish white, grey orange, yellowish green, green, orange),

leaf shape (entire, trilobate, pentalobate, 3-palmately lobed, 5-palmately lobed, other), fruit rind color (creamy white, yellow, yellow green, orange, other), fruit patches (present or absent), fruit peduncle at maturity (slipable or non-slipable), fruit shape at peduncle end (pointed, rounded and truncate), fruit shape at blossom end, fruit surface (grooved or smooth), fruit sutures (present or absent), fruit surface netting (absent, moderate or dense), flesh texture (mealy, intermediate or crispy), total soluble solids (brix), pH, titrable acidity (%), β -carotene (mg /100 gm of fresh fruit weight) and ascorbic acid content (mg/100 g of fresh fruit weight) was recorded and average of four plants was used for statistical analysis.

Table 3.1: Details of accessions used for genetic divergence analysis

S. No.	Genotypes	Code	Group	Source
1	Afghanistan Collection -1	M1	<i>Inodorus</i>	PAU, Ludhiana
2	Afghanistan Collection -2	M2	<i>Inodorus</i>	PAU, Ludhiana
3	Canary Yellow-1	M3	<i>Inodorus</i>	PAU, Ludhiana
4	Carabean Gold	M4	<i>Inodorus</i>	United states of America
5	Durgapura Madhu	M5	<i>Cantalupensis</i>	TNAU, Coimbatore
6	Dissected Leaf	M6	<i>Cantalupensis</i>	PAU, Ludhiana
7	Durasol	M7	<i>Inodorus</i>	Harris seeds, Rochester, New York, USA
8	Green Flesh	M8	<i>Inodorus</i>	PAU, Ludhiana
9	Hara Madhu	M9	<i>Reticulatus</i>	PAU, Ludhiana
10	Hemed	M10	<i>Cantalupensis</i>	ARO, Volcani Centre Israel
11	Honey Dew	M11	<i>Inodorus</i>	PAU, Ludhiana
12	IC-267375	M12	<i>Cantalupensis</i>	PAU, Ludhiana
13	IC-274034	M13	<i>Momordica</i>	PAU, Ludhiana
14	IIVM-3	M14	<i>Cantalupensis</i>	IIVR, Varanasi
15	MM Selection-103	M15	<i>Cantalupensis</i>	PAU, Ludhiana
16	Kajri-1	M16	<i>Cantalupensis</i>	PAU, Ludhiana
17	Kajri-2	M17	<i>Cantalupensis</i>	PAU, Ludhiana
18	Kajri Doctor	M18	<i>Cantalupensis</i>	Doctor Seeds Pvt. Ltd.
19	Khasta Kharbooza	M19	<i>Cantalupensis</i>	PAU, Ludhiana
20	MC-2012-6	M20	<i>Cantalupensis</i>	PAU, Ludhiana
21	MC-2013-2	M21	<i>Cantalupensis</i>	PAU, Ludhiana
22	MC-2014-2	M22	<i>Cantalupensis</i>	PAU, Ludhiana
23	MM-105	M23	<i>Cantalupensis</i>	PAU, Ludhiana
24	MM-105-1	M24	<i>Cantalupensis</i>	PAU, Ludhiana
25	MM-2012-4	M25	<i>Cantalupensis</i>	PAU, Ludhiana
26	MM-2014-11	M26	<i>Cantalupensis</i>	PAU, Ludhiana
27	MM-2014-13	M27	<i>Cantalupensis</i>	PAU, Ludhiana
28	MM-2014-2	M28	<i>Cantalupensis</i>	PAU, Ludhiana
29	MM-2014-2-1	M29	<i>Cantalupensis</i>	PAU, Ludhiana

S. No.	Genotypes	Code	Group	Source
30	MM-2014-3	M30	<i>Cantalupensis</i>	PAU, Ludhiana
31	MM-3864	M31	<i>Cantalupensis</i>	PAU, Ludhiana
32	MM-3965	M32	<i>Cantalupensis</i>	PAU, Ludhiana
33	MM-4226	M33	<i>Cantalupensis</i>	PAU, Ludhiana
34	MM-4279	M34	<i>Cantalupensis</i>	PAU, Ludhiana
35	MM-4305	M35	<i>Cantalupensis</i>	PAU, Ludhiana
36	MM Var-3	M36	<i>Cantalupensis</i>	PAU, Ludhiana
37	MM var-4	M37	<i>Cantalupensis</i>	PAU, Ludhiana
38	MS-3	M38	<i>Reticulatus</i>	PAU, Ludhiana
39	Natal	M39	<i>Inodorus</i>	United states of America
40	PAUS-11	M40	<i>Reticulatus</i>	PAU, Ludhiana
41	PAUS-12	M41	<i>Reticulatus</i>	PAU, Ludhiana
42	PAUS-13	M42	<i>Reticulatus</i>	PAU, Ludhiana
43	PAUS-14	M43	<i>Reticulatus</i>	PAU, Ludhiana
44	PAUS-15	M44	<i>Reticulatus</i>	PAU, Ludhiana
45	PAUS-16	M45	<i>Reticulatus</i>	PAU, Ludhiana
46	PAUS-17	M46	<i>Reticulatus</i>	PAU, Ludhiana
47	PAUS-17-1	M47	<i>Reticulatus</i>	PAU, Ludhiana
48	PAUS-18	M48	<i>Reticulatus</i>	PAU, Ludhiana
49	PAUS-19	M49	<i>Reticulatus</i>	PAU, Ludhiana
50	PAUS-26	M50	<i>Reticulatus</i>	PAU, Ludhiana
51	PAUS-27	M51	<i>Reticulatus</i>	PAU, Ludhiana
52	PAUS-27-1	M52	<i>Reticulatus</i>	PAU, Ludhiana
53	PAUS-28	M53	<i>Reticulatus</i>	PAU, Ludhiana
54	PAUS-30	M54	<i>Reticulatus</i>	PAU, Ludhiana
55	PAUS-31	M55	<i>Reticulatus</i>	PAU, Ludhiana
56	PAUS-4	M56	<i>Reticulatus</i>	PAU, Ludhiana
57	PAUS-8	M57	<i>Reticulatus</i>	PAU, Ludhiana
58	PAUS-9	M58	<i>Reticulatus</i>	PAU, Ludhiana
59	PAUS-9-1	M59	<i>Reticulatus</i>	PAU, Ludhiana
60	Punjab Sunheri	M60	<i>Reticulatus</i>	PAU, Ludhiana
61	Riogold	M61	<i>Reticulatus</i>	United states of America
62	SM-2013-18	M62	<i>Momordica</i>	PAU, Ludhiana
63	SM-2013-2	M63	<i>Momordica</i>	PAU, Ludhiana
64	SM-2013-5	M64	<i>Momordica</i>	PAU, Ludhiana
65	SM-2013-9	M65	<i>Momordica</i>	PAU, Ludhiana
66	SM-2014-7	M66	<i>Momordica</i>	PAU, Ludhiana
67	SM-2015-2	M67	<i>Momordica</i>	PAU, Ludhiana
68	WM-11	M68	<i>Callosus</i>	PAU, Ludhiana
69	WM-2013-2	M69	<i>Callosus</i>	PAU, Ludhiana
70	Canary Yellow-2	M70	<i>Inodorus</i>	PAU, Ludhiana

3.1.3 Morphological characters

I) Vegetative characters

a) Vine length (cm)

After 60 days of sowing, vine length was measured as length of primary branch.

b) Shape of leaf

Leaf shape was recorded after 60 days of sowing as entire (1), trilobate (2), pentalobate(3), 3- palmately lobed (4) and 5- palmately lobed (5).

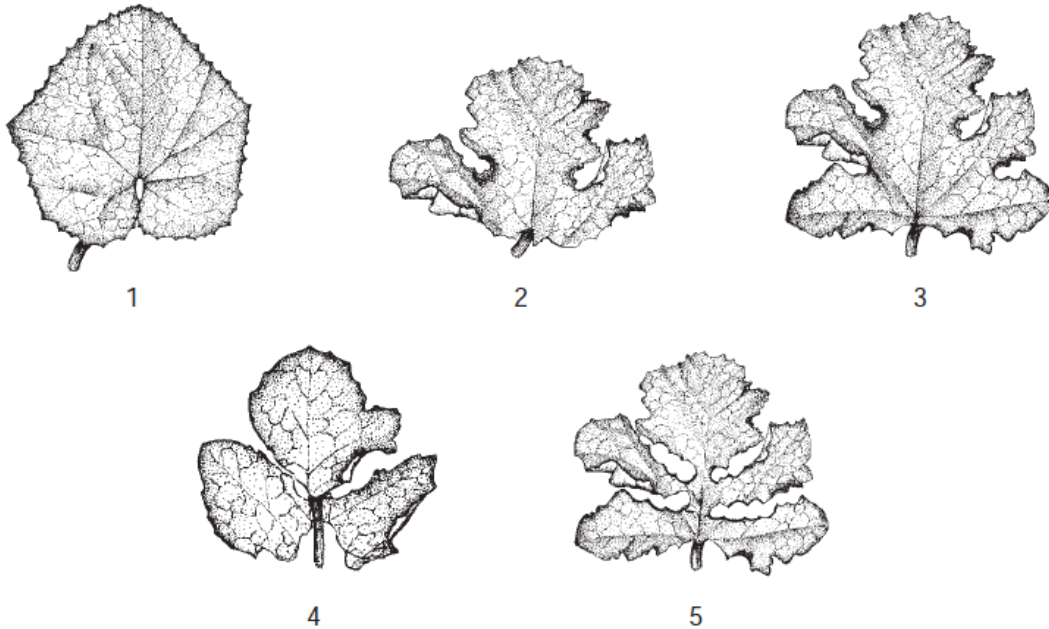


Figure 3.1: Leaf shapes mentioned in IPGRI melon descriptors

c) Days to first female flower appearance

Days to 1st female flower appearance was noted as number of days elapsed from sowing to the appearance of first female.

d) Days to first fruit harvest

Days to first fruit harvest was noted as the number of days elapsed from date of sowing to 1st fruit harvest.

e) Days to last fruit harvest

Days to last fruit harvest was recorded as the number of days elapsed from date of sowing to last fruit harvest.

f) Fruit peduncle at maturity

Fruit peduncle at maturity was recorded as, slipable and non-slipable.

g) Fruit shape at peduncle end

Fruit shape at peduncle end was visually recorded as pointed, rounded and truncate.

h) Fruit shape at blossom end

Fruit shape at blossom end was visually recorded as pointed and intermediate.

i) Fruit surface

Fruit surface was recorded as, grooved or smooth.

j) Fruit suture

Fruit sutures were recorded as, present or absent.

k) Fruit surface netting

Fruit surface netting was recorded as, absent, moderate or dense.

l) Fruit rind color

Fruit rind color was visually observed, as creamy white, yellow, yellow green, orange, and other.

m) Fruit patches

Fruit patches were recorded as, present or absent.

ii) Fruit yield and component traits

a) Fruit weight (g)

Fruits were harvested at full slip stage from four randomly selected plants. The weight (g) of each fruit was recorded and mean was calculated after final harvest.

b) Number of fruits/vine

Thirty days after transplanting, the numbers of fruits per vine were counted on four plants in each replication.

c) Yield/ plant (kg)

Fruits were harvested at full slip stage from four randomly selected plants in each replication. At each harvest fruits were counted and weight was recorded. After final harvest, cumulative yield was compiled to calculate average yield per plant (kg/ plant).

d) Polar diameter of fruit (cm)

Polar diameter of harvested fruits was measured as the distance from blossom end to the stem end.

e) Equatorial diameter of fruit (cm)

The fruits used to measure equatorial diameter were also used for measuring fruit width. Fruit width was recorded as the distance between two distal ends horizontally at the middle of fruit.

f) Fruit shape index (FSI)

Fruit shape index was calculated by dividing polar diameter by fruit equatorial diameter. FSI value equal to 1, less than 1 and more than 1, indicates round, depressed and oblong fruit shape.

g) Seed cavity length (cm)

Seed cavity length of harvested fruits was measured as the distance from blossom end to the stem end.

h) Seed cavity breadth (cm)

Seed cavity breadth was recorded as the distance between two distal ends horizontally at the middle of fruit.

i) Seed cavity size (length × breadth cm²)

Seed cavity was measured longitudinally and equatorially with a measuring scale. The seed cavity size was calculated as a product of length and width dimensions.

j) Seed cavity index

Seed cavity index was calculated by dividing polar diameter by fruit equatorial diameter. SCI value equal to 1, less than 1 and more than 1, indicates round, depressed and oblong fruit shape.

k) Flesh thickness (cm)

The fruits were longitudinally cut into two halves and flesh thickness was measured as distance from rind to seed cavity.

l) Rind thickness (mm)

Rind thickness was recorded as pericarp thickness by using Vernier Callipers.

iii) Fruit quality traits

a) Total soluble solids (%)

The TSS content of fresh juice extracted from fully ripened fruits was estimated by using a hand refractometer.

b) β-carotene (mg/100 g of fresh fruit weight)

For the beta carotene estimation 5g fresh sample was crushed in 97% petroleum ether+ 3% acetone with help of pestle mortar. After this extract was transferred to a separating funnel for an hour and same amount of water was added to it. Extract was collected after removal of water. A total 50 ml final volume was made up with petroleum ether and OD was recorded at 452 nm. (Mc Collum 1955).

c) Ascorbic acid (mg/100 g of fresh fruit weight)

Ascorbic acid content was determined using a procedure described by Heinze *et al* (1944). Two ml fresh extracted juice was added to an equal volume of the metaphosphoric acid and acetic acid solution in the conical flask and the mixture titrated with standardized dye solution (dichlorophenol indophenol dye). Ascorbic acid was calculated as described as following:

$$\text{Ascorbic acid content (mg/ 100 ml fruit juice)} = (Y/X) \times (100/Z)$$

Where, Y= Amount of the dye used in titration of “Z” volume of fresh extracted juice

X= Amount of the dye used in titration of 1 mg of ascorbic acid

Z= Volume of fruit juice taken for titration

d) Titrable acidity (%)

For estimating titrable acidity 2 ml fresh juice extracted from ripened fruits was neutralized with N/10 NaOH. Phenolphthalein indicator was used for recording the end point of titration. Titrable acidity was calculated as: 0.0064 (g anhydrous citric acid/ 100 ml juice).

e) pH

pH of fresh juice extracted from fully ripened fruits was estimated by using a pH meter

f) Fruit Firmness (lb/inch²)

Firmness of cut-fruits was estimated using hand-held penetrometer (Model FT-327, USA). The probe (11 mm) was inserted into flesh of fruits and firmness was recorded in lb/inch²).

g) Flesh color

Fruit flesh color was scored visually as white, light green, cream, light orange, orange, and dark orange.

i) Flesh texture

Flesh texture was recorded as, mealy, intermediate or crispy.

3.2 Statistical analysis

a) Analysis of variance

For quantitative parameters mean values of four plants from each replication was used for statistical analysis. Data of all variables was subjected to analysis of variance (ANOVA) using randomized block design following generalized linear model procedures of SAS (Version 9.3). The analysis of variance for randomized block design was carried out by using the following model.

$$Y_{ijk} = m + g_{ij} + b_k + e_{ijk}$$

Where,

Y_{ijk} = phenotypic value of the ij^{th} genotype grown in the k^{th} replication

m = general population mean

g_{ij} = effect of the ij^{th} genotype, where $i, j, = 1 \dots g$

b_k = effect of the k^{th} replication, where $k = 1 \dots r$

e_{ijk} = environmental effect

Analysis of variance based on the above model led to the following components of variance.

Analysis of variance

Source of variation	df	SS	MSS		F value
			Observed	Expected	
Replications	r-1	$S_r = \frac{\sum r^2}{g} - \frac{(\sum x)^2}{N}$	$M_r = S_r / r-1$	$\sigma_e + g\sigma_r$	M_r / M_e
Genotypes	g-1	$S_g = \frac{\sum g^2}{r} - \frac{(\sum x)^2}{N}$	$M_g = S_g / g-1$	$\sigma_e + r\sigma_g$	M_g / M_e
Error	(r-1)(g-1)	$S_e = S_t - S_r - S_g$	$M_e = S_e / (r-1)(g-1)$	σ_e	
Total	gr-1	S_t			

Where,

r = number of replications

g = number of genotypes

N = total number of observations

S_r = replication sum of squares

S_g = genotype sum of squares

S_e = error sum of squares

S_t = total sum of squares

σ_r = replication variance

σ_g = genotypic variance

σ_e = error variance

The genotypic variance was tested against error variance by 'F' test for (g-1) and (r-1)(g-1) degree of freedom. Similarly the replication variance was tested against error variance for (r-1) and (r-1)(g-1) degree of freedom.

The standard error of difference between the genotypic means is based on r replications. It was estimated as follows:

$$SD(d) = \pm \sqrt{\frac{2M_e}{r}}$$

Least significance (LS) = SE (d) x t_{(r-1)(g-1)} at 5% level of significance.

b) Principle Component Analysis (PCA)

Principal components analysis was performed using 17 quantitative traits for analyzing similarity between the genotypes SAS software. A biplot between PC1 and PC2 was constructed to elucidate phenotypic grouping.

c) Coefficient of variability

Coefficient of variability was calculated by the formula suggested by Burton and DeVane (1953) at phenotypic and genotypic levels.

i) Phenotypic coefficient of variance (PCV)

$$PCV = \sqrt{\frac{\text{Phenotypic variance } (\sigma^2_p)}{\text{General mean of population } (X)}} \times 100$$

ii) Genotypic coefficient of variance (GCV)

$$GCV = \sqrt{\frac{\text{Genotypic variance } (\sigma^2_g)}{\text{General mean of population } (X)}} \times 100$$

a) Phenotypic variance (V_P)

$$V_P = V_G + V_E$$

Where, V_G is the genotypic variance and V_E is the environmental variance

b) Genotypic variance (V_G)

$$V_G = V_A + V_D + V_I$$

Where, V_A is the additive genetic variance, V_D is the dominance variance, and V_I is the interaction or epistatic variance

iii) Heritability (%)

Heritability (broad sense) was calculated as per formula given by Burton and De Vane (1953) and Johnson *et al* (1995).

$$Hb_s = \frac{(\sigma^2_g)}{(\sigma^2_p)} \times 100$$

Where, Hb_s = Heritability (broad sense)

(σ^2_g) = Genotypic variance

(σ^2_p) = Phenotypic variance

3.3 Experiment 2: Characterization of melon (*Cucumis melo* L.) accessions using molecular markers

a) Genomic DNA extraction

Young leaves were collected from 15-20 days old seedlings of each melon genotype. Leaves collected from five plants were pooled together, cleaned with the ethanol and 100 mg leaf sample used for the extraction of genomic DNA. DNA was extracted with help of standard Cetyl Trimethyl Ammonium Bromide (CTAB) procedure which was given by Doyle and Doyle (1990) with some changes. To obtain high quality genomic DNA treatment of polyvinyl pyrrolidone was given for removal of polyphenols, thereby preventing their interaction with DNA.

1. Leaf samples were crushed to form fine powder using liquid nitrogen with the help of pestle and mortar.

2. Fine powder of leaf samples was collected in 2 ml centrifuge tubes 800 µl of pre-heated (65°C) CTAB buffer was appended and thoroughly mixed.
3. Tubes were incubated at the temperature (65°C) for one hour in water bath. During the incubation period, contents were mixed 4-5 times by inverting tubes lightly.
4. In the tubes, mixture of 800 µl of chloroform and iso-amyl alcohol (24: 1 v/v) was added, contents were mixed by inverting tubes for 5 minutes and placed for 15-20 min. on shaker.
5. At the room temperature, mixture was centrifuged for 10 minutes at 13000rpm.
6. Then without any disturbance to interface, upper aqueous phase (supernatant) was transferred into the new tubes.
7. Near about 2/3 volumes of chilled iso-propanol was appended and mix it by inverting tubes.
8. In the refrigerator samples were stored at the temperature of -20°C for 2 hours, then centrifuged at 13000rpm for 10 min. which results into the precipitation of nucleic acid.
9. After the completion of the centrifugation process, the supernatant was removed by inverting the tubes and pellet genomic DNA was reserved.
10. Pellet of genomic DNA was cleaned with the 300 µl 70% of ethanol twice and centrifuged for 3 min. at 13000 rpm.
11. Pellet of genomic DNA was dried at room temperature and DNA pellet was dissolved in 100 µl of TE buffer.

Table 3.2: Composition of CTAB buffer (100 ml)

Component	Quantity	Final concentration
1M Tris, pH-8.0	20.0 ml	100 mM
5M NaCl	28.0 ml	1.4 M
0.5M EDTA	4.0 ml	20 mM
10% CTAB	20.0 ml	2.0%
Sodium bisulphate	0.5 g	0.5%
Mercaptoethanol	1.0 g	1.0%
dd H ₂ O	28.0 ml	-

Table 3.3: Composition of TE buffer

Components	Final concentration	Volume per 100 ml
1M Tris-Hcl	10mM	1.0 ml
0.5M EDTA	1mM	0.2 ml
Double distilled water	-	To make the volume 100 ml

b) Estimation of Quantity of genomic DNA

DNA was quantified using Thermo Scientific NanoDrop™ 1000 Spectrophotometer. The instrument was standardized with double distilled water and blank value was calibrated using 1X TE. After that 1 µl DNA sample was placed onto the fibre optic cable end, which is called as receiving fibre. A 2nd fibre optic cable, which is called source fibre brought into contact with liquid sample by closing breach between the ends of fibre optic. The spectrometer used a pulsed xenon flash lamp as source of light and calculated the ratio of absorbance at 260 and 280 nm which was used to assess the purity of DNA. The instrument was restrained by computer software, and data logged in archive file on computer. Pure DNA has ratio of ~1.8, whereas pure RNA has a ratio of ~2.0. If the ratio deviates to either side, it indicates the presence of proteins, phenol, RNA or other contaminants. The absorbance ratio at 260 and 280 nm, which is the secondary measure for testing purity of nucleic acid, are often higher than the respective 260/280 values. The ratio 260/280 ranges from 1.8 - 2.2 for good quality DNA samples. The absorbance at 260 and 280 nm measures the concentration of DNA and RNA respectively in ng/µl. The DNA was normalized at a conc. of 50 ng per µl for individual sample.

c) Estimation of Quality of genomic DNA

Purity of isolated genomic DNA was estimated with help of agarose gel electrophoresis. For agarose gel electrophoresis, in 100 ml of 1X TBE electrophoresis buffer 0.8g of agarose was diluted (1mM EDTA, 45mM Boric acid and 45mM Tris base). Solution was heated till it became transparent and agarose get dissolved completely. At the temp. of 60°C, it was cooled with continuously stirring and the 5µl of ethidium bromide (10 mgml⁻¹) was dissolved to final the conc. of 0.5 µg per µl buffer. Prepare a gel mould with the combs and pour agarose solution into it and placed it for 30 to 40 minutes to solidify.

Loading dye (6X) was added to DNA samples which consists of 0.4% w per v sucrose, 0.25% w per v bromophenol blue or 30% glycerol in sterile water) so that the final conc. of loading dye was 1X. After the solidification of gel, it was placed in the electrophoresis tank. Micropipette is used to load the DNA samples into wells. DNA of known concentrations (100 ng per µl; 200 ng per µl; 400 ng per µl; 500 ng per µl and 1000 ng per µl) also loaded along with DNA samples. After loading the DNA samples, gel was run about 2 to 3 hours at stable volt of 5V per cm. Then, gel was visualized under the UV trans-illuminator. DNA samples photographed with help of photo gel-documentation system. To ascertain DNA conc. of each sample intensity of fluorescence of individual sample compared with known DNA conc. Estimation of quality of DNA done on the bases that whether the DNA formed individual high molecular weight band i.e good quality or smear band (poor / degraded quality).

d) PCR amplification

Thirty SSR markers amplified DNA through polymerase chain reaction. PCR amplification of 20 µl total volume was performed with 1.25 µl of each of forward and reverse primer (5 µM), 2.5µl of 1mM dNTPs, 2.0 µl of 10X PCR buffer, 4.0 µl of DNA (50ng), 0.25 µl of Taq polymerase (5 units/µl) and double distilled H₂O with help of thermal cycler. PCR profile accorded initial denaturation at temp. of 94°C for 4 minutes and later thirty-five cycles each of with denaturation at temp. of 94°C for 1 minute, marker annealing at temp. of 48-57°C for 1minute and marker extension at temp. of 72°C for 1 minute. The last step of extension done at temp. of 72°C for 7 minutes. For the optimization of reaction conditions, annealing temperature get modified for the individual marker. Before the analysis polymerase chain reaction products (PCR) stored at the temp. of 4°C. All the polymerase chain reaction run in duplicate. Amplified DNA fragments separated on a 1.5% gel which contain 1X TBE (1 mM EDTA, 45 mM Tris-borate) and 0.5 µg per ml aqueous sol. of the ethidium bromide. At the constant voltage of 100V for 2 to 3 hours in 0.5× TBE buffer, gel was run. Gel visualized under the ultra-violet light and photographed with the photo-documentation system.

e) Microsatellite Markers

For the assessment of genetic variability in melon accessions, thirty SSR markers were selected which were previously described and utilized by Chiba *et al* 2003. Following markers were custom synthesized from Integrated DNA Technologies Canada for in vitro DNA amplification. Below mentioned markers were used for evaluation and identification of genetic variability presented in Table 3.4.

Table 3.4: Microsatellite markers used in diversity analysis

	Locus	Primer sequence (5'-3')		Annealing temp. (°C)
		Forward Primer	Reverse Primer	
1	EMS50	TCAACCGTCTTCTCTCCACA	GTCATCGTTGAGTGCCAGAG	57
2	EMS51	TTCAAGCCTAGTTGTTTCTTGAT	TGTAATCGGGTTGAGTAAACAGGA	58
3	EMS61	TTTCAAAAAGCGAACCAGCTA	TCGGACTCGATTACCAAACA	54
4	EMS65	ACGACCTTCTCCTCCTCCTC	ACCGATTGAAGGTTGGATT	54
5	EMS70	TCCCTACCAATGAGGGGACT	TCAAACAAGATACATAGCCAATGAAA	57
6	EMS80	CGTCCCCTTGTTACTACCTCA	AAATCCTCCCTACATATATTATGCATT	52
7	EMS85	AGACAGCGGAGCTTTTCTT	TGAAATCGAAACGTCCTCTGAA	54
8	EMS109	CCCCCTTTTCTCCTTCTTCTT	GCTCTCATGGGAAACAGAGG	58
9	EMS124	GCGTCTAAAAAGGGATAAGG	ATTTTCACAAAAGGGGGAGAG	55
10	EMS125	GGAAACGCAAATCAGTGAG	CTGAACGTGGACGACATTTTT	55

	Locus	Primer sequence (5'-3')		Annealing temp. (°C)
		Forward Primer	Reverse Primer	
11	EMS129	TCAGACTCCATTTTCAGAGCCTA	CTTCAACCCCATTTTCTCACA	57
12	EMS130	CATTGGGAAAAAGGGTATGGA	CTGGCTCCTTCACATTGTTGT	55
13	EMS133	AAACATCAACACACACCCACA	TCAGCGACGGTCATCTATTTT	55
14	EMS134	TCTTTCCTCTGAAAATCCTTCT	TGCTTTGCTACATGCTETCCT	58
15	EMS178	CATAGAGCATTGCGGAGT	TGAAAAGCTAGCATGGATTGG	55
16	EMS182	TTCTTCATAATTCTAAATTTTCCATC	CCAGGTGGAAGTTTT	54
17	CMMS14-1	CATTGCTACTATTGTCGTCGTTGCT	GCTTCTTCTTTCTTTTCCGTATCCATTTT	60
18	CMMS1-3	TTGAATGATTGGAGGGAAGATAACG	CAAATATTGATGGATTTAATATATT	46
19	CMMS3-1	AAATATAAGCAAACCAAAGTTGACC	CCGGGATATACGGACATACACACAC	60
20	CMMS30-3	TTCCCACCAGCCCAACGGACACACT	GAGATACAGAAACGACGACTAACCT	60
21	CMMS33-1	TGTAATAGGARTGACCAAGGGGAGTT	TTCAGGAGCTACAACAAGATTTCAA	58
22	CMMS004	GCCCAACGGACACACTCACTCACAC	GAGGGAGTAAGAATAAGAAGAAGAA	58
23	CMGA127	GAACTAAGACTCTCCAATTAA	ATGTCCTAACTGCCAAACATA	46
24	CMGA128	ATGAAGAAGGGATATTCAAAG	ACTCCATTGTTGCTAACCTTT	53
25	CMTCN8	CCTCCGCCACATATTACAAT	TTCATCTTGACACETAAGAG	48
26	CMTCN38	TAAAACACTCTCGTGACTCC	GATCTGAGGTTGAAGCAAAG	55
27	CMTCN21	GCTGTAAAACGAAACGGAGA	CGATCTTCTTTATTCTTCGCC	55
28	CMT13	TGGATGGATAAGGTGGTAAG	TTCCCCTAGTCGCTCTC	46
29	CMAG59	TTGGGTGGCAATGAGGAA	ATATGATCTTCCATTTC	46
30	CMMS35-4	ACGGATACATCGAGGAGACTTCATG	GTCAGCTCAACCCTTACTTTTTC	60

Table 3.5: Polymerase chain reaction (PCR) mixture

Component	Stock conc.	Final conc.	Volume
DNA sample	25.0 g	50.0 ng	3.5
Polymerase chain reaction buffer	5X	1.0X	4.0
MgCl ₂	25.0 mM	1.5 mM	1.2
dNTP mix	10.0 mM	0.5 mM	4.0
Forward marker	5.0 mM	0.25 mM	1.0
Reverse marker	5.0 mM	0.25 mM	1.0
Taq polymerase	5 units	1 unit	0.2
Nuclease free water			5.1
Total			20.0

Table 3.6: Profile for Polymerase chain reaction (PCR)

Step no.	Cycling conditions	Temperature and Time
1 st	Initial Denaturation	94°C for 4 minutes
2 nd	Denaturation	94 °C for 1 minute
3 rd	Annealing	46-60°C for 1minute
4 th	Extension	72 °C for 2 minutes
5 th	Go to 2 nd	35 cycles
6 th	Final extension	72 °C for 7 minute
7 th	Hold	4 °C

f) Recording of SSR alleles

In each of the genotype, observations recorded in the binary format. Simple sequence repeat alleles were recorded for absence (0) and presence (1) of the band. Those bands considered as missing (9) which were difficult to score or diffused. Consistently produced and well resolved fragments obtained through amplification were considered and scored manually. In each accession unique or specific alleles along with the total alleles were also identified.

g) Statistical analysis

Polymorphic information content values were estimated by using equation of Anderson *et al* (1993) which provides an estimation of discriminatory locus or loci power. By taking into account, not only number of alleles which were expressed, also the relative frequencies of alleles.

$$PIC = \sum_{i=1}^n (P_{ij})^2$$

Here,

P_{ij} is the frequency of j^{th} allele in i^{th} marker and summation extends to “n” pattern.

NTSYS-PC version 2.02e software package was used for assessment for Genetic diversity among parental lines based on SSR markers.

h) Number of alleles / locus

Number of alleles (A) observed and effective no. of alleles (Ae) / locus was estimated by below mentioned formula

$$Ae = 1 / \sum p_i^2,$$

Where p_i is the frequency of the i^{th} allele)

i) Analysis using NTSYS-PC version 2.02e

Molecular data was analyzed with the help of computer baesd programme Numerical Taxonomical and Multivariate analysis system (NTSYS-PC version 2.02e rohlf, 1998). To determine the similarity on the basis of no.of amplified bands, data from 30 primers was used.

Estimation of similarities was done by using SIMQUAL function of NTSYS which computes a variety of similarities coefficient for qualitative data (nominal data).

j) Analysis using DARwin 6.0 software package

The genetic diversity among the germplasm lines was computed using PC based DARwin Programme (Perrier and Jacquemoud-Collet 2006). For the generate of dendrogram, data was contented to unweighted pair group's method with arithmetic mean (UPGMA) analysis. Data of 30 markers used to estimate the dissimilarity content on the basis of no. of amplified bands.

$$d_{ij} = \frac{b+c}{2a+(b+c)}$$

where, d_{ij} : dissimilarity between units i and j

a : no. of the variables, where $x_i = \text{presence}$ and $x_j = \text{presence}$

b : no. of the variables, where $x_i = \text{presence}$ and $x_j = \text{absence}$

c : no. of the variables, where $x_i = \text{absence}$ and $x_j = \text{presence}$

Here 'a' described matched fragments, b and c represented unmatched fragments. While, $2a+(b+c)$ are total no. of fragments amplified in specific set.

For the estimation of dissimilarity coefficients allelic data developed from 30 SSR primers by using software DARwin 5 as presented below:

$$D_{ij} = 1 - \frac{1}{L} \sum_{i=1}^L \frac{m_i}{\pi}$$

Where, D_{ij} : Dissimilarity between i and j allele

L : Number of loci

π : Poloidy

m_i : Number of matching alleles for locus i

Tree was constructed using neighbor joining on the basis of UPGMA

k) Genetic diversity (GD)

Genetic diversity was estimated by the formula given by Nei (1987).

$$GD = n(1 - p^2)/(n-1)$$

Here, n = no. of samples

P = frequency of single allele

l) Heterozygosity

Measurement of heterozygosity among loci represents the genetic variation. Each diploid cell has 2 alleles, of which single was inherited from individual parent. If particular has 2 diff. alleles at specific locus, particular is heterozygous at that locus and if 2 alleles are duplicate, the peculiar is homozygous. High heterozygosity delineates greater genetic variability and vice versa. The observed level of heterozygosity was compared with the expected by Hardy-Weinburg equilibrium. If observed heterozygosity is less than expected, it

can be fingerprinted to contrast forces like inbreeding depression. If observed heterozygosity is more than that of expected heterozygosity, then it can be suspect an isolate breaking effect i.e. the mixing of two-isolated population has occurred. Observed heterozygosity (H_0) and the expected heterozygosity (H_e) were estimated (Marshall *et al* 1998).

Where H_0 = no. of hetrozygous peculiar / no. scored

Expected heterozygosity (H_e) estimated by below mentioned formula

$$H_e = 1 - \sum p_i^2$$

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled “Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits” was conducted at Vegetable Research Farm, Department of Vegetable Science and Molecular Biological Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during the cropping season 2016. Experimental results for morphological and molecular characterization have been presented under following headings:

4.1 Morphological characterization of muskmelon germplasm

4.2 Molecular characterization of muskmelon germplasm with simple sequence repeats markers

4.1 Morphological characterization of muskmelon germplasm

Results of the experiment have been discussed in the light of relevant literature under the following sub-heads:

- i) Vegetative characters
- ii) Fruit yield and component traits
- iii) Fruit quality traits

i) **Vegetative characters**

Data pertaining to vine length, leaf shape, days to first female flower appearance, days to first fruit harvest, days to last fruit harvest are presented in the Table 4.2, 4.4 and 4.5.

a) **Vine length (cm)**

In present investigation, vine length showed significant variations among melon genotypes (Table 4.1). The maximum vine length was recorded in Hara Madhu (162.2 cm), followed by Riogold (161.3 cm), MM-4226 (156.8). However, minimum vine length observed in genotype PAUS-15 (61.2 cm), followed by WM-2013-2 (64.1 cm), SM-2013-18 (65.7cm) (Table 4.2). Box plot analysis indicated maximum variability for vine length in group *reticulatus* and minimum in *callosus* group (Fig. 4.1). However, Koli *et al* (2013) reported large variation in vine length ranging 101 cm to 220 cm.

b) **Leaf Shape**

Leaf shape was classified into entire, trilobate, pentalobate, 5-palmately lobed. Forty-two (60.0%) had pentalobate shape, twenty-four (34.3%) possessed entire leaf shape, three genotypes had 5-palmately lobed and one genotype (1.4%) possessed trilobate leaf shape (Table 4.5).

c) **Days to first female flower appearance**

Days to first female flower appearance is a desirable trait for obtaining early yield, which can fetch high market prices. Significant differences among genotypes were observed

for days to first female flower appearance (Table 4.1). Minimum number of days to appearance of first female flower were recorded in PAUS-13 (56.5 days) followed by SM-2013-2 (57 days) and Dissected leaf (57.5 days). While, maximum number of days to appearance of first female flower were exhibited in SM-2015-2 (71 days) these were statistically at par with genotype MM-105-1 (71 days) and PAUS-30 (71 days) (Table 4.4). Box plot analysis indicated maximum variability for days to first female flower appearance in group *reticulatus* and minimum in *callosus* group (Fig. 4.2)

Table 4.1: Analysis of variance for different characters in melons

Source of variation	Genotypes	Error
d.f	69	
Vine Length (cm)	1167.6*	22.9
Days to first female flower appearance	24.8*	2.9
Days to first fruit harvest	27.6*	11.8
Days to last fruit harvest	16.6*	3.9
Fruit Weight (g)	301935.2*	6138.5
Number of fruits per vine	6.5*	0.12
Yield (Kg/plant)	2.9*	0.06
Fruit Length (cm)	15.74 *	0.56
Fruit breadth (cm)	7.5*	0.27
Fruit Shape Index	0.11*	0.002
Seed Cavity Length (cm)	10.0*	0.35
Seed Cavity Width (cm)	2.1 *	0.13
Seed Cavity size	585.40*	24.3
Flesh Thickness (cm)	0.77*	0.034
Seed cavity index	0.30*	0.015
Rind thickness (mm)	3.9*	0.17
TSS (Brix)	11.12*	0.41
Beta- carotene (mg/100 g)	0.99*	0.004
Ascorbic acid (mg/100 g)	68.9*	2.7
Titration acidity (%)	0.04*	0.0003
pH	1.58*	1.6
Firmness	9.5*	0.024

*Significant at $P \leq 0.01$

d) Days to first fruit harvest

Significant differences were observed for days to first fruit harvest (Table 4.1). In the present investigation, earliest fruits were harvested from genotype MM-4279 (80.50 days after sowing) followed by PAUS-17 (89.50 days) and PAUS-17-1. However, genotype MM-2012-4 took maximum days (105 days) to first fruits harvest followed by Carabean gold (101 days) and PAUS-31 (100 days) with average of 95.0 days (Table 4.4). Box-plot analysis indicated maximum variability for days to first fruit harvest in group *cantalupensis* and minimum in *callosus* group (Fig. 4.2). The results are in consonance with those of Rakhi and Rajamony (2005), Lotti *et al* (2008), Fergany *et al* (2011) and Malik *et al* (2012) .

e) Days to last fruit harvest

There was significant difference reported for days to last fruit harvest (Table 4.1). Minimum number of days to last harvest was recorded in genotype SM-2013-9 (98.5 days) of followed by WM-2013-2 (99 days) and Dissected leaf (100 days). However, last fruits of genotype IIVM-3 were harvested after 115.5 of sowing followed by Kajri Doctor (111 days) and MM1204 (110 days), respectively (Table 4.4). Box-plot analysis indicated maximum variability for days to last fruit harvest in group *cantalupensis* and minimum in *callosus* group (Fig. 4.2) Similarly, Malik *et al* (2012) reported days to last fruit harvest ranged from 98.83 to 110.85 with overall mean 105.16.

f) Fruit peduncle at maturity

Fruit peduncle at maturity was recorded as, slipable and non-slipable. Fifty-nine were slipable (84%), while eleven genotypes (16%), Afghanistan collection-1, Afghanistan collection-2, Khasta Kharbooza, Green flesh, Canary yellow-1, Hara Madhu, and Canary yellow-2 were observed to be non-slipable (Table 4.5).

g) Fruit shape at peduncle end

Sixty four (91%) were rounded at peduncle base. While, six genotypes (8.5%) possessed pointed shape at peduncle end (Table 4.6).

h) Fruit shape at blossom end

Sixty four (91%) were intermediate at peduncle base. While, six genotypes (8.5%) possessed pointed shape at peduncle end (Table 4.6).

i) Fruit surface

Surface of fruit was classified as grooved or smooth. Out of total seventy genotypes, sixty-five (93%) were found to have smooth surface, while the remaining five (7%) genotypes were identified, as grooved (Table 4.6).

j) Fruit suture

For this trait, genotypes were classified on the basis on presence or absence of sutures. Sixty-two (89%) were non-sutured, while sutures were present in eight (11%) genotypes (Table 4.6).

k) Fruit surface netting

Netting on fruit surface was classified into absent, moderate, and dense. Forty-six genotypes (66%) had no-netting, one (1.4%) had moderate netting and twenty-three (33%) were found to be densely netted (Table 4.6).

l) Fruit rind color

Fruit rind color was an important fruit trait for consumer preference. Great variability was observed for fruit rind color viz., creamy white, yellow, yellow green, orange and other., Four genotypes (5.7%) had yellow rind color, eleven genotypes (15.7%) had yellow green, thirteen genotypes (18.6%) possessed creamy white rind color and forty-one genotypes (58.6%) had orange rind color and one genotype had other color of rind (Table 4.5).

m) Fruit patches

Less variation was observed for fruit patches. Out of seventy genotypes only three genotypes (4.3%), Afghanistan collection-1, Afghanistan collection-2 and Khasta Kharbooza had fruit patches (Table 4.5).

ii) Fruit yield and component traits

a) Fruit weight (g)

Fruit weight is an important determinant of yield in muskmelon. Considerable variation was observed in fruit weight parameter (Table 4.1), ranging from 92.5 to 2000 g with overall mean of 821.25 g. The maximum fruit weight observed in genotype Honey dew (2000.0 g), followed by Khasta kharbooza (1900.0 g) and Afghanistan collection-2 (1550.8 g). Whereas, minimum fruit weight recorded in WM-2013-2 (92.5 g) followed by WM-11 (117.3 g) and MC-2012-6 (268.8 g) (Table 4.2). Box plot analysis indicates maximum variability for fruit weight was observed in group *inodorous*, while minimum in *callosus* group (Fig. 4.1). In a muskmelon diversity study, Dhillon *et al* (2007) reported minimum and maximum fruit weight 0.239 g and 1400 g, respectively. Similarly, Fergeny *et al* (2011) reported that average fruit weight of muskmelon genotypes ranged from 0.175 to 1735 g. Further, Zhang *et al* (2012) reported that fruit weight in muskmelon ranged from 330 g to 1350 g.

b) Number of fruits per plant

There was significant variation for number of fruits per plant among muskmelon genotypes (Table 4.1). Number of fruits per plant varied from 1.3 to 8.5, with mean value of 3.6. Maximum number of fruits per plant were recorded in genotype MM-4226 (8.5) followed

by WM-11 (7.8) and WM-2013-2 (7.8). However, minimum number of fruits per plant found in genotype Canary yellow-2 (1.3) which was statistically at par with genotype MS-3 (1.3) and PAUS-12 (1.3) (Table 4.2). Box-plot analysis indicated maximum variability for number of fruits per vine in group *cantalupensis* and minimum in *callosus* group (Fig. 4.2). In muskmelon diversity study Fergany *et al* (2011) also reported number of fruit per plant from 2.8 to 9. Also, Prasad *et al* (2004), Dhillon *et al* (2009), Fergany *et al* (2011) found significant variations for number of fruits per plant.

c) Yield/ plant (kg)

There was significant difference reported for Yield (kg/plant) (Table 4.1). Yield (kg/plant) varied from 0.7 to 6.5 yield/ plant (kg) with mean value of 2.6 kg/plant. Fruit yield (kg/ plant) was reported minimum in wild melon cultivars WM-2013-2 (0.72 kg/plant) followed by PAUS-12 (0.73 kg/plant) and WM-11 (0.91 yield/ plant (kg) .However, maximum yield was exhibited in genotype Honey Dew (6.5 kg per plant) followed by MM-2014-11 (5.3 yield/ plant (kg)) and MM Selection-103 (4.9 yield/ plant (kg)) (Table 4.2). Box-plot analysis indicated maximum variability for yield in group *inodorous* and minimum in *callosus* group (Fig. 4.3). Reddy *et al* (2013) observed highest phenotypic coefficient of variation for fruit yield (28.30%).

d) Fruit length (cm)

All the melon accessions had a significant variation for fruit length (Table 4.1). Maximum fruit length was observed in genotype Afghanistan collection-1 (21.0 cm) followed by Afghanistan collection-2 (18.4 cm) and MM-2014-11 (17.3 cm). However, minimum found in MC-2012-6 (6.8 cm) followed by WM-2013-2 (7.3 cm) (Table 4.2). Box-plot analysis indicated maximum variability for fruit length in group *inodorous* and minimum in *callosus* group (Fig. 4.1). Szamosi *et al* (2010) reported that fruit length of muskmelon genotypes ranged from 7.9 to 29.8 cm and 5.5 to 26.7 cm in fifty eight Hungarian and Turkish *Cucumis melo* accessions, respectively.

e) Fruit breadth (cm)

Significant differences were found for fruit breadth among all the genotypes (Table 4.1). Fruit breadth ranged from 4.8 cm to 14.9 cm with average fruit breadth of all genotypes 10.82 cm. Genotype Green flesh exhibited maximum fruit breadth (14.96 cm) which was statistically at par with genotypes MM-2014-2 (14.38 cm) and MM-2014-2-1 (14.2 cm) (Table 4.2). Box-plot analysis indicated maximum variability for fruit breadth in group *inodorous* and minimum in *callosus* group (Fig. 4.1). Similarly, Koli *et al* (2013) assessed the

phenotypic diversity among thirty three landraces of *C. melo* and reported that fruit breadth ranged from 7 cm to 12 cm.

f) Fruit shape index

Fruit shape index is important horticultural trait from consumer point of view. When fruit shape index is close to one, fruit is round and when value deviated from one fruit shape change to oval or elongate as in case of snapmelon. In the present investigation, fruit shape index value varied from 0.80 to 1.96 with mean value of 1.12. Mostly, genotypes were round, except Durpapura Madhu, Afghanistan collection-1, Afghanistan collection-2, Canary yellow group and snapmelon genotypes which were observed to be oval or elongated as presented in table 4.3. Box-plot analysis indicated maximum variability for vine length in group *cantalupensis* and minimum in *callosus* group (Fig. 4.1).

g) Seed cavity length (cm)

There were significant differences for seed cavity length among all the genotypes (Table 4.1). Seed cavity length varies from 4.3 cm to 15.3 cm with average value of all genotypes 7.5. Maximum seed cavity length was found in genotype Afghanistan collection-1 (15.3 cm) followed by Afghanistan collection-2 (12.8 cm) and SM1502 (12.5 cm). However, minimum seed cavity length found in MC-2012-6 (4.3 cm) followed by MM-3864 (4.7 cm) and MM3965 (4.7 cm) (Table 4.3). Similarly, Szamosi *et al* (2010) reported length of seed cavity varied from 4 cm to 12.7 cm in another study.

h) Seed cavity breadth (cm)

Significant differences were observed for seed cavity breadth (Table 4.1). Seed cavity breadth ranged from 3.4 cm to 7.6 cm with mean value of 5.7 cm. Maximum seed cavity breadth found in genotype MM-2014-3 (7.8 cm) followed by Kajri-1 (7.4 cm) and MM-105 (7.3 cm). However, minimum seed cavity breadth reported in WM-11 (3.4 cm) followed by WM-2013-2 (3.6 cm) and natal (4.0 cm) (Table 4.3).

i) Seed cavity area (cm²)

Seed cavity area (SCA) is important fruit trait. Smaller the seed cavity area more will be the thick flesh and vice versa. Significant differences were observed for seed cavity area (Table 4.1). SCA ranged from 19.3 cm² to 108.8 cm² with mean value of 43.6. Genotype Afghanistan collection-1 (108.8 cm²) recorded maximum seed cavity size followed by Afghanistan collection-2 (92.4 cm²) and Honey dew (74.5 cm²). However, minimum seed cavity area observed in WM-11 (19.3 cm²) followed by WM-2013-2 (20.3 cm²) and MC-2012-6 (21.0 cm²) (Table 4.3). Box plot analysis indicated maximum variability for seed cavity area in group *inodorous* and minimum in *callosus* group (Fig. 4.1).



Plate 1: Variation for fruit type among melon accessions



Plate 2: Fruits of *cantalupensis* group



Plate 3: Fruit of *Inodorus* group



Plate 4: Fruits of *momordica* group

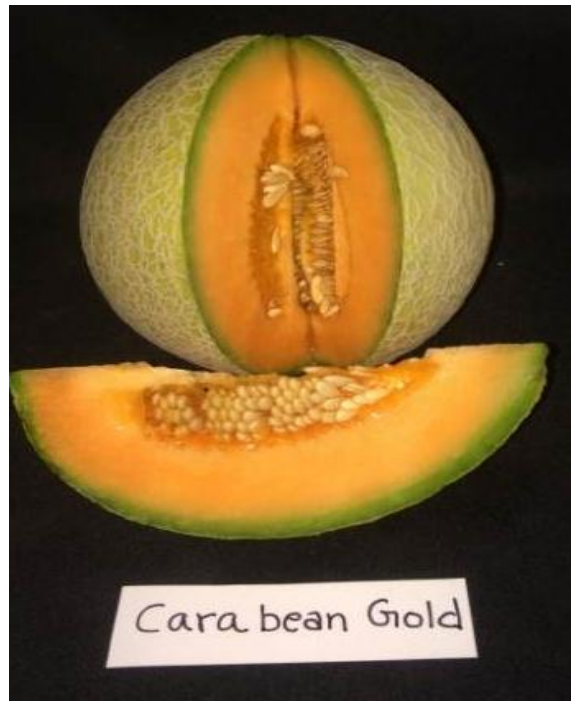


Plate 5: Fruits of *reticulatus* group

j) Seed cavity index (SCI)

When SCI is close to one, cavity is round and when value deviated from one cavity shape changes to oval or elongate. In the present investigation, seed cavity index value varied from 0.69 to 2.31 with mean value of 1.36. Eleven percent of the genotypes had round seed cavity (Table 4.3). Box plot analysis indicated maximum variability for seed cavity area in group *inodorous* and minimum in *callosus* group (Fig. 4.2).

k) Flesh thickness (cm)

Significant variations were found among all the genotypes for flesh thickness (Table 4.1). Maximum flesh thickness was observed in genotype Green flesh (3.4 cm) followed by MM-2014-2-1 (3.3 cm) and Canary yellow-2 (3.3 cm). Genotype WM-2013-2 (0.8 cm) had minimum flesh thickness followed by WM-11 (1.0 cm) and SM-2013-9 (1.04 cm). Flesh thickness ranged from 0.78 cm to 3.4 cm with a mean value of 2.4 cm (Table 4.4). Box-plot analysis indicated maximum variability for vine length in group *cantalupensis* and minimum in *callosus* group (Fig. 4.1).

k) Rind thickness (mm)

Rind thickness is a desired horticultural trait for improving shelf life of fruit. Snap melon genotypes were found with minimum rind thickness i.e SM-2013-2 (1.0 mm) which was statistical at par with the rind thickness of genotypes SM-2013-9 (1.0 mm), SM-2014-7 (1.0 mm) and MC-2012-6 (1.0 mm). However, maximum rind thickness was reported in genotype PAUS-30 (7.1 mm) followed by PAUS-9-1 (5.5 mm) and MM-2014-13 (5.3 mm). Rind thickness ranged from 1 mm to 7.13 mm with mean value of 3.61 mm (Table 4.4). Box-plot analysis indicated maximum variability for rind thickness in group *reticulatus* and minimum in *callosus* group (Fig. 4.1). Similarly, Malik *et al* (2014) observed rind thickness among Indian accessions belonging to *momordica*, *cantalupensis* and *reticulatus* groups ranged from 1.5 to 3.1 mm and 3.2 to 3.7 mm for eight USA *reticulatus* cultivars which can be positively correlated with storage life.

Table 4.2: Mean performance of melon accessions for various morphological characters

S. No.	Genotypes	Code	Vine length (cm)	Fruit weight (g)	Number of fruits per vine	Yield / Plant (Kg)	Fruit length (cm)	Fruit breadth (cm)
1	Afghanistan Collection -1	M1	119.1	1543.8	2.8	4.2	21.0	12.9
2	Afghanistan Collection -2	M2	108.0	1550.8	2.1	3.3	18.4	12.4
3	Canary Yellow-1	M3	123.2	950.0	1.3	1.2	12.1	11.4
4	Carabean Gold	M4	67.6	601.3	2.0	1.2	10.7	10.8
5	Durgapura Madhu	M5	128.0	600.0	3.4	2.0	15.6	9.0
6	Dissected Leaf	M6	107.6	1168.8	1.9	2.2	12.8	12.1
7	Durasol	M7	95.3	678.8	4.3	2.9	11.8	9.6
8	Green Flesh	M8	103.5	1406.3	3.5	4.9	15.0	14.9
9	Hara Madhu	M9	162.2	656.3	6.5	4.3	10.3	11.3
10	Hemed	M10	93.7	937.5	1.6	1.5	12.3	12.0
11	Honey Dew	M11	138.7	2000.0	3.3	6.5	16.7	11.3
12	IC-267375	M12	152.4	613.8	3.9	2.4	9.3	11.0
13	IC-274034	M13	117.5	387.5	4.8	1.8	10.0	8.7
14	IIVM-3	M14	119.7	925.0	4.5	4.2	10.5	11.6
15	MM selection-103	M15	126.0	987.5	5.0	4.9	9.8	12.0
16	Kajri-1	M16	140.0	787.5	4.3	3.3	10.4	12.8
17	Kajri-2	M17	145.4	780.8	4.5	3.5	10.0	11.3
18	Kajri Doctor	M18	140.7	768.8	4.8	3.7	19.1	13.9
19	Khasta Kharbooza	M19	100.3	1900.0	2.1	4.0	6.8	7.9
20	MC-2012-6	M20	128.3	268.8	7.5	2.0	8.8	8.4
21	MC-2013-2	M21	76.5	396.3	3.8	1.5	15.0	11.4
22	MC-2014-2	M22	113.0	1387.5	2.5	3.5	9.6	10.5
23	MM-105	M23	118.7	837.5	3.5	2.9	9.6	10.6

Table 4.2 Cont.

S. No.	Genotypes	Code	Vine length (cm)	Fruit weight (g)	Number of fruits per vine	Yield (Kg/Plant)	Fruit length (cm)	Fruit breadth (cm)
24	MM-105-1	M24	123.8	850.0	3.4	2.9	10.9	10.4
25	MM-2012-4	M25	109.2	543.8	2.1	1.2	17.3	13.3
26	MM-2014-11	M26	127.3	1450.0	3.6	5.3	12.7	12.1
27	MM-2014-13	M27	127.6	1043.8	2.8	2.9	14.6	14.4
28	MM-2014-2	M28	114.9	1275.0	3.6	4.6	14.8	14.2
29	MM-2014-2-1	M29	108.0	1293.8	3.0	3.9	10.9	13.4
30	MM-2014-3	M30	97.2	1283.8	3.4	4.3	8.4	10.5
31	MM-3864	M31	133.4	531.3	7.5	4.0	8.8	11.1
32	MM-3965	M32	103.5	683.8	4.0	2.7	8.3	8.8
33	MM-4226	M33	156.8	362.5	8.5	3.1	10.9	10.2
34	MM-4279	M34	151.8	687.5	4.3	2.9	10.0	10.2
35	MM-4305	M35	133.7	537.5	4.9	2.6	10.4	12.5
36	MM Var-3	M36	122.9	925.0	3.5	3.2	10.7	12.5
37	MM var-4	M37	88.6	443.8	5.9	2.6	9.3	9.3
38	MS-3	M38	121.0	843.8	1.3	1.1	12.8	12.1
39	Natal	M39	84.1	401.3	2.5	1.0	9.5	8.5
40	PAUS-11	M40	109.5	1000.0	2.1	2.1	11.1	11.1
41	PAUS-12	M41	100.6	581.3	1.3	0.7	10.7	10.4
42	PAUS-13	M42	121.6	850.0	2.5	2.1	12.0	11.7
43	PAUS-14	M43	108.9	737.5	2.0	1.5	12.0	11.7
44	PAUS-15	M44	61.3	693.8	1.6	1.1	10.5	10.5
45	PAUS-16	M45	126.0	950.0	2.5	2.4	14.7	10.8
46	PAUS-17	M46	73.0	806.3	2.3	1.8	12.4	11.4
47	PAUS-17-1	M47	77.8	818.8	2.5	2.0	12.4	11.4
48	PAUS-18	M48	120.3	812.5	2.4	1.9	10.7	9.9
49	PAUS-19	M49	125.4	806.3	1.6	1.3	11.9	11.3

Table: 4.2 Cont.

S. No.	Genotypes	Code	Vine length (cm)	Fruit weight (g)	Number of fruits per vine	Yield (Kg/Plant)	Fruit length (cm)	Fruit breadth (cm)
50	PAUS-26	M50	89.9	712.5	1.9	1.3	12.5	11.3
51	PAUS-27	M51	88.6	800.0	2.0	1.6	11.3	11.3
52	PAUS-27-1	M52	97.2	793.8	2.4	1.9	11.5	11.1
53	PAUS-28	M53	89.9	662.5	3.6	2.4	10.4	10.3
54	PAUS-30	M54	113.3	1106.3	2.3	2.5	14.6	11.5
55	PAUS-31	M55	83.5	712.5	4.0	2.9	11.0	10.9
56	PAUS-4	M56	115.9	650.0	3.4	2.2	10.3	10.8
57	PAUS-8	M57	84.1	725.0	1.9	1.4	11.0	10.9
58	PAUS-9	M58	134.0	1128.8	1.5	1.7	14.3	12.3
59	PAUS-9-1	M59	118.7	1168.8	1.8	2.0	14.0	12.4
60	Punjab Sunheri	M60	96.8	407.5	5.9	2.4	9.4	9.1
61	Riogold	M61	161.3	956.3	4.1	3.9	13.6	11.4
62	SM-2013-18	M62	65.7	643.8	3.9	2.5	13.0	10.0
63	SM-2013-2	M63	131.8	297.5	7.3	2.2	10.3	7.0
64	SM-2013-5	M64	147.6	662.5	5.3	3.5	11.3	10.1
65	SM-2013-9	M65	132.4	302.5	5.8	1.7	10.9	6.9
66	SM-2014-7	M66	68.3	281.3	5.5	1.5	10.5	7.4
67	SM-2015-2	M67	109.2	460.0	4.8	2.2	15.3	7.9
68	WM-11	M68	81.0	117.3	7.8	0.9	7.6	5.4
69	WM-2013-2	M69	64.1	92.5	7.8	0.7	7.3	4.8
70	Canary Yellow-2	M70	113.3	1475.0	1.3	1.8	16.8	12.1
	LSD ($P \leq 0.05$)		9.5	15 6.3	0.7	0.5	1.5	1.0
	CV (%)		4.3	9.5	9.7	9.8	6.3	4.9

Table 4.3: Mean performance of melon accessions for various morphological characters

S. No.	Genotypes	Code	Fruit shape index	Seed cavity length (cm)	Seed cavity breadth (cm)	Seed cavity area	Seed cavity index
1	Afghanistan Collection -1	M1	1.6	15.3	7.0	108.8	2.2
2	Afghanistan Collection -2	M2	1.5	12.8	7.2	92.4	1.8
3	Canary Yellow-1	M3	1.1	6.8	5.5	37.0	1.2
4	Carabean Gold	M4	1.0	6.1	5.8	35.6	1.1
5	Durgapura Madhu	M5	1.7	10.6	4.9	52.6	2.2
6	Dissected Leaf	M6	1.0	7.6	6.2	47.9	1.2
7	Durasol	M7	1.2	7.0	4.6	33.6	1.5
8	Green Flesh	M8	1.0	7.9	7.1	56.9	1.1
9	Hara Madhu	M9	0.9	5.5	6.8	37.0	0.8
10	Hemed	M10	1.0	7.4	5.5	41.2	1.3
11	Honey Dew	M11	1.5	11.9	6.1	74.5	1.9
12	IC-267375	M12	0.9	4.8	6.0	29.7	0.8
13	IC-274034	M13	1.2	6.4	5.3	33.8	1.2
14	IIVM-3	M14	0.9	7.8	7.3	56.9	1.1
15	MM selection-103	M15	0.9	5.4	7.1	38.6	0.8
16	Kajri-1	M16	0.8	5.4	7.4	38.9	0.7
17	Kajri-2	M17	0.8	5.5	7.0	39.8	0.8
18	Kajri Doctor	M18	0.9	10.8	6.4	39.0	1.7
19	Khasta Kharbooza	M19	1.4	4.3	4.9	69.4	0.9
20	MC-2012-6	M20	0.9	5.0	4.3	21.1	1.2
21	MC-2013-2	M21	1.1	10.3	6.4	21.8	1.6
22	MC-2014-2	M22	1.3	6.4	7.3	69.8	0.9
23	MM-105	M23	0.9	6.3	7.0	46.4	0.9
24	MM-105-1	M24	0.9	6.6	5.1	44.3	1.3
25	MM-2012-4	M25	1.1	11.1	6.4	33.0	1.7

Table 4.3 Cont.

S. No.	Genotypes	Code	Fruit shape index	Seed cavity length (cm)	Seed cavity breadth (cm)	Seed cavity area	Seed cavity index
26	MM-2014-11	M26	1.3	9.7	5.7	70.5	1.7
27	MM-2014-13	M27	1.1	9.1	6.8	54.8	1.4
28	MM-2014-2	M28	1.0	9.0	6.6	62.3	1.4
29	MM-2014-2-1	M29	1.0	6.3	7.8	61.6	0.8
30	MM-2014-3	M30	0.8	4.7	6.0	49.3	0.8
31	MM-3864	M31	0.8	4.7	6.8	28.6	0.7
32	MM-3965	M32	0.8	4.9	5.2	33.8	0.9
33	MM-4226	M33	0.9	6.7	5.6	25.6	1.2
34	MM-4279	M34	1.1	6.4	6.3	39.9	1.0
35	MM-4305	M35	1.0	5.8	6.5	40.2	0.9
36	MM Var-3	M36	0.9	5.9	6.6	39.8	0.9
37	MM var-4	M37	1.0	5.4	5.0	29.6	1.1
38	MS-3	M38	1.0	7.6	5.2	40.3	1.5
39	Natal	M39	1.1	5.8	4.0	24.3	1.4
40	PAUS-11	M40	1.0	7.4	4.5	33.7	1.6
41	PAUS-12	M41	1.0	6.2	4.8	29.6	1.3
42	PAUS-13	M42	1.0	7.2	5.6	41.4	1.3
43	PAUS-14	M43	1.0	7.5	5.2	39.4	1.4
44	PAUS-15	M44	1.0	5.4	4.3	23.1	1.3
45	PAUS-16	M45	1.4	10.3	4.8	49.6	2.1
46	PAUS-17	M46	1.1	7.9	5.0	39.2	1.6
47	PAUS-17-1	M47	1.1	7.9	5.0	39.4	1.6
48	PAUS-18	M48	1.1	6.6	4.4	28.6	1.5
49	PAUS-19	M49	1.1	6.7	5.0	33.5	1.3
50	PAUS-26	M50	1.1	7.5	5.6	42.2	1.3
51	PAUS-27	M51	1.0	6.7	5.1	34.8	1.3
52	PAUS-27-1	M52	1.0	6.4	4.9	31.5	1.3

Table 4.3 Cont.

S. No.	Genotypes	Code	Fruit shape index	Seed cavity length (cm)	Seed cavity breadth (cm)	Seed cavity area	Seed cavity index
53	PAUS-28	M53	1.0	6.1	4.9	31.3	1.2
54	PAUS-30	M54	1.3	10.6	6.0	64.1	1.8
55	PAUS-31	M55	1.0	6.1	5.0	31.0	1.2
56	PAUS-4	M56	1.0	6.1	4.9	30.7	1.2
57	PAUS-8	M57	1.0	6.0	4.2	25.7	1.4
58	PAUS-9	M58	1.2	9.3	6.4	60.3	1.4
59	PAUS-9-1	M59	1.1	8.8	6.0	52.8	1.5
60	Punjab Sunheri	M60	1.0	5.7	4.5	25.6	1.3
61	Riogold	M61	1.2	8.0	4.4	35.3	1.9
62	SM-2013-18	M62	1.3	10.6	5.9	63.3	1.8
63	SM-2013-2	M63	1.5	8.3	4.9	41.4	1.7
64	SM-2013-5	M64	1.1	8.5	6.9	60.3	1.2
65	SM-2013-9	M65	1.6	9.3	4.6	43.4	2.0
66	SM-2014-7	M66	1.4	8.2	4.6	37.5	1.8
67	SM-2015-2	M67	2.0	12.5	5.4	67.0	2.3
68	WM-11	M68	1.5	5.6	3.4	19.3	1.7
69	WM-2013-2	M69	1.6	5.5	3.6	20.3	1.6
70	Canary Yellow-2	M70	1.4	10.5	6.2	66.3	1.7
	LSD ($P \leq 0.05$)		0.08	1.1	0.7	9.8	0.2
	CV (%)		4.0	7.8	6.5	11.3	9.0

Table 4.4: Mean performance of melon accessions for various morphological characters

S. No.	Genotypes	Code	Flesh thickness (cm)	Rind thickness (mm)	Days to 1 st female flower appearance	Days to 1 st fruit harvest	Days to last fruit harvest
1	Afghanistan Collection -1	M1	2.9	5.0	65.0	98.5	106.0
2	Afghanistan Collection -2	M2	2.2	4.8	70.0	95.0	106.0
3	Canary Yellow-1	M3	2.8	3.6	64.5	100.0	104.0
4	Carabean Gold	M4	2.3	4.4	60.5	101.0	105.0
5	Durgapura Madhu	M5	2.0	2.8	61.0	93.5	102.0
6	Dissected Leaf	M6	2.6	4.3	57.5	91.0	100.0
7	Durasol	M7	2.5	2.5	67.5	93.5	105.5
8	Green Flesh	M8	3.4	4.9	61.0	91.0	105.0
9	Hara Madhu	M9	1.9	1.5	68.5	100.0	107.0
10	Hemed	M10	3.0	4.1	59.0	95.5	103.0
11	Honey Dew	M11	2.4	4.9	63.5	100.0	107.0
12	IC-267375	M12	2.0	2.1	64.5	96.0	105.0
13	IC-274034	M13	1.6	1.4	64.0	90.5	106.0
14	IIVM-3	M14	2.5	4.5	68.5	100.0	115.5
15	MM selection-103	M15	2.8	4.8	68.0	93.0	106.0
16	Kajri-1	M16	2.1	3.0	67.5	94.5	108.5
17	Kajri-2	M17	2.4	3.0	66.5	94.0	108.5
18	Kajri Doctor	M18	2.0	3.0	67.5	96.5	111.0
19	Khasta Kharbooza	M19	3.0	4.3	58.5	94.5	106.5
20	MC-2012-6	M20	1.5	1.0	63.5	92.0	107.0
21	MC-2013-2	M21	1.9	4.8	64.0	99.0	108.0
22	MC-2014-2	M22	2.3	4.8	64.0	92.5	105.0
23	MM-105	M23	2.0	3.3	69.0	99.0	105.0
24	MM-105-1	M24	2.1	3.4	71.0	99.5	104.5
25	MM-2012-4	M25	2.0	4.1	68.5	105.0	110.0

Table 4.4 Cont.

S. No.	Genotypes	Code	Flesh thickness (cm)	Rind thickness (mm)	Days to 1 st female flower appearance	Days to 1 st fruit harvest	Days to last fruit harvest
26	MM-2014-11	M26	3.3	4.6	67.5	97.5	105.5
27	MM-2014-13	M27	2.7	5.3	65.0	94.5	104.5
28	MM-2014-2	M28	3.3	4.8	65.0	94.0	103.0
29	MM-2014-2-1	M29	3.3	4.8	66.5	95.0	107.5
30	MM-2014-3	M30	2.8	5.0	67.0	95.5	105.5
31	MM-3864	M31	2.2	2.0	67.0	91.5	107.5
32	MM-3965	M32	2.0	2.0	61.5	93.0	106.5
33	MM-4226	M33	1.4	2.4	64.0	91.0	103.0
34	MM-4279	M34	2.4	3.5	64.5	80.5	109.5
35	MM-4305	M35	1.6	3.1	67.0	93.5	106.5
36	MM Var-3	M36	2.4	3.9	66.5	98.5	106.5
37	MM var-4	M37	2.0	2.0	64.0	92.5	107.0
38	MS-3	M38	3.1	4.8	65.5	96.5	109.5
39	Natal	M39	2.1	3.5	61.5	99.0	107.0
40	PAUS-11	M40	2.9	4.6	65.5	92.5	104.5
41	PAUS-12	M41	2.9	3.4	63.0	96.0	100.0
42	PAUS-13	M42	2.6	4.6	56.5	92.0	104.0
43	PAUS-14	M43	2.9	5.0	65.0	95.5	107.5
44	PAUS-15	M44	2.7	4.8	64.0	92.5	102.0
45	PAUS-16	M45	2.9	3.4	65.0	97.5	109.5
46	PAUS-17	M46	2.9	3.6	63.0	89.5	105.0
47	PAUS-17-1	M47	2.9	3.3	64.0	90.0	104.0
48	PAUS-18	M48	2.4	4.6	67.0	93.0	102.5
49	PAUS-19	M49	2.9	4.9	61.0	93.5	102.0
50	PAUS-26	M50	2.4	3.8	62.5	94.0	102.0

Table 4.4 Cont.

S. No.	Genotypes	Code	Flesh thickness (cm)	Rind thickness (mm)	Days to 1 st female flower appearance	Days to 1 st fruit harvest	Days to last fruit harvest
51	PAUS-27	M51	2.7	5.0	60.0	96.0	105.0
52	PAUS-27-1	M52	2.9	5.1	61.0	95.5	105.5
53	PAUS-28	M53	2.3	3.8	61.5	98.5	105.5
54	PAUS-30	M54	2.2	7.1	70.5	99.0	104.5
55	PAUS-31	M55	2.7	4.5	68.5	100.0	105.5
56	PAUS-4	M56	2.5	3.3	66.0	91.5	107.0
57	PAUS-8	M57	2.7	3.9	64.5	91.5	107.0
58	PAUS-9	M58	2.6	5.3	68.5	96.0	104.5
59	PAUS-9-1	M59	2.7	5.5	65.0	97.0	105.0
60	Punjab Sunheri	M60	2.0	3.3	69.5	99.0	109.5
61	Riogold	M61	3.2	5.3	59.0	97.0	104.0
62	SM-2013-18	M62	1.7	1.0	68.0	91.5	105.0
63	SM-2013-2	M63	1.1	1.0	57.0	91.0	101.5
64	SM-2013-5	M64	1.8	1.6	65.0	91.0	102.0
65	SM-2013-9	M65	1.0	1.0	61.0	91.5	98.5
66	SM-2014-7	M66	1.1	1.0	64.0	97.5	104.0
67	SM-2015-2	M67	1.2	1.5	71.0	99.0	104.0
68	WM-11	M68	1.0	1.3	70.0	93.0	101.0
69	WM-2013-2	M69	0.8	1.5	61.5	92.5	99.0
70	Canary Yellow-2	M70	3.3	3.6	58.5	95.5	103.0
	LSD ($P \leq 0.05$)		0.4	0.8	3.4	6.8	3.9
	CV (%)		7.8	11.3	2.7	3.6	1.9

iii) Fruit quality traits

a) Total soluble solids (%)

Significant differences were observed for TSS content (Table 4.1). Genotype MM var-4 possessed the highest total soluble solid content among all the seventy genotypes which was (11.3) followed by MM-4279 (10.6) and MM selection-103 (10.1). However, snapmelon genotypes reported least total soluble solid content found in genotype SM-2013-2 (1.6) which was statistically at par with genotypes IC-274034 (1.8) and SM-2014-7 (1.8). Average value for TSS for all the genotypes reported was 7.4 (Table 4.7). Box-plot analysis indicated maximum variability for TSS content in group *cantalupensis* and minimum in *momordica* group (Fig. 4.2). Reddy *et al* (1990) examined melon varieties comprising desert and non-desert types for TSS variation and reported that medium TSS varieties had very high variability for TSS content. Szamosi *et al* (2010) reported among Hungarian melon TSS ranged from 5.5 to 15.5 and in Turkish melon varied from 2.7 to 12.7.

b) β - carotene (mg/100g of fresh fruit flesh)

Muskmelon contains substantial amount of β -carotene content which is the important components of a healthy human diet. Significant variability was reported in all the melon genotypes for β -carotene content (Table 4.1). β -carotene content ranged from 0.02 to 3.32 mg/100 gm. Maximum β -carotene was reported in orange fleshed genotypes such as, MS-3 (3.3 mg/100g), PAUS-11 (2.5 mg/100g) and PAUS-31 (2.1 mg/100 g). However, white fleshed genotypes reported minimum β -carotene i.e. Natal (0.02 mg/100 g) followed by Canary yellow-2 (0.03 mg/ 100 g) and WM-2013-2 (0.06 mg/100 g) as presented in Table 4.7. Box-plot analysis indicated maximum variability for β -carotene content in group *reticulatus* and minimum in *callosus* group (Fig. 4.3). In another study, Watanabe *et al* (1991) reported that β -carotene content varied from 9.2 to 18.0 $\mu\text{g g}^{-1}$ depending upon the varieties. Similarly, Crosby *et al* (2007) also reported that carotenoid content changed with the flesh colour and ranged from 0 in white-fleshed to 40 $\mu\text{g g}^{-1}$ in dark orange-fleshed genotypes.

c) Ascorbic acid content (mg/100 g of fresh fruit flesh)

There existed a significant variability for ascorbic acid content (Table 4.1). Ascorbic acid content ranged from 14.5 to 46.3 mg/ 100 g with mean value of 26.8 mg/ 100 g. Maximum ascorbic acid content was found in genotype Punjab Sunhehri (46.3 mg/ 100 g) followed by MM selection-103 (38.8 mg/ 100 g) and MM-105-1 (36.3 mg/ 100 g). However, genotype MM-2014-2 (14.5 mg/ 100 g) possessed minimum ascorbic acid content followed by PAUS-8 (15.3 mg/ 100 g) and Canary Yellow-1 (15.8 mg/ 100 g) as presented in table 4.7. Box-plot analysis indicated maximum variability for ascorbic acid content in group *reticulatus* and minimum in *callosus* group (Fig. 4.3). However, Sharma and Lal (2004) analyzed ten varieties of muskmelon for physiochemical constituents and reported that vitamin C content ranged from 8.3 to 23.1 mg/100 g of fresh weight.

d) Titrable acidity (g anhydrous citric acid/100 ml fruit juice)

The sugar/acid ratio determines the flavour of fruit. Titrable acidity (TA) of all the genotypes ranged from 0.09 to 0.63 with mean value of 0.22. Snapmelon genotypes possessed maximum TA SM-2013-2 (0.63) which was statistically at par with the TA of genotypes SM-2013-9 (0.62) and SM-2014-7 (0.60) as depicted in table 4.5. However, minimum was reported in MM-4279 (0.10) which was statistically at par with genotypes MM-4226 (0.10) and Hemed (0.11) (Table 4.7). Box-plot analysis indicated maximum variability for ascorbic acid content in group *cantalupensis* and minimum in *momordica* group (Fig. 4.3). Similar results were reported by Fergany *et al* (2011) , Dhillon *et al* (2009) and Dhillon *et al* (2007) with a range from 0.12 to 0.57%, 0.03 to 0.65% and 0.08 to 0.61% respectively.

e) pH

Value of pH varied from 3.9 to 7.0 with mean value of 5.9. Maximum pH value was observed in genotype PAUS-8 (7.0) which was statistically at par with genotypes MM-2014-11 (7.0), MM-3965 (7.0) and PAUS-31 (7.0). However, minimum was reported in MC-2012-6 (3.9) which was statistically at par with genotypes SM-2014-7 (4.0) and SM-2013-18 (4.0) (Table 4.7). Box-plot analysis indicated maximum variability for pH in group *cantalupensis* and minimum in *callosus* group (Fig. 4.3). According to Bianchi *et al* (2016) the pH of melon significant varies from 5.2 to 6.5 depending on the cultivar used. Parveen *et al* (2012) noted variations in pH (5.4 to 5.6) with *Ravi* melon variety. However, Beaulieu *et al* (2007) reported a slightly higher pH value (6.8) with *Cantaloupe* variety.

f) Firmness (lb/inch²)

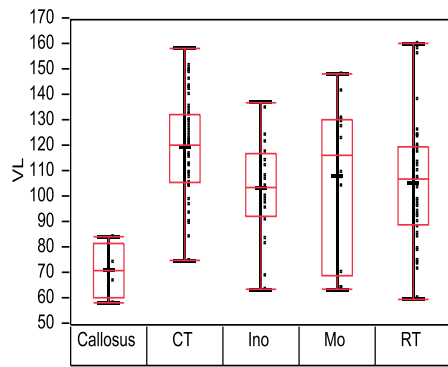
Firmness is an important trait for maintaining the quality of fruits. Genotype Hara Madhu (2.4 lb/inch²) possessed minimum firmness followed by MC-2012-6 (2.5 lb/inch²) and SM-2013-9 (3.8 lb/inch²). However, PAUS-9-1 (10.5 lb/inch²) genotype showed maximum firmness which was statistically at par with genotypes PAUS-9 (10.5 lb/inch²), Durgapura Madhu (10.5 lb/inch²) and Green flesh (10.5 lb/inch²) (Table 4.7). Box-plot analysis indicated maximum variability for firmness in group *reticulatus* and minimum in *callosus* group (Fig. 4.3).

g) Flesh color

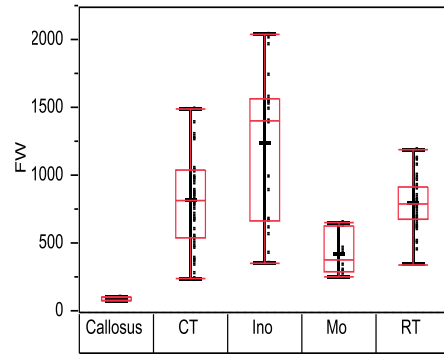
Great variability was recorded for color of flesh among all the genotypes viz., creamish-white, yellowish-green, green, and orange. Sixteen genotypes (22.9%) had creamish white flesh color, four genotypes (5.7%) had yellowish green color, eleven (15.7%) genotypes recorded green flesh color, and thirty-nine (55.74%) genotypes possessed orange flesh color (Table 4.5).

h) Flesh texture

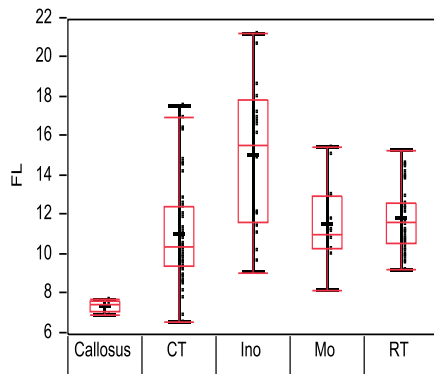
Sixty-one, sixteen and nine percent genotypes had intermediate, mealy and crispy texture, respectively as depicted in table 4.6.



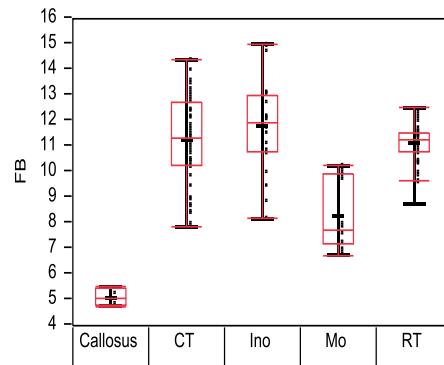
(A) Vine length



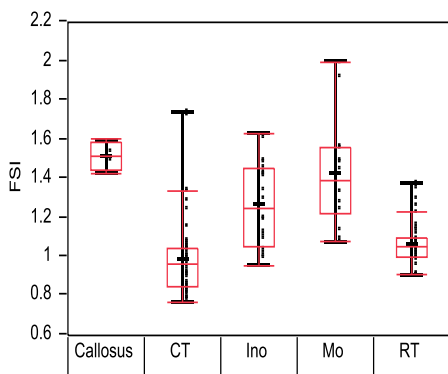
(B) Fruit weight



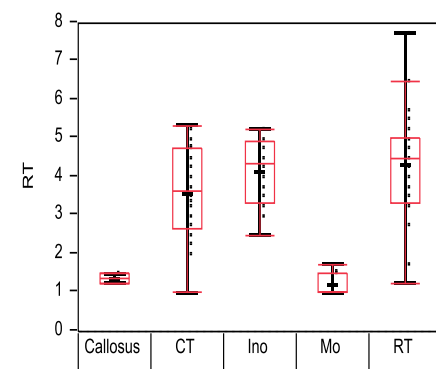
(C) Fruit length



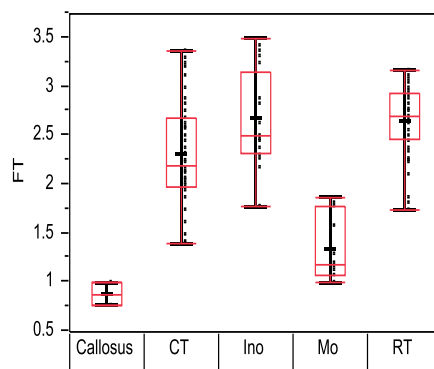
(D) Fruit weight



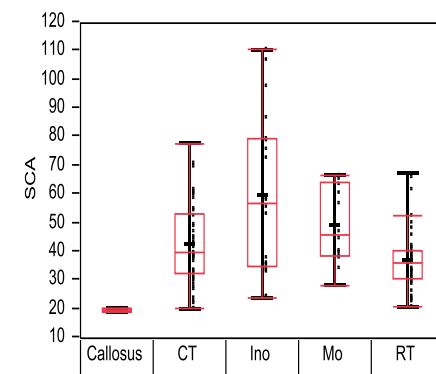
(E) Fruit shape index



(F) Rind thickness

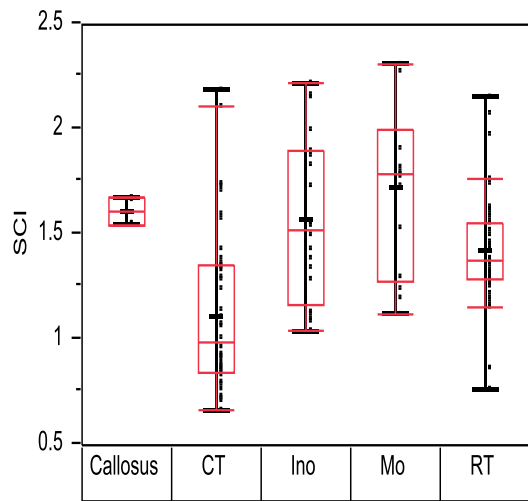


(G) Flesh thickness

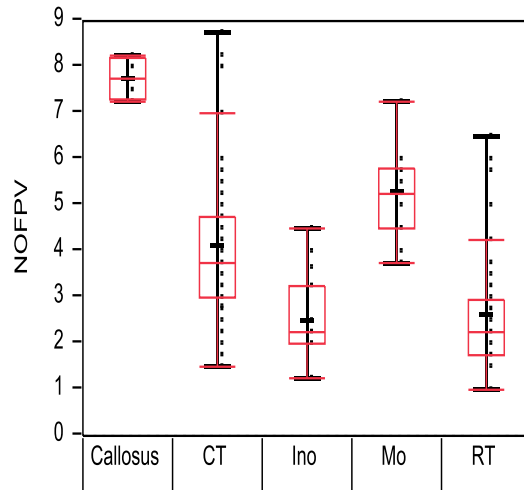


(H) Seed cavity area

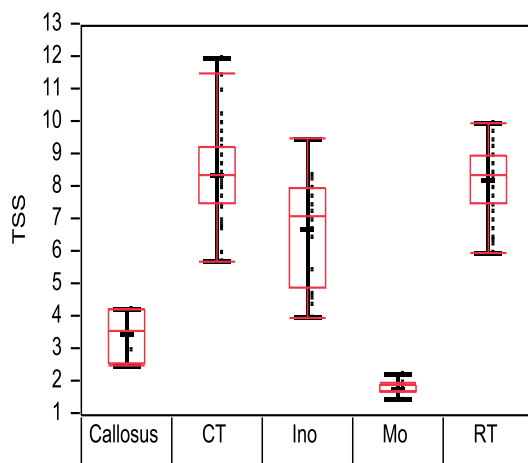
Figure 4.1: Box-plot analysis of five melon groups



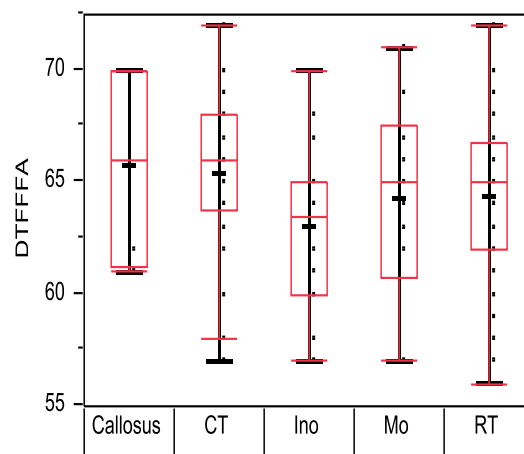
(A) Seed cavity index



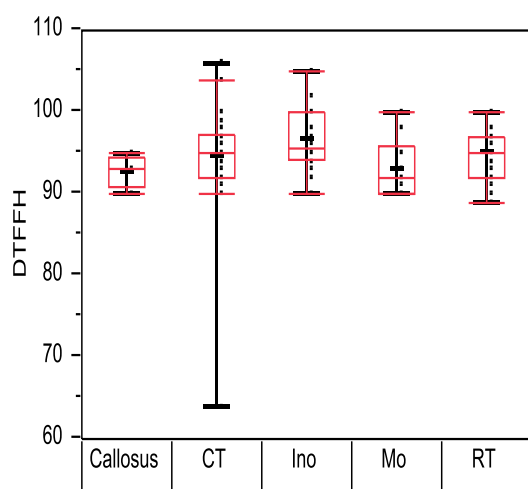
(B) Number of fruits per vine



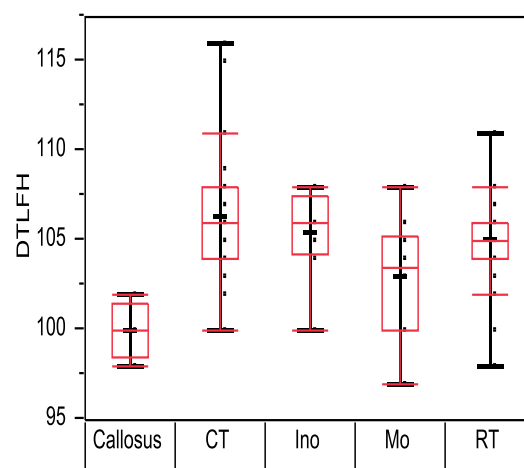
(C) TSS content



(D) Days to first female flower appearance

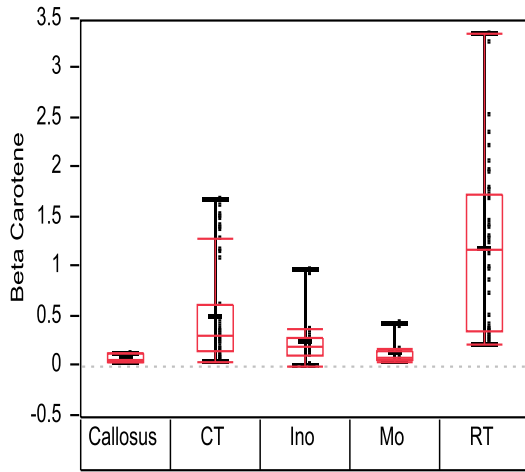


(E) Days to first fruit harvest

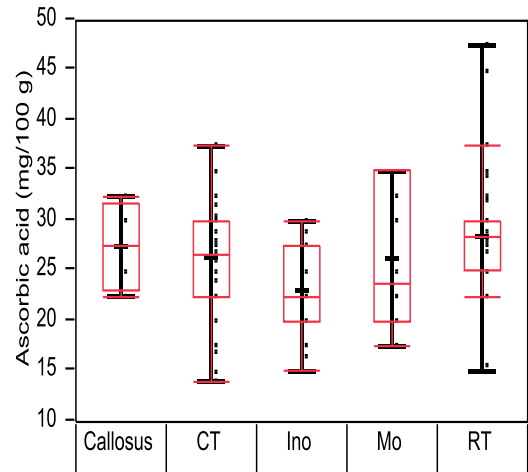


(F) Days to last last fruit harvest

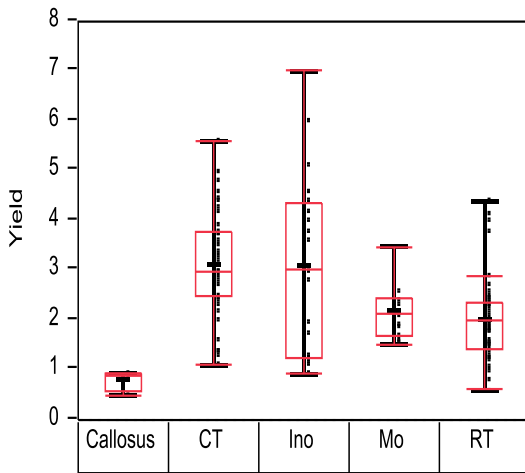
Figure 4.2: Box-plot analysis of five melon groups



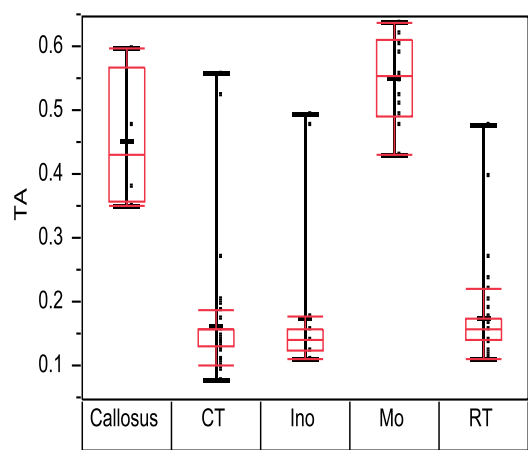
(A) β -carotene content



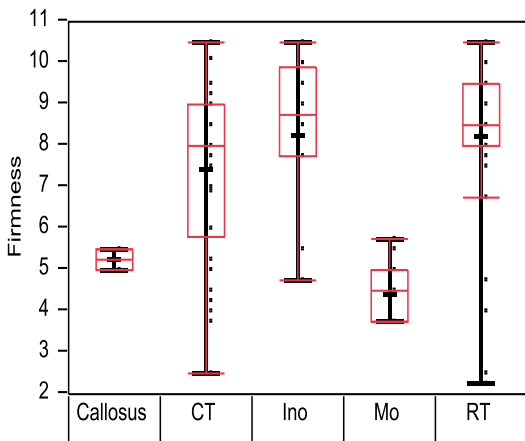
(B) Ascorbic acid content



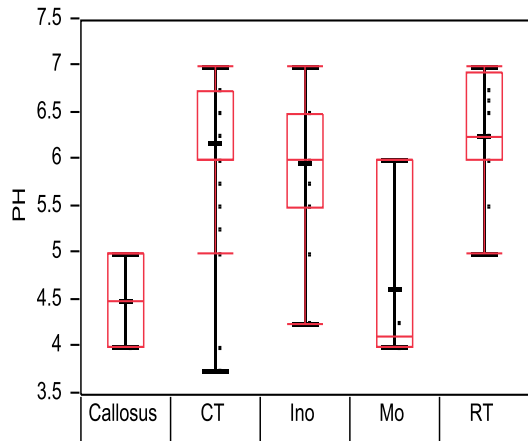
(C) Yield



(D) Titrable acidity



(E) Firmness



(F) pH

Figure 4.3: Box-plot analysis of five melon groups

Table 4.5: Brief morphological description of different melon accessions studied

S. No.	Genotypes	Fruit flesh color	Leaf Shape	Rind color	Fruit patches	Fruit peduncle at maturity
1	Afghanistan Collection -1	Creamish white	Pentalobate	Yellow Green	Present	Non-slipable
2	Afghanistan Collection -2	Creamish white	Entire	Yellow Green	Present	Non-slipable
3	Canary Yellow-1	Orange	5-palmately lobed	Yellow	Absent	Non-slipable
4	Carabean Gold	Creamish white	Entire	Yellow	Absent	Non-slipable
5	Durgapura Madhu	Orange	5-palmately lobed	Orange	Absent	Slipable
6	Dissected Leaf	Yellowish green	Trilobate	Yellow Green	Absent	Slipable
7	Durasol	Orange	Entire	Yellow	Absent	Non-slipable
8	Green Flesh	Green	Pentalobate	Yellow	Absent	Non-slipable
9	HaraMadhu	Green	Pentalobate	Yellow Green	Absent	Non-slipable
10	Hemed	Orange	Pentalobate	Orange	Absent	Slipable
11	Honey Dew	Orange	Pentalobate	Orange	Absent	Non-slipable
12	IC-267375	Yellowish green	Pentalobate	Creamy white	Absent	Slipable
13	IC-274034	Creamish	Entire	Creamy white	Absent	Slipable
14	IIVM-3	Orange	Entire	Yellow Green	Absent	Slipable
15	MM selection-103	Green	Entire	Orange	Absent	Slipable
16	Kajri-1	Green	Entire	Yellow Green	Absent	Slipable
17	Kajri-2	Green	Entire	Yellow Green	Absent	Slipable
18	Kajri Doctor	Green	Entire	Yellow Green	Absent	Slipable
19	Khasta Kharbooza	Creamish white	Pentalobate	Yellow Green	Present	Non-slipable
20	MC-2012-6	Creamish white	Pentalobate	Creamy white	Absent	Slipable
21	MC-2013-2	Orange	Pentalobate	Creamy white	Absent	Slipable
22	MC-2014-2	Orange	Pentalobate	Creamy white	Absent	Slipable
23	MM-105	Green	Entire	Orange	Absent	Slipable
24	MM-105-1	Green	Entire	Yellow Green	Absent	Slipable

Table 4.5: Cont...

S. No.	Genotypes	Fruit flesh color	Leaf Shape	Rind color	Fruit patches	Fruit peduncle at maturity
25	MM-2012-4	Orange	Pentalobate	Orange	Absent	Slipable
26	MM-2014-11	Orange	Entire	Orange	Absent	Slipable
27	MM-2014-13	Orange	Pentalobate	Orange	Absent	Slipable
28	MM-2014-2	Orange	Pentalobate	Orange	Absent	Slipable
29	MM-2014-2-1	Orange	Pentalobate	Yellow Green	Absent	Slipable
30	MM-2014-3	Orange	Pentalobate	Orange	Absent	Slipable
31	MM-3864	Yellowish green	Entire	Orange	Absent	Slipable
32	MM-3965	Orange	Pentalobate	Orange	Absent	Slipable
33	MM-4226	Orange	Entire	Orange	Absent	Slipable
34	MM-4279	Green	5-Palmately lobed	Yellow Green	Absent	Slipable
35	MM-4305	Green	Entire	Orange	Absent	Slipable
36	MM Var-3	Green	Pentalobate	Orange	Absent	Slipable
37	MM var-4	Yellowish green	Pentalobate	Orange	Absent	Slipable
38	MS-3	Orange	Entire	Orange	Absent	Slipable
39	Natal	Creamish white	Pentalobate	Orange	Absent	Non-Slipable
40	PAUS-11	Orange	Pentalobate	Orange	Absent	Slipable
41	PAUS-12	Orange	Entire	Orange	Absent	Slipable
42	PAUS-13	Orange	Pentalobate	Orange	Absent	Slipable
43	PAUS-14	Orange	Entire	Orange	Absent	Slipable
44	PAUS-15	Orange	Pentalobate	Orange	Absent	Slipable
45	PAUS-16	Orange	Entire	Orange	Absent	Slipable
46	PAUS-17	Orange	Pentalobate	Orange	Absent	Slipable
47	PAUS-17-1	Orange	Pentalobate	Orange	Absent	Slipable
48	PAUS-18	Orange	Pentalobate	Orange	Absent	Slipable

Table 4.5: Cont...

S. No.	Genotypes	Fruit flesh color	Leaf Shape	Rind color	Fruit patches	Fruit peduncle at maturity
49	PAUS-19	Orange	Entire	Orange	Absent	Slipable
50	PAUS-26	Orange	Pentalobate	Orange	Absent	Slipable
51	PAUS-27	Orange	Pentalobate	Orange	Absent	Slipable
52	PAUS-27-1	Orange	Pentalobate	Orange	Absent	Slipable
53	PAUS-28	Orange	Pentalobate	Orange	Absent	Slipable
54	PAUS-30	Orange	Pentalobate	Orange	Absent	Slipable
55	PAUS-31	Orange	Pentalobate	Orange	Absent	Slipable
56	PAUS-4	Orange	Entire	Orange	Absent	Slipable
57	PAUS-8	Orange	Pentalobate	Orange	Absent	Slipable
58	PAUS-9	Orange	Pentalobate	Orange	Absent	Slipable
59	PAUS-9-1	Creamish white	Pentalobate	Orange	Absent	Slipable
60	Punjab Sunheri	Orange	Entire	Orange	Absent	Slipable
61	Riogold	Orange	Pentalobate	Orange	Absent	Slipable
62	SM-2013-18	Creamish white	Pentalobate	Creamy white	Absent	Slipable
63	SM-2013-2	Creamish white	Pentalobate	Creamy white	Absent	Slipable
64	SM-2013-5	Creamish white	Entire	Creamy white	Absent	Slipable
65	SM-2013-9	Creamish white	Entire	Creamy white	Absent	Slipable
66	SM-2014-7	Creamish white	Pentalobate	Creamy white	Absent	Slipable
67	SM-2015-2	Orange	Pentalobate	Creamy white	Absent	Slipable
68	WM-11	Creamish white	Pentalobate	Creamy white	Absent	Slipable
69	WM-2013-2	Creamish white	Pentalobate	Creamy white	Absent	Slipable
70	Canary Yellow-2	Creamish white	Pentalobate	Yellow	Absent	Non-slipable

Table 4.6: Brief morphological description of different melon accessions studied

S. No.	Genotypes	Fruit shape at peduncle end	Fruit shape at blossom end	Fruit surface	Fruit sutures	Fruit netting	Flesh texture
1	Afganistan Collection -1	Pointed	Pointed	Grooved	Absent	Absent	Crispy
2	Afganistan Collection -2	Pointed	Pointed	Grooved	Absent	Absent	Crispy
3	Canary Yellow-1	Pointed	Pointed	Grooved	Absent	Absent	Crispy
4	Carabean Gold	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
5	Durgapura Madhu	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
6	Dissected Leaf	Pointed	Intermediate	Smooth	Absent	Absent	Mealy
7	Durasol	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
8	Green Flesh	Rounded	Intermediate	Smooth	Absent	Absent	Crispy
9	Hara Madhu	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
10	Hemed	Rounded	Intermediate	Smooth	Present	Absent	Mealy
11	Honey Dew	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
12	IC-267375	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
13	IC-274034	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
14	IIVM-3	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
35	MM selection-103	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
15	Kajri-1	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
16	Kajri-2	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
17	Kajri Doctor	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
18	Khasta Kharbooza	Pointed	Pointed	Grooved	Absent	Absent	Crispy
19	MC-2012-6	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
20	MC-2013-2	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
21	MC-2014-2	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
22	MM-105	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
23	MM-105-1	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate

Table 4.6: Cont

S. No.	Genotypes	Fruit shape at peduncle end	Fruit shape at blossom end	Fruit surface	Fruit sutures	Fruit netting	Flesh texture
24	MM-2012-4	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
25	MM-2014-11	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
26	MM-2014-13	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
27	MM-2014-2	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
28	MM-2014-2-1	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
29	MM-2014-3	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
30	MM-3864	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
31	MM-3965	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
32	MM-4226	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
33	MM-4279	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
34	MM-4305	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
36	MM Var-3	Rounded	Intermediate	Smooth	Present	Absent	Intermediate
37	MM var-4	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
38	MS-3	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
39	Natal	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
40	PAUS-11	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
41	PAUS-12	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
42	PAUS-13	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
43	PAUS-14	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
44	PAUS-15	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
45	PAUS-16	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
46	PAUS-17	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
47	PAUS-17-1	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
48	PAUS-18	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate

Table 4.6: Cont

S. No.	Genotypes	Fruit shape at peduncle end	Fruit shape at blossom end	Fruit surface	Fruit sutures	Fruit netting	Flesh texture
49	PAUS-19	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
50	PAUS-26	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
51	PAUS-27	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
52	PAUS-27-1	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
53	PAUS-28	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
54	PAUS-30	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
55	PAUS-31	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
56	PAUS-4	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
57	PAUS-8	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
58	PAUS-9	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
59	PAUS-9-1	Rounded	Intermediate	Smooth	Absent	Dense	Intermediate
60	Punjab Sunheri	Rounded	Intermediate	Smooth	Absent	Moderate	Intermediate
61	Riogold	Rounded	Intermediate	Smooth	Absent	Absent	Intermediate
62	SM-2013-18	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
63	SM-2013-2	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
64	SM-2013-5	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
65	SM-2013-9	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
66	SM-2014-7	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
67	SM-2015-2	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
68	WM-11	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
69	WM-2013-2	Rounded	Intermediate	Smooth	Absent	Absent	Mealy
70	Canary Yellow-2	Pointed	Pointed	Grooved	Absent	Absent	Crispy

Table 4.7: Mean performance of melon accessions for various biochemical traits

S. no.	Genotypes	Code	TSS (°Brix)	β -carotene (mg/100gm)	Ascorbic acid (mg/100 gm)	Titration acidity (g anhydrous citric acid/ 100 ml fruit juice)	pH	Firmness (lb/ inch ²)
1	Afghanistan Collection -1	M1	4.0	0.21	28.8	0.2	7.0	9.0
2	Afghanistan Collection -2	M2	4.7	0.16	30.0	0.1	5.8	10.0
3	Canary Yellow-1	M3	7.8	0.13	15.8	0.2	5.0	7.8
4	Carabean Gold	M4	8.1	0.96	20.0	0.1	7.0	4.8
5	Durgapura Madhu	M5	8.5	0.21	22.5	0.2	6.0	10.5
6	Dissected Leaf	M6	7.2	0.31	26.3	0.2	6.5	7.6
7	Durasol	M7	7.4	0.37	28.3	0.2	6.3	9.8
8	Green Flesh	M8	6.0	0.22	26.3	0.1	6.5	10.5
9	Hara Madhu	M9	7.6	0.37	23.8	0.2	7.0	2.4
10	Hemed	M10	6.8	0.15	21.3	0.1	6.6	7.3
11	Honey Dew	M11	6.8	0.28	18.8	0.2	6.5	9.0
12	IC-267375	M12	7.9	0.48	33.8	0.1	6.8	3.9
13	IC-274034	M13	1.8	0.44	32.5	0.5	4.1	5.6
14	IIVM-3	M14	9.5	1.57	29.5	0.2	6.9	9.4
15	MM selection-103	M15	10.1	1.62	30.8	0.1	7.0	9.8
16	Kajri-1	M16	8.6	0.36	25.5	0.2	5.3	7.2
17	Kajri-2	M17	8.9	0.33	25.8	0.2	5.4	7.3
18	Kajri Doctor	M18	8.9	0.33	26.3	0.2	5.8	7.5
19	Khasta Kharbooza	M19	6.1	0.22	21.3	0.1	6.0	7.8
20	MC-2012-6	M20	6.4	0.22	28.8	0.5	3.9	2.5
21	MC-2013-2	M21	7.6	1.42	24.5	0.1	6.0	5.0
22	MC-2014-2	M22	5.9	0.10	17.3	0.2	6.0	9.5
23	MM-105	M23	7.8	0.45	36.3	0.2	6.3	8.8

Table 4.7 Cont.

S. no.	Genotypes	Code	TSS (°Brix)	β-carotene (mg/100gm)	Ascorbic acid (mg/100 gm)	Titration acidity (g anhydrous citric acid/ 100 ml fruit juice)	pH	Firmness (lb/ inch²)
24	MM-105-1	M24	7.9	0.37	36.3	0.2	6.1	8.0
25	MM-2012-4	M25	9.4	1.24	23.8	0.2	6.0	8.5
26	MM-2014-11	M26	7.8	0.62	23.8	0.1	7.0	8.5
27	MM-2014-13	M27	8.9	1.14	26.0	0.2	6.0	8.0
28	MM-2014-2	M28	9.3	0.08	14.5	0.2	6.0	9.5
29	MM-2014-2-1	M29	9.4	0.10	17.3	0.2	6.4	8.8
30	MM-2014-3	M30	7.5	1.46	29.0	0.2	5.4	5.6
31	MM-3864	M31	9.1	0.15	28.8	0.1	6.0	4.6
32	MM-3965	M32	8.5	0.31	31.3	0.1	7.0	8.0
33	MM-4226	M33	8.4	0.23	29.5	0.1	7.0	9.0
34	MM-4279	M34	10.6	0.26	31.3	0.1	7.0	4.5
35	MM-4305	M35	6.4	0.13	17.3	0.1	6.8	5.3
36	MM Var-3	M36	8.3	0.15	30.3	0.2	6.0	8.5
37	MM var-4	M37	11.3	0.07	25.5	0.2	6.3	8.0
38	MS-3	M38	8.9	3.32	30.0	0.2	5.0	8.5
39	Natal	M39	8.2	0.02	23.8	0.2	5.5	5.5
40	PAUS-11	M40	8.9	2.46	29.5	0.2	5.5	8.0
41	PAUS-12	M41	8.1	0.37	34.8	0.3	6.0	10.0
42	PAUS-13	M42	8.0	1.19	32.3	0.1	6.0	10.0
43	PAUS-14	M43	8.5	1.46	28.8	0.2	6.0	8.5
44	PAUS-15	M44	6.4	0.48	23.8	0.2	7.0	9.5
45	PAUS-16	M45	8.8	1.39	31.3	0.2	6.1	8.0
46	PAUS-17	M46	9.9	0.30	25.0	0.2	6.4	9.0
47	PAUS-17-1	M47	9.6	0.24	25.0	0.2	6.3	9.0
48	PAUS-18	M48	8.9	1.75	27.5	0.1	5.5	9.5
49	PAUS-19	M49	8.6	0.34	29.0	0.1	6.4	9.5
50	PAUS-26	M50	8.4	2.02	26.0	0.2	6.3	9.0
51	PAUS-27	M51	8.3	1.13	28.8	0.2	7.0	8.0

Table 4.7 Cont.

S. no.	Genotypes	Code	TSS (°Brix)	β-carotene (mg/100gm)	Ascorbic acid (mg/100 gm)	Titration acidity (g anhydrous citric acid/ 100 ml fruit juice)	pH	Firmness (lb/ inch ²)
52	PAUS-27-1	M52	9.5	1.23	27.5	0.2	7.0	8.5
53	PAUS-28	M53	9.3	1.57	33.8	0.2	6.7	7.9
54	PAUS-30	M54	6.5	0.87	26.3	0.4	5.0	9.5
55	PAUS-31	M55	7.5	2.12	32.5	0.2	7.0	8.5
56	PAUS-4	M56	7.3	0.94	28.8	0.2	6.0	4.0
57	PAUS-8	M57	6.9	1.11	15.3	0.2	7.0	4.8
58	PAUS-9	M58	6.9	0.30	22.5	0.2	6.3	10.5
59	PAUS-9-1	M59	7.3	0.28	22.5	0.2	6.3	10.5
60	Punjab Sunheri	M60	8.8	1.72	46.3	0.2	6.8	7.5
61	Riogold	M61	8.9	1.77	33.8	0.1	6.0	6.8
62	SM-2013-18	M62	1.8	0.08	17.5	0.5	4.0	3.8
63	SM-2013-2	M63	1.6	0.09	33.8	0.6	4.3	4.5
64	SM-2013-5	M64	1.8	0.08	35.0	0.5	6.0	3.8
65	SM-2013-9	M65	2.1	0.10	20.0	0.6	6.0	3.8
66	SM-2014-7	M66	1.8	0.11	23.8	0.6	4.0	4.5
67	SM-2015-2	M67	1.8	0.18	21.3	0.5	4.0	5.0
68	WM-11	M68	4.3	0.13	23.8	0.5	5.0	5.5
69	WM-2013-2	M69	2.8	0.06	31.3	0.4	4.0	5.0
70	Canary Yellow-2	M70	8.3	0.04	18.8	0.5	4.3	8.5
	LSD ($P \leq 0.05$)			0.13	3.3	0.04	0.22	0.3
	CV (%)			9.6	6.2	9.0	1.8	2.1

4.1.1 Principal component analysis (PCA)

Cluster diagram was generated using principal component analysis (PCA). Eigen values and contribution percentage of each principal component axis were calculated using the correlation matrix. In present study 70 melon genotypes belonging *reticulatus*, *cantaloupensis*, *inodorous*, *momordica* and *callosus* horticultural groups were evaluated which were collected from different regions around the world. Thirty-three qualitative and quantities characters were assessed and large genetic variability was observed in melon genotypes with respect to β -carotene content, titrable acidity, number of fruits per vine, fruit weight, yield, firmness, etc (Table 4.9) . Principal component analysis was used to describe the variation in melon accessions. The percentage of variation explained by the first seven components was 29.2%, 17.3%, 12.4%, 9.3%, 6.2%, 4.4% and 3.9% respectively (Table 4.8 and Fig. 4.2). The principal components with higher eigenvalues that delineated the accessions into separate groups in the first seven components are presented in Table 4.8.

Table 4.8: Principle component analysis

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigen values	5.2	3.1	2.2	1.7	1.1	0.8	0.7
% variance	29.2	17.3	12.4	9.3	6.2	4.4	3.9
Cumulative % variance	29.2	46.4	55.8	65.1	71.3	75.6	79.5
VL	0.051	-0.024	0.447	-0.221	0.270	-0.221	-0.321
FW	0.316	0.360	0.120	-0.055	0.045	-0.140	0.012
NOFPV	-0.290	-0.170	0.361	-0.122	0.055	0.165	0.033
Yld	0.166	0.139	0.531	-0.144	0.041	-0.010	0.013
FSI	-0.171	0.422	-0.054	0.146	0.184	0.153	-0.105
SCA	0.133	0.494	0.228	0.068	0.105	-0.004	0.044
FT	0.379	0.073	-0.130	-0.099	0.017	-0.264	0.102
RT	0.377	0.093	-0.140	0.120	0.102	-0.019	0.003
DTFFFA	0.011	-0.068	0.350	0.465	-0.151	0.269	0.517
DTFFH	0.107	0.017	0.050	0.585	-0.096	0.227	-0.648
DTLFH	0.163	-0.202	0.328	0.277	-0.289	-0.334	0.077
TSS	0.310	-0.306	-0.024	-0.099	-0.146	-0.021	0.057
Bcaro	0.172	-0.262	-0.128	0.355	0.364	-0.332	-0.021
AA	-0.015	-0.313	0.096	0.066	0.723	0.129	0.064
TA	-0.347	0.189	-0.035	0.172	0.117	-0.214	0.105
PH	0.278	-0.168	0.058	-0.237	-0.078	0.495	-0.237
Firm	0.302	0.127	-0.135	0.029	0.228	0.396	0.323

Legend: VL: Vine length, FW: Fruit weight, NOFPV: Number of fruit per vine, Yld: Yield (kg/plant), FSI: Fruit shape index, SCA: Seed cavity area, FT: Flesh thickness, RT: Rind thickness, DTFFFA: Days to 1st female flower appearance, DTFFH: Days to 1st fruit harvest, DTLFH: Days to last fruit harvest, TSS: Total soluble solids, Bcaro: β -carotene (mg/100 gm), AA: Ascorbic acid (mg/100 gm), TA: Titrable acidity, PH: pH, Firm: Firmness.

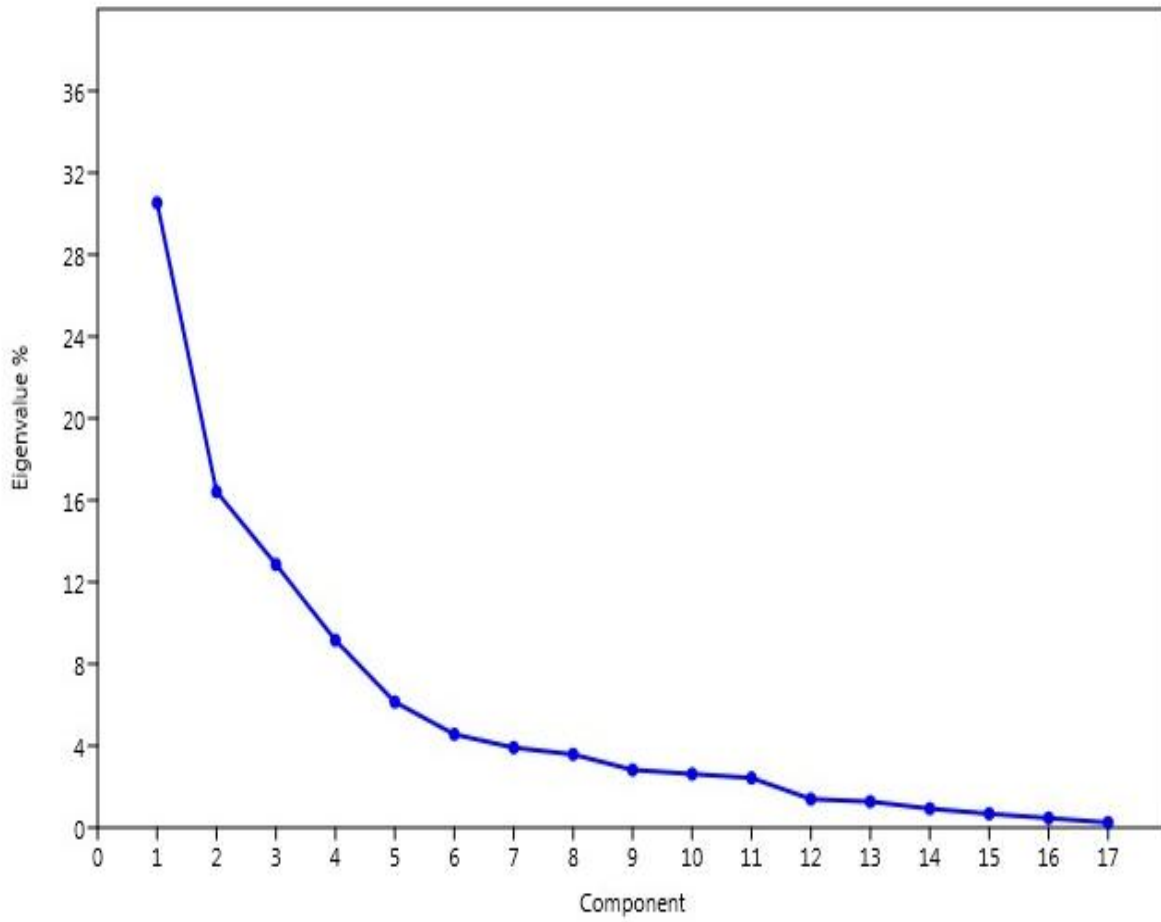


Figure 4.4: Screeplot of PCA of muskmelon genotypes

Table 4.9: Descriptive analysis of twenty-one variable of seventy muskmelon genotypes

S. No.	Variable	N	Mean	Minimum	Maximum	Range	Skewness	Kurtosis	CV (%)	Std dev	SE	Variance
1	VL	140	44.1	23.5	64.3	40.8	-0.1	-0.5	22.1	9.7	0.8	94.6
2	FW	140	821.5	85.0	2050.0	1965.0	0.8	0.7	47.6	391.1	33.1	152934.0
3	FL	140	11.9	6.6	21.3	14.6	1.0	1.2	23.9	2.8	0.2	8.1
4	FB	140	10.8	4.8	15.0	10.3	-0.7	1.0	18.1	2.0	0.2	3.9
5	FSI	140	1.1	0.8	2.0	1.2	1.2	1.5	21.4	0.2	0.0	0.1
6	SCL	140	7.5	4.1	15.4	11.3	1.1	1.1	30.1	2.3	0.2	5.1
7	SCB	140	5.6	3.4	7.9	4.5	0.1	-0.8	18.7	1.1	0.1	1.1
8	SCA	140	43.5	19.1	110.8	91.6	1.3	2.2	40.0	17.4	1.5	302.7
9	FT	140	2.4	0.8	3.5	2.7	-0.5	-0.2	26.8	0.6	0.1	0.4
10	RT	140	3.6	1.0	7.8	6.8	-0.3	-0.6	39.4	1.4	0.1	2.0
11	TSS	140	7.3	1.5	12.0	10.5	-1.1	0.6	32.8	2.4	0.2	5.7
12	NOFPV	140	3.6	1.0	8.8	7.8	0.9	0.2	50.5	1.8	0.2	3.3
13	DTFFFA	140	64.6	56.0	72.0	16.0	-0.4	-0.4	5.8	3.7	0.3	13.8
14	DTFFH	140	95.0	64.0	106.0	42.0	-2.1	15.9	4.7	4.4	0.4	19.7
15	DTLFH	140	105.3	97.0	116.0	19.0	0.1	0.9	3.0	3.2	0.3	10.2
16	Yld	140	2.6	0.5	7.0	6.5	0.8	0.5	47.3	1.2	0.1	1.5
17	Bcaro	140	0.7	0.0	3.4	3.4	1.5	1.9	105.7	0.7	0.1	0.5
18	AA	140	26.7	14.0	47.5	33.5	0.2	0.6	22.4	6.0	0.5	35.6
19	TA	140	0.2	0.1	0.6	0.6	1.8	1.8	67.3	0.1	0.01	0.02
20	PH	140	6.0	3.8	7.0	3.3	-0.9	0.2	14.8	0.9	0.1	0.8
21	Firm	140	7.5	2.3	10.5	8.3	-0.6	-0.8	29.2	2.2	0.2	4.7

Legend: VL: Vine length, FW: Fruit weight, NOFPV: Number of fruit per vine, Yld: Yield (kg/plant), FSI: Fruit shape index, FL: Fruit length, FB: Fruit breadth, SCA: Seed cavity area, SCL: Seed cavity length, SCB: Seed cavity breadth, FT: Flesh thickness, RT: Rind thickness, DTFFFA: Days to 1st female flower appearance, DTFFH: Days to 1st fruit harvest, DTLFH: Days to last fruit harvest, TSS: Total soluble solids, Bcaro: β -carotene (mg/100 gm), AA: Ascorbic acid (mg/100 gm), TA: Titrable acidity, PH: pH, Firm: Firmness.

4.1.2 Biplot analysis of PC1 and PC2

A biplot was constructed between PC1 and PC2, explaining 46.4% of variation. The clusters formed are mainly related to variation in fruit weight, number of fruits per vine, flesh thickness, rind thickness, TSS, Titrable acidity, pH, firmness. From Fig. 4.5, it was clear that the four clusters were formed with one un-clustered melon genotype contributed significantly to PC1. Similarly, fruit weight, fruit shape index, seed cavity area, TSS and ascorbic acid contributed to PC2. Thus, in the biplot genotypes were clustered on the basis of fruit size and biochemical characters. Thus, snapmelon accessions (SM-2015-2, SM-2013-18, SM-2013-5, SM-2013-9, SM-2014-7, SM-2013-2, IC-274034) having oval fruit shape, maximum number of fruits per plant, minimum flesh thickness, maximum titrable acidity were clustered in the left in cluster I. While, Cluster II consists of two wild melon genotypes (WM-2013-2 and WM-11) having maximum number of fruits per plant, minimum vine length belonging to group *callosus*. Cluster III had accessions (Afganistan Collection-1, Durgapura Madhu, Dissected Leaf, Green Flesh Hara Madhu, Hemed, Afganistan Collection-2, Honey Dew, Canary Yellow-1, Canary Yellow-2, MM-2014-2, PAUS-30, Khasta kharbooza, PAUS-9 MC-2014-2, MM-2014-11, MM-2014-2-1, MM-2014-13, PAUS-9-1) with highest fruit weight, fruit length, fruit breadth, seed cavity area, flesh thickness and rind thickness were on the right upper part of the scatter diagram. Thus, cluster three is related to fruit weight and size traits. Cluster IV had accessions (IIVM-3, MM selection-103, Kajri-, Kajri-2, Kajri Doctor, Khasta Kharbooza, MC-2014-2, MM-105, MC-2013-2, MM-105-1 MM-2012-4, MM-2014-11, MM-2014-13, MM-2014-2, MM-2014-2-1, MM-2014-3, MM-3864, MM-3965, MM-4226, MM-4279, MM-4305, MM Var-3, MM var-4, MS-3, Natal, PAUS-11, PAUS-12, PAUS-13, PAUS-14, PAUS-15, PAUS-16, PAUS-17, PAUS-17-1, PAUS-18, PAUS-19, PAUS-26, PAUS-27, PAUS-27-1, PAUS-28, PAUS-30, PAUS-31, PAUS-4, PAUS-8, PAUS-9, PAUS-9-1, Punjab Sunheri, Durasol, Carabean gold) with high β -carotene, TSS, pH and firmness, the trait related to fruit quality. One accession MC-2012-6, remained un-clustered, which had low values of rind thickness, flesh thickness, fruit weight and fruit length.

4.1.3 D² analysis

On the basis of 17 morphological descriptors Euclidean's inter and intra-cluster distance matrices of 70 melon genotypes are presented in Figure 4.6, where each circle is represented by a cluster number. Numerical within the circle represent intra-cluster distance, whereas figures on the connecting lines denote the inter-cluster distances. The inter-cluster distance varied from 759.6 between clusters 1 and 2 to 5194.5 between clusters 4 and 8. This indicated that high level of genetic divergence between any two accessions of melon. The maximum intra-cluster distance matrix was observed in cluster 9 (728.5) followed by clusters

followed by cluster 1 (588.3) and 7 (454.6). Cluster 6 exhibited the minimum intra-cluster variation (344.0). Maximum number of genotypes was grouped in cluster 1 and 7 i.e 15 in each group. However, individual genotype “Canary Yellow-2” of *inodorous* group was present in cluster 4 which did not grouped in any cluster. Clustering pattern of 70 melon genotypes based on the euclidean’s analysis is given in table 4.10.

The distribution of accessions into clusters by diversity analysis is independent to the horticulture group and geographic origin of the accessions with exception of cluster 9, in which all accessions belonging to *momordica* group were clustered with two accessions of *collosus* group and individual genotype of *cantalupensis* group.

Table 4.10: Clustering pattern of 70 melon genotypes on the basis of the Euclidean’s analysis

Cluster No.	No. of genotype(s)	Name of accession(s)
Cluster I	15	Afghanistan Collection -1, MM-2014-11, Honey Dew, Afghanistan Collection -2, MC-2014-2, Durgapura Madhu, PAUS-9, PAUS-9-1, PAUS-30, Dissected Leaf, Hemed, Khasta Kharboosa, MM-2014-2, MM-2014-2-1, Green Flesh
Cluster II	12	Durasol, PAUS-15, PAUS-17, PAUS-17-1, PAUS-12, PAUS-19, MM-105, MM-105-1, MM Var-3, MM-3965, MM-4226, MM var-4
Cluster III	5	Canary Yellow-1, Natal, Kajri-1, Kajri-2, Kajri Doctor
Cluster IV	1	Canary Yellow-2
Cluster V	5	Carabean Gold, MC-2013-2, MM-2014-3, PAUS-4, PAUS-8
Cluster VI	5	Hara Madhu, IC-267375, MM-3864, MM-4279, MM-4305
Cluster VII	15	IIVM-3, MM Selection-103, PAUS-28, PAUS-31, Punjab Sunheri, PAUS-14, MM-2012-4, MM-2014-13, PAUS-16, PAUS-27, PAUS-27-1, PAUS-18, PAUS-26, PAUS-13, Riogold
Cluster VIII	2	MS-3, PAUS-11
Cluster IX	10	SM-2013-18, SM-2013-2, SM-2013-5, SM-2013-9, SM-2014-7, SM-2015-2, WM-11, WM-2013-2, IC-274034, MC-2012-6

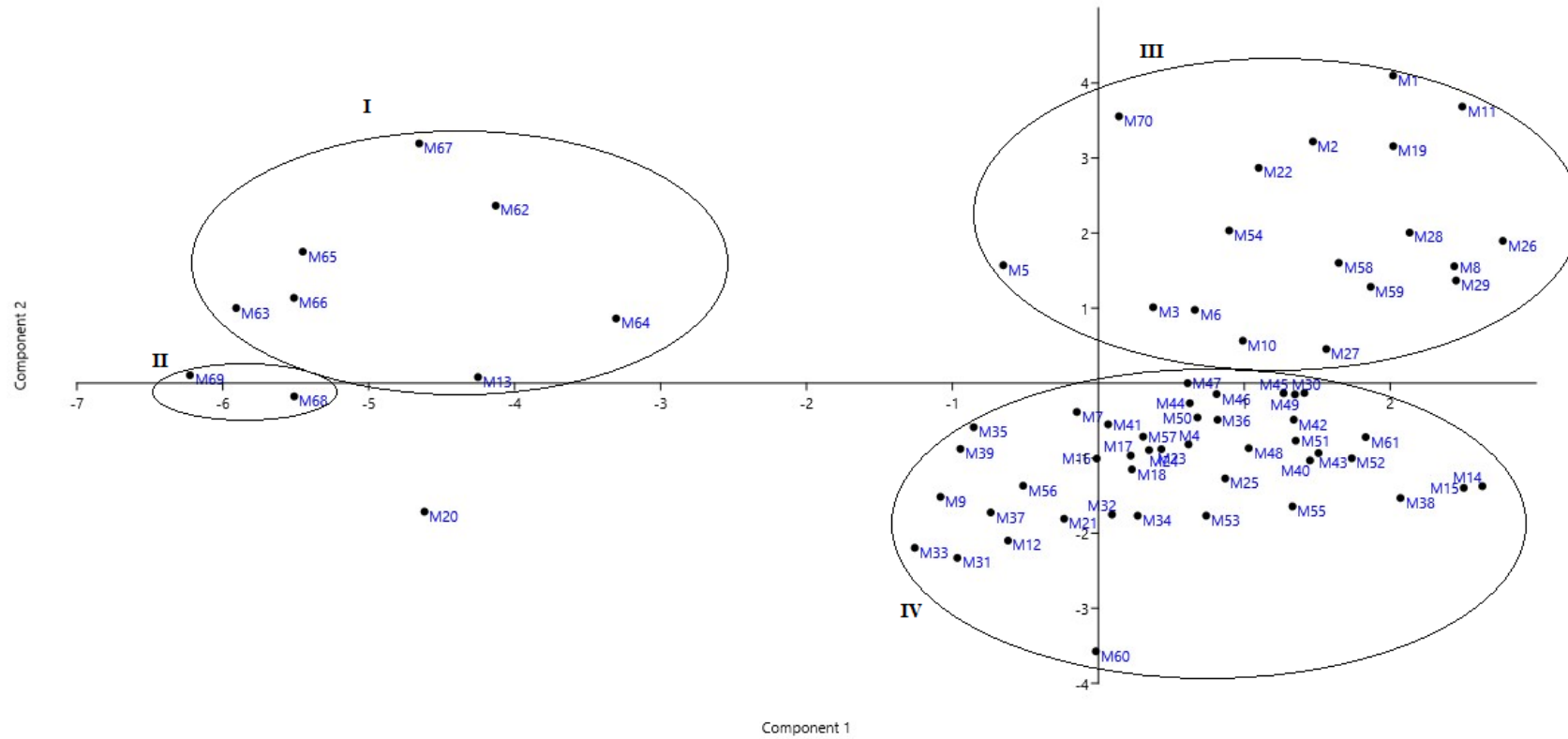


Figure 4.5: Cluster diagram constructed on the basis of biplot analysis of seventy melon genotypes (M1 to M70) as presented in table 3.1

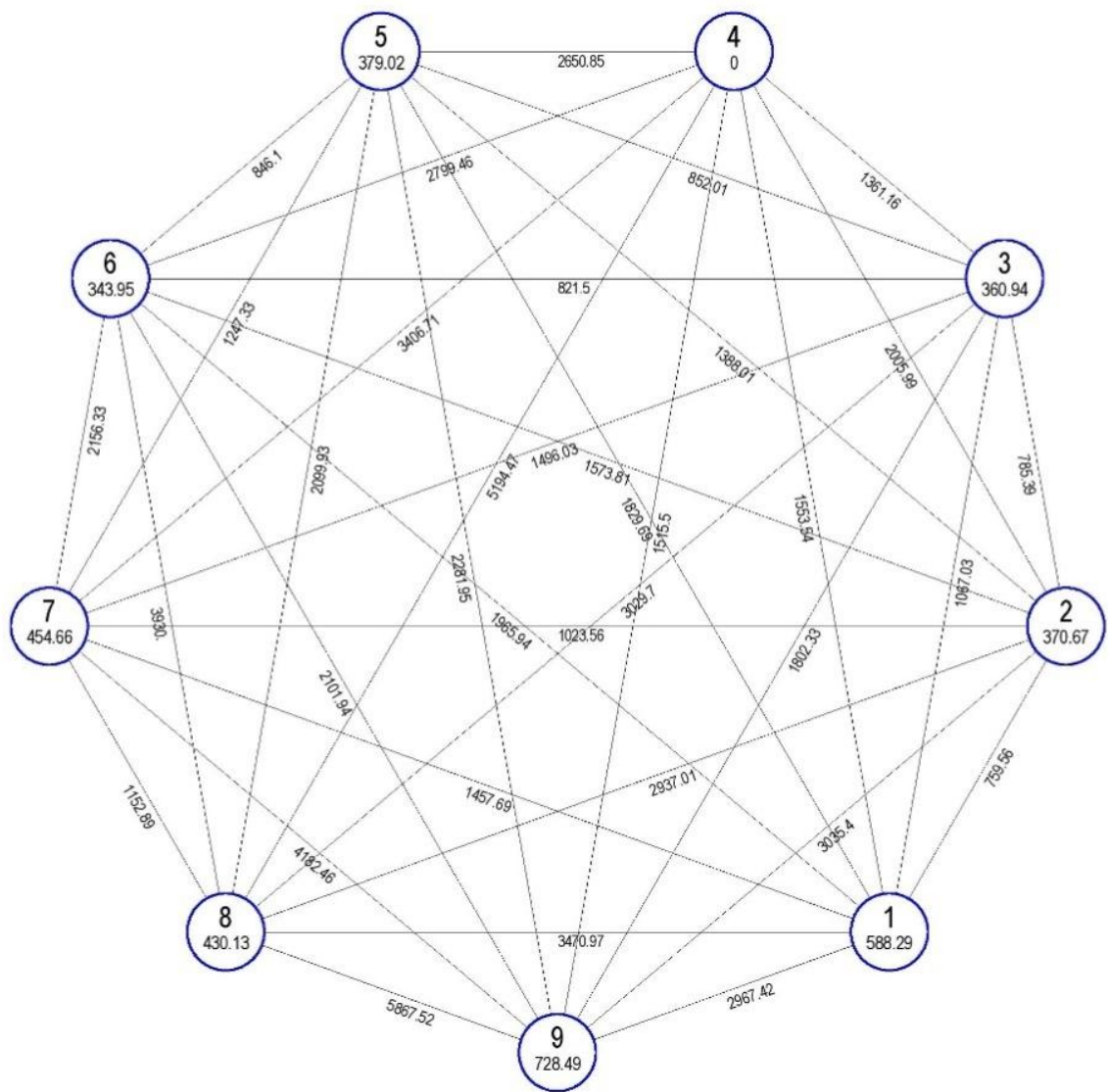


Figure 4.6: Euclidean distance (not to the scale) showing cluster analysis of the melon genotypes on basis of their phenotypic characters

4.1.4 Correlation coefficients

In plant breeding, degree of relationship of plant characters has always been useful in selection. Existence of association between the different characters is determined by studying the correlation existing among them. For this purpose, it is important to know genetic correlation among different plant characters, which may provide information regarding the correlated response to selection. Correlation coefficients between different plant characters are presented below Table 4.11.

a) Vine length

Vine length had significant and positive correlation with yield (0.46) and number of fruits per vine. While, it shows negative correlation with fruit shape index (-0.12).

b) Fruit weight

Fruit weight showed positive correlation with fruit yield (0.60), seed cavity area (0.75), flesh thickness (0.69), rind thickness (0.66), firmness (0.53). However, fruit weight had significantly negative correlation with number of fruits per vine (-0.55), ascorbic acid (-0.27) and titratable acidity (-0.38).

c) Number of fruits per vine

Number of fruits per vine had significantly negative correlation with seed cavity area (-0.29), flesh thickness (-0.71), rind thickness (-0.70), TSS (-0.30), β -carotene (-0.26), and firmness (-0.53). While, number of fruits per vine positive correlation with ascorbic acid (0.24) and titratable acidity (0.36).

d) Yield (kg/ plant)

Fruit yield given positive and significant correlation with seed cavity area (0.52), flesh thickness (0.25), days to 1st female flower appearance (0.23) and pH (0.29).

e) Fruit shape index

Fruit shape index had positive and significant correlation with seed cavity area (0.46) and titratable acidity (0.49), however negative correlation with flesh thickness (-0.30), TSS (-0.61), β -carotene (-0.26), ascorbic acid (-0.24), pH (-0.40).

f) Seed cavity area

Seed cavity area had positive and significant correlation with flesh thickness (0.27), rind thickness (0.32) and firmness (0.31). However, seed cavity area had negative and significant correlation with TSS (-0.26) and β -carotene (-0.24).

g) Flesh thickness

Flesh thickness shows positive and significant correlation with rind thickness (0.74), TSS (0.57), β -carotene (0.31), pH (0.43) and firmness (0.57) however, negative correlation with titratable acidity (-0.57).

h) Rind thickness

Rind thickness had positive and significant correlation with days to last fruit harvest (0.16), TSS (0.47), β -carotene (0.43), pH (0.42) and firmness (0.65). However, rind thickness gives significant and negative correlation with titrable acidity (-0.57).

i) Days to 1st female flower appearance

Days to 1st female flower appearance shows positive and significant correlation with days to 1st fruit harvest (0.29) and days to last fruit harvest (0.39).

j) Days to 1st fruit harvest

Days to 1st fruit harvest shows positive and significant correlation days to last fruit harvest (0.29), TSS (0.09), β -carotene (0.24). However, days to 1st fruit harvest negative and significant correlation with titrable acidity (-0.08).

k) Days to last fruit harvest

Days to last fruit harvest shows positive and significant correlation TSS content (0.41), β -carotene content (0.28) and pH (0.20). However, Days to last fruit harvest shows negative correlation with Titrable acidity (-0.34).

l) β -carotene content

β -carotene content shows positive and significant correlation with ascorbic acid (0.35) and negative correlation with titrable acidity (-0.27).

m) Total soluble solid content

Total soluble solid content shows significantly positive correlation with β -carotene (0.37), pH (0.53) and firmness (0.43). However, it depicts negative correlation with Titrable acidity (-0.72).

n) Ascorbic acid content

Ascorbic acid shows negative correlation with Titrable acidity (-0.06) and significantly positive correlation with pH (0.09).

o) Titrable acidity

Titrable acidity gives negative and significant correlation pH (-0.72). pH gives positive and significant correlation with firmness (0.33).

p) pH

pH gives significant and positive correlation with firmness (0.33).

q) Firmness

Firmness shows significantly positive correlation with fruit weight (0.53), flesh thickness (0.57), seed cavity area (0.31) and rind thickness (0.65). However, it depicts negative correlation with number of fruits per vine (-0.53) and titrable acidity (-0.43).

Table 4.11: Correlation among morphological traits of 70 melon accessions

	VL	FW	NOFPV	Yld	FSI	SCA	FT	RT	DTFFFA	DTFFH	DTLFH	TSS	Bcaro	AA	TA	PH	Firm	
VL		0.17	0.22	0.46*	-0.12	0.19	0.01	-0.03	0.11	-0.06	0.17	0.13	-0.07	0.17	-0.14	0.10	-0.05	
FW			-0.55*	0.60*	0.06	0.75*	0.69*	0.66*	-0.04	0.14	0.13	0.18	0.00	-0.27*	-0.38*	0.25*	0.53*	
NOFPV				0.21	0.05	-0.29*	-0.71*	-0.70*	0.18	-0.22	0.00	-0.30*	-0.26*	0.24*	0.36*	-0.22	-0.53*	
Yld					-0.10	0.52*	0.25*	0.18	0.23*	0.04	0.33	0.11	-0.10	-0.01*	-0.28	0.29*	0.13	
FSI						0.46*	-0.30*	-0.18	-0.07	0.03*	-0.36	-0.61*	-0.26*	-0.24*	0.49*	-0.40*	-0.04	
SCA							0.27*	0.32*	0.12	0.13	0.04	-0.26*	-0.23*	-0.27	0.03	0.00	0.31*	
FT								0.74*	-0.17	0.06	0.18	0.57*	0.31*	-0.11	-0.57*	0.43*	0.57*	
RT									-0.01	0.27	0.16*	0.47*	0.43*	-0.09	-0.57*	0.42*	0.65*	
DTFFFA										0.29*	0.39*	0.02	0.10	0.07	0.01	-0.03	0.01	
DTFFH											0.27*	0.09*	0.24*	-0.01	-0.08*	0.05	0.15	
DTLFH												0.41	0.28*	0.09	-0.34*	0.20*	-0.01	
TSS													0.37	0.09	-0.72	0.53	0.43	
Bcaro														0.35*	-0.27*	0.16	0.16	
AA															-0.06	0.09	0.02	
TA																	-0.72*	-0.43
PH																		0.33*
Firm																		

* Correlation is significant at the 0.05 level.

Legend: VL: Vine length, FW: Fruit weight, NOFPV: Number of fruit per vine, Yld: Yield (kg/plant), FSI: Fruit shape index, SCA: Seed cavity area, FT: Flesh thickness, RT: Rind thickness, DTFFFA: Days to 1st female flower appearance, DTFFH: Days to 1st fruit harvest, DTLFH: Days to last fruit harvest, TSS: Total soluble solids, Bcaro: β -carotene (mg/100 gm), AA: Ascorbic acid (mg/100 gm), TA: Titrable acidity, PH: pH, Firm: Firmness.

4.1.5 Components of variation and correlation coefficients

There were narrow differences between magnitude of phenotypic and genotypic coefficient of variation for all the characters studied, indicating low environment effect on expression on these character, which implies that phenotypic variability is reliable is measure of genotypic variability. Highest phenotypic coefficient of variation was exhibited for β -carotene content (149.7%), titrable acidity (95.0%), number of fruits per vine (70.9%), fruit yield (66.5%), fruit weight (66.9%), vine length (31.0%), rind thickness (54.7%), seed cavity area (55.7%), TSS (45.7%), seed cavity length (42%), firmness (41.3%), seed cavity index (40.2%), flesh thickness (37.2%), fruit length (33.4%), fruit shape index (30.1%), seed cavity breadth (25.7%), fruit breadth (25.2%) and pH (21%), Further, days to first female flower appearance (7.7%), days to first fruit harvest (5.5%) and days to last fruit harvest (3.9%) displayed comparatively low phenotypic coefficient of variation.

Table 4.12: Estimation of phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and heritability (broad sense)

S. No.	Trait	Mean	GCV (σ^2_g)	PCV (σ^2_p)	Heritability (%)
1	Vine length	110.2	30.7	31.0	98.0
2	Fruit weight	821.5	66.2	66.9	98.0
3	Fruit length	11.9	32.8	33.4	96.4
4	Fruit breadth	10.8	24.8	25.2	96.3
5	Fruit shape index	1.1	29.8	30.1	98.2
6	Seed cavity length	7.5	41.3	42.0	96.5
7	Seed cavity breadth	5.6	24.9	25.7	93.6
8	Seed cavity area	43.5	54.5	55.7	95.8
9	Flesh thickness	2.4	36.4	37.2	95.6
10	Rind thickness	3.6	53.5	54.7	95.7
11	TSS	7.3	44.8	45.7	96.3
12	NOFPV	3.6	70.2	70.9	98.1
13	DTFFFA	64.6	7.2	7.7	88.0
14	DTFFH	95.0	4.2	5.5	57.0
15	DTLFH	105.3	3.4	3.9	76.2
16	Yield	2.6	65.7	66.5	97.8
17	Bcaro	0.7	149.4	149.7	99.6
18	Ascorbic acid	26.7	30.5	31.1	96.0
19	Titrable acidity	0.2	94.6	95.0	99.1
20	pH	6.0	20.9	21.0	99.2
21	Firmness	7.5	41.3	41.3	99.7
22	SCI	1.4	39.2	40.2	95.0

Legend: VL: Vine length, FW: Fruit weight, NOFPV: Number of fruit per vine, Yld: Yield (kg/plant), FSI: Fruit shape index, FL: Fruit length, FB: Fruit breadth, SCA: Seed cavity area, SCL: Seed cavity length, SCB: Seed cavity breadth, FT: Flesh thickness, RT: Rind thickness, DTFFFA: Days to 1st female flower appearance, DTFFH: Days to 1st fruit harvest, DTLFH: Days to last fruit harvest, TSS: Total soluble solids, Bcaro: β -carotene (mg/100 gm), AA: Ascorbic acid (mg/100 gm), TA: Titrable acidity, PH: pH, Firm: Firmness.

Maximum genotypic coefficient of variation was observed in β -carotene content (149.4%), titrable acidity (94.6%), number of fruits per vine (70.2%), yield (65.7%), fruit weight (66.2%), vine length (30.7%), rind thickness (53.5%), seed cavity area (54.5%), TSS (44.8%), seed cavity length (41.3%), firmness (41.3%), seed cavity index (439.2%), flesh thickness (36.4%), fruit length (32.8%), fruit shape index (29.8%), seed cavity breadth (24.9%), fruit breadth (24.8%) and pH (20.9%). However, days to first female flower appearance (7.2%), days to first fruit harvest (4.2%) and days to last fruit harvest (3.4%) displayed comparatively low phenotypic coefficient of variation (Table 4.12).

4.2 Molecular characterization of muskmelon germplasm with simple sequence repeats markers

a) Allelic amplification in melon germplasm

The analysis of genetic diversity is a classical application of SSR markers. In the present investigation, genetic divergence among seventy melon genotypes was assessed with thirty SSR primers. Earlier also it is reported that molecular markers are powerful tools for elucidating genetic diversity, determining parentage and revealing phylogenetic relationships among various melon accessions (Roy *et al* 2012, Fergany *et al* 2011, Zhang *et al* 2012, Kacar *et al* 2012, Malik *et al* 2014). All the microsatellites analyzed showed amplification and out of 30 amplified primers, 24 markers (80 per cent) exhibited polymorphism and showed greater genetic diversity while remaining 6 (20 per cent) were monomorphic. A total of 67 alleles were amplified by 24 polymorphic primers and eleven alleles were amplified by six monomorphic primers. The number of alleles amplified ranged from 2 to 4 with an average of 2.8 alleles per locus (Table 4.15). In similar study, Lopez-sese *et al* (2002) found (2.4) alleles per locus and Tzitzikas *et al* (2009) found 2.47 alleles per locus. Four markers amplified four alleles, eleven revealed three alleles and for the remaining, nine amplified two alleles (Table 4.15). The variation in the number of alleles produced by SSR markers demonstrates heterozygosity in different alleles at a given locus in which the heterozygosity could reflect greatly the state of genetic variability.

b) Percent polymorphism

Thirty SSR primers amplified total 3230 alleles across all the germplasm set with an average of 107.67 alleles per genotype. Average number of amplified fragments for polymorphic markers was 84.93, while monomorphic markers it was 22.73. The maximum number of alleles (58) was amplified in accession IC-274034 while minimum (36) in Riogold. However, percentage of polymorphic marker was highest in accession PAUS-19 (86.96) followed by 86.05 per cent in Afghanistan Collection-1 (Table 4.16). Percentage of polymorphism of polymorphic marker ranged from 50 to 100. Out of 24 primers, three exhibited 100 percent polymorphism, four was having 75 percent, ten with 66.7 percent and

the remaining seven exhibited 50 percent polymorphism. Average polymorphism (%) of all the polymorphic primer pairs across all the genotypes was 67.4 (Table 4.15).

c) Specific alleles

Seven SSR primers were found to have higher discriminating potential for differentiation of the genotypes as they uncovered 7 unique/specific alleles in twenty-three genotypes. These primers amplified more than one allele. However, one allele was amplified in few accessions that differentiate these genotypes from other. Therefore, twenty five genotypes can be differentiated from each other using the markers which revealed unique alleles. Most of the accessions identified by specific alleles were of *reticulatus* and *cantalupensis* group. One accession of *callosus* group (WM-2013-2) and one of *momordica* (SM-2015-2) were identified by unique alleles amplified by CMMS1-3 and EMS65 markers respectively. While, accession of *inodorous* group was not identified by any unique allele (Table 4.14).

One marker CMTCN38 uncovered specific alleles in maximum number (6) of genotypes. Two markers, EMS 65 and EMS 178 each revealed unique alleles in four genotypes whereas CMTC 13, CMAG59 and CMTCN21 revealed in three genotypes and CMMS1-3 identified only three genotypes. Data revealed that the markers with maximum number of alleles (4) had proved its ability to differentiate cultivars through specific alleles except one (CMMS3-1) (Table 4.14).

d) Polymorphic information content

The polymorphic information content value which is a measure of allelic diversity ranged from 0.090 (CMTC13) to 0.614 (EMS182) with a mean value of 0.43 across all genotypes (Table 4.15). Similar range of PIC value of SSR markers was observed in muskmelon by Ning *et al* (2014). Primers CMGA59, CMTCN38, CMMS3-1 and EMS178 amplified maximum 4 alleles and have PIC values 0.550, 0.600, 0.442, 0.534, respectively. Ten SSR primers revealed PIC value more than 0.50. It has been observed that marker CMMS3-1 amplified four alleles and had PIC value of 0.44 while EMS125 amplified three alleles and had PIC value of 0.61. Similarly, CMTCN21 amplified three alleles and had PIC value of 0.34 while, EMS182 amplified two alleles and had PIC value of 0.50. Therefore, there was no strong correlation between the PIC value and the number of alleles amplified by a marker. Genetic diversity was ranged from 0.091 (CMTC13) to 0.623 (EMS125). The average value of genetic diversity across all the primers was 0.436 (Table 4.15).

e) Heterozygosity of SSR markers

Observed and expected heterozygosity were calculated to examine the organization of genetic diversity within the melon accessions. Thus, the measure of the amount of heterozygosity across loci is used as a general indicator of the amount of genetic variability. The present set of SSR markers was having a lot of genetic variability as the heterozygosity

value was very high (0.99). High heterozygosity is observed in melons due to their cross-pollinating behavior. In the present study, the observed heterozygosity (H_0) ranged from 0.04 (CMT13) to 0.99 (CMMS35-4, CMMS30-3, CMAG59) with an average of 0.53 at the locus. Whereas, the values of expected heterozygosity (H_e) ranged from 0.10 (CMT13) to 0.81 (EMS125) with an average value of 0.48 (Table 4.15). Hence, the average observed heterozygosity (53%) within a melon germplasm was higher as compared to the average expected heterozygosity (48%). Often the observed level of heterozygosity was compared to what was expected under Hardy-Weinberg equilibrium (HWE). In the present study, observed heterozygosity is higher than expected, therefore it is suspect of an isolate breaking effect i.e. the mixing of two isolated populations.

f) Cluster analysis and similarity coefficient

The dendrogram (Fig.4.6) depicting the genetic relationships as revealed by DARwin 6.0 classified the genotypes into three major clusters, Cluster I, Cluster II, and Cluster III. The cluster I, which was having 23 genotypes was further sub-divided into two sub clusters IA and IB with seventeen and six genotypes (Table 4.13) respectively. Similarly, Cluster II consisted of 36 genotypes which was further divided into two sub clusters IIA and IIB with 23 and 13 genotypes respectively. While, eleven genotypes were clustered in cluster III with 9 in IIIA and 2 genotypes in IIIB sub cluster. Hence, maximum number of genotypes was clustered in Cluster II.

Both the genotypes belonging to *callosus* group were clustered in cluster IIA. Of the total nine genotypes belonging to *inodorus* group, six were clustered in cluster IIB, two grouped in cluster IIIB and single genotype (Durasol) grouped with the genotypes of cluster IA. All the genotypes of *momordica* group were clustered randomly within three major clusters. Similarly, accessions of *reticulatus* and *cantalupensis* group were frequently grouped in cluster I, II and III. Out of the four accessions from USA, genotype Durasol was grouped in cluster I and Carabean gold grouped in cluster IIB. While, two genotypes Natal and Riogold of different horticultural groups were grouped in cluster II. Hence, the distribution of accessions into clusters by diversity analysis is independent to the horticulture group and geographic origin of the accessions which is similar to the results of Escribano and Lazaro (2009). Similarly, Kacar *et al* (2012) and Sensoy *et al* (2007) also determined that distinction among group *inodorus* and group *cantalupensis* (sweet) genotypes was also not very significantly different in their evaluations. Malik *et al* (2014) also reported intermixing of some accessions of *cantalupensis* and *reticulatus* while analyzing genetic diversity in melon accessions using SSR markers. Hence, intermixing of accessions of *reticulatus*, *cantalupensis*, *inodorus*, *callosus* and *momordica* within clusters showed that classification of muskmelon groups on morphological does not necessarily correspond to the genetic relationship shown by molecular analysis. The out crossing behavior may be the cause of genetic introgression in melons.

The similarity coefficient based on DNA amplification of 70 melon accessions using microsatellite markers was estimated by dice similarity coefficient. The highest similarity coefficient of 0.91 was observed in genotypes MM selection-103 and Punjab Sunhehri (cluster IIA) followed by 0.84 in PAUS-13 and Durgapura Madhu (cluster IIB) (Table 4.17). Although, these accessions belonged to different groups, *reticulatus* and *cantalupensis* but were closely related as these showed the high genetic similarity coefficient.

Table 4.13: Cluster analysis on basis of molecular markers

Cluster	Sub cluster	Genotypes
I	IA (17)	PAUS-11, MM-2014-3, PAUS-8, MM-105-1, MM-3965, PAUS-18, Kajri1, Durasol, PAUS-14, PAUS-9-1, Dissected leaf, PAUS-19, SM-2013-9, PAUS-9, Hemed, MC-2013-2, Carabean gold
	IB (6)	PAUS-17-1, PAUS-27, MM-2014-13, MM-4279, IIVM-3, Kajri
II	IIA (23)	MM selection-103, Punjab sunhehri, SM-2013-5, WM-11, MM-2012-4, MM-4305, PAUS-31, Hara madhu, SM-2013-18, WM-2013-2, MC-2012-6, SM-2013-2, PAUS-4, MM-2014-11, PAUS-12, PAUS-28, PAUS-16, MS-3, MM var-4, MM-2014-2-1, Riogold, PAUS-17, PAUS-15
	IIB (13)	PAUS-27-1, Carabean gold, PAUS-13, Durgapura madhu, MM-2014-2, Natal, Khasta kharbooza, Kajri Doctor, MM var-3, Green flesh, Canary yellow-1, Canary yellow-2, Honey dew
III	IIIA (9)	SM-2014-7, SM-2015-2, IC-274034, MM-105, IC-267375, MM-3864, MC-2014-2, MM-4226, PAUS-30,
	IIIB (2)	Afghanistan collection-2, Afghanistan collection- 1

Table 4.14: Specific/unique alleles detected by SSR primers and identified melon genotypes

S. No.	SSR markers	Total no. of alleles detected	Specific /unique alleles	Genotype identified
1	CMTC13	2	1	Dissected leaf, WM-2013-2, Hara Madhu
2	CMAG59	4	1	MM-4279, Kajri, MM-3864
3	CMTCN38	4	1	MM-2014-2-1, PAUS-12, MM var-4, MS-3, PAUS-16, PAUS-28
4	CMMS1-3	3	1	WM-2013-2, PAUS-14
5	CMTCN21	3	1	PAUS-15, MM-2014-13, IIVM-3
6	EMS65	3	1	SM-2015-2, IC-274034, MM-3864, MC-2014-2
7	EMS178	4	1	Kajri Doctor, PAUS-9, IC-267375, MM-105

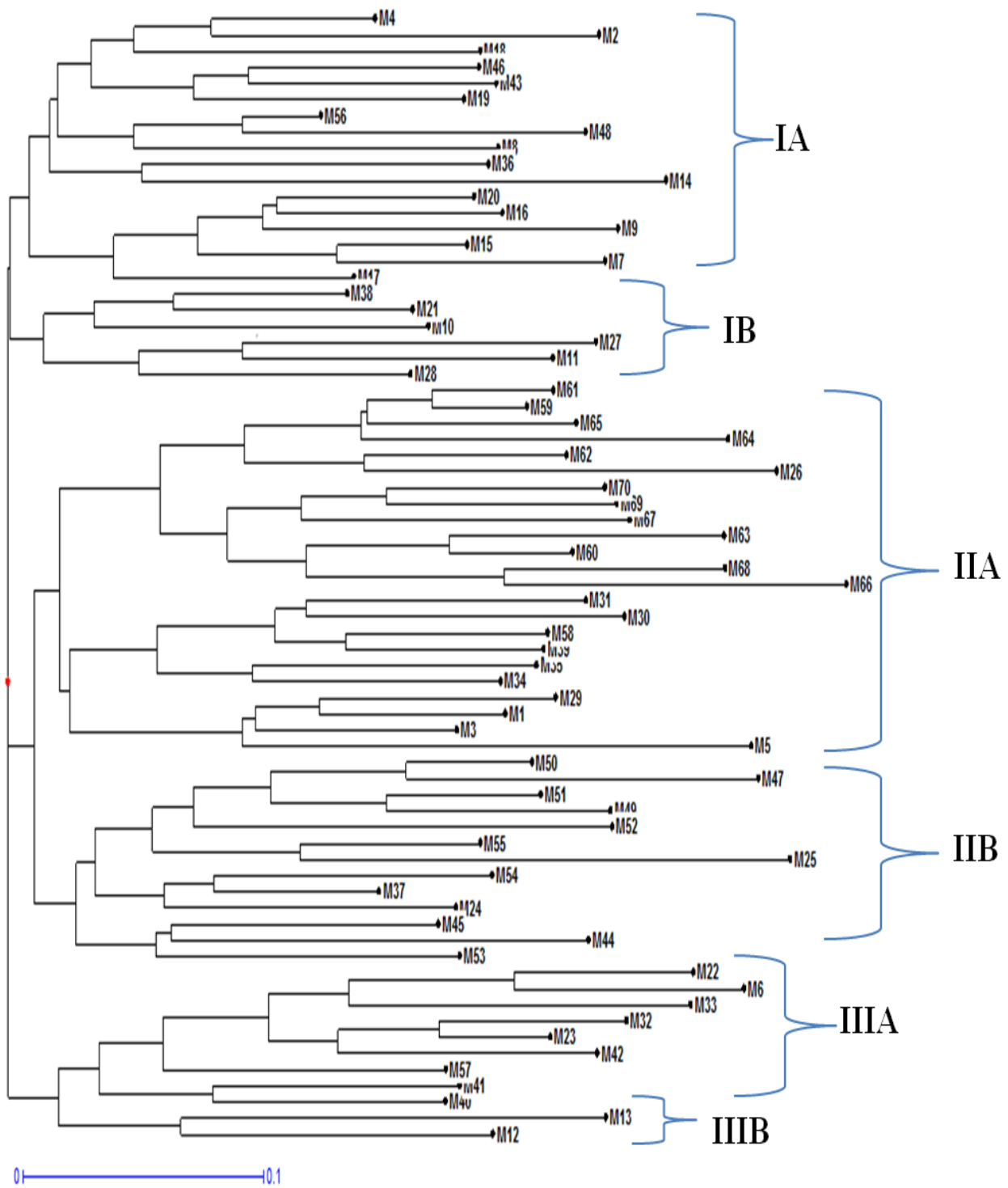


Figure 4.6: Cluster analysis of 70 melon accessions (M1 to M70) by using DARwin 6 software as presented in table 4.16

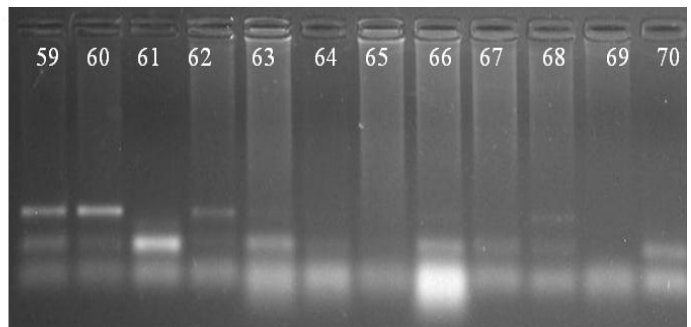
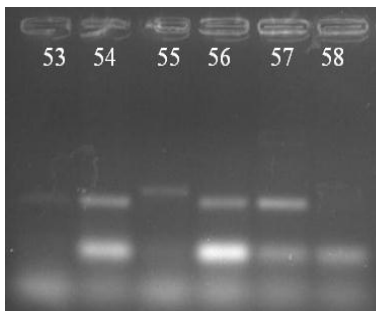
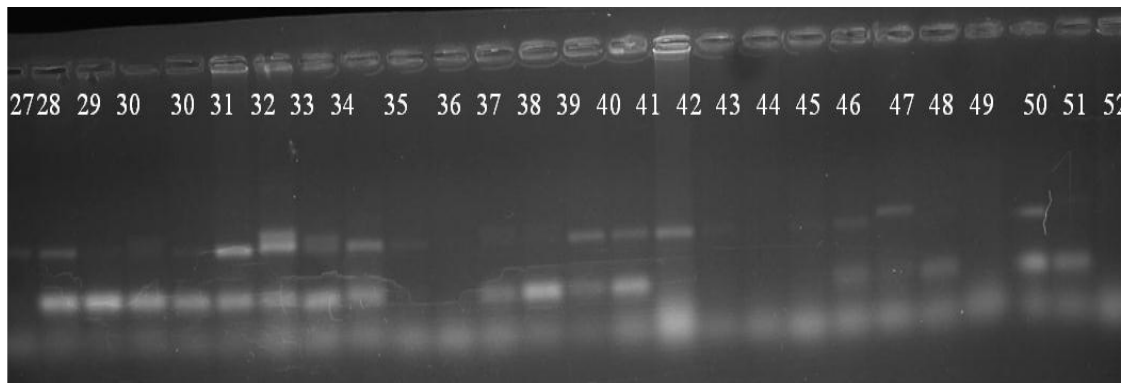
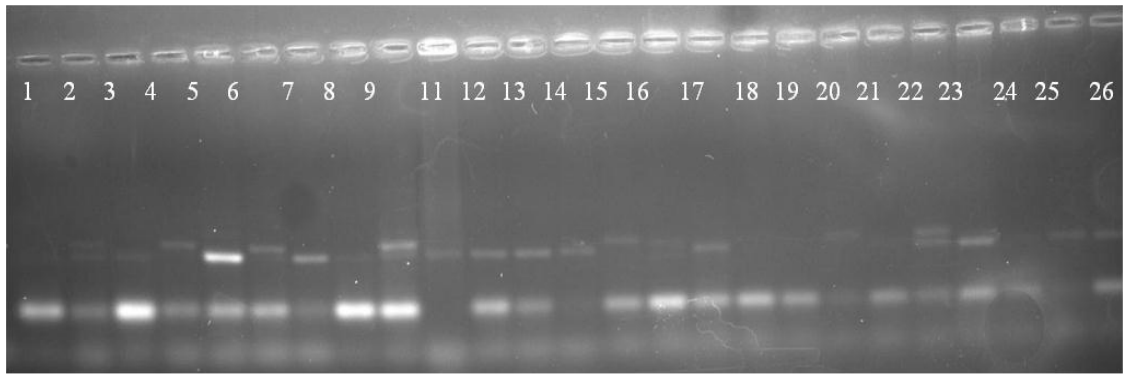


Plate 6: Amplification profile generated by SSR primer CMMS1-3 in 70 melon accessions (M1 to M70) as presented in table 4.15

Table 4.15: Number of alleles amplified, polymorphism (%), Polymorphic Information Content (PIC) value, genetic diversity and heterozygosity of SSR markers

S. No.	Primers	Total no. of alleles	Monomorphic alleles	Polymorphic alleles	Polymorphism (%)	PIC	Genetic diversity	Heterozygosity (o)	Expected (He)
1	CMTC13	2.0	1.0	1.0	50.0	0.090	0.09	0.04	0.10
2	CMAG59	4.0	1.0	3.0	75.0	0.550	0.56	0.99	0.80
3	CMTCN38	4.0	1.0	3.0	75.0	0.600	0.61	0.67	0.79
4	CMMS004	2.0	1.0	1.0	50.0	0.433	0.44	0.44	0.50
5	CMMS33-1	2.0	1.0	1.0	50.0	0.315	0.32	0.24	0.37
6	CMMS3-1	4.0	1.0	3.0	75.0	0.442	0.45	0.36	0.56
7	CMMS1-3	3.0	1.0	2.0	66.7	0.457	0.46	0.51	0.53
8	CMGA127	3.0	1.0	2.0	66.7	0.417	0.42	0.34	0.46
9	CMMS35-4	3.0	1.0	2.0	66.7	0.541	0.55	0.99	0.31
10	CMTCN21	3.0	1.0	2.0	66.7	0.340	0.34	0.25	0.41
11	CMTCN8	3.0	1.0	2.0	66.7	0.613	0.62	0.83	0.82
12	CMMS14-1	2.0	1.0	1.0	50.0	0.502	0.51	0.97	0.06
13	CMMS30-3	3.0	1.0	2.0	66.7	0.598	0.61	0.99	0.68
14	EMS51	3.0	1.0	2.0	66.7	0.520	0.53	0.57	0.71
15	EMS130	2.0	0.0	2.0	100.0	0.281	0.29	0.10	0.32
16	EMS182	2.0	0.0	2.0	100.0	0.500	0.51	0.94	0.11
17	EMS125	3.0	0.0	3.0	100.0	0.614	0.62	0.73	0.81
18	EMS65	3.0	1.0	2.0	66.7	0.277	0.28	0.18	0.32
19	EMS85	3.0	1.0	2.0	66.7	0.424	0.43	0.39	0.59
20	EMS109	2.0	1.0	1.0	50.0	0.184	0.19	0.89	0.20
21	EMS178	4.0	1.0	3.0	75.0	0.534	0.54	0.46	0.76
22	EMS61	3.0	1.0	2.0	66.7	0.457	0.46	0.42	0.65
23	EMS124	2.0	1.0	1.0	50.0	0.235	0.24	0.16	0.27
24	EMS134	2.0	1.0	1.0	50.0	0.400	0.41	0.39	0.47
	Total	67.0	21.0	46.0	1616.7	10.32	10.47	12.84	11.58
	Mean	2.8	0.9	1.9	67.4	0.430	0.44	0.53	0.48

Table 4.16: Total number of alleles amplified in each of seventy genotypes using thirty SSR markers

S. No.	Genotypes	Code	Number of amplified alleles		Total	Poly-morphism (%)
			Monomorphic markers	Polymorphic markers		
1	Riogold	M1	6	30	36	83.33
2	MM-2014-3	M2	8	34	42	80.95
3	PAUS-17	M3	11	31	42	73.81
4	PAUS-11	M4	11	36	47	76.60
5	PAUS-15	M5	9	33	42	78.57
6	SM-2015-2	M6	11	42	53	79.25
7	MC-2013-2	M7	9	34	43	79.07
8	PAUS-14	M8	11	33	44	75.00
9	PAUS-9	M9	9	38	47	80.85
10	MM-2014-13	M10	9	36	45	80.00
11	IIVM-3	M11	8	42	50	84.00
12	Afghanistan Collection-1	M12	6	37	43	86.05
13	Afghanistan Collection-2	M13	10	33	43	76.74
14	Dissected Leaf	M14	6	32	38	84.21
15	Hemed	M15	11	34	45	75.56
16	SM-2013-9	M16	8	38	46	82.61
17	PAUS-26	M17	10	39	49	79.59
18	PAUS-8	M18	10	33	43	76.74
19	PAUS-18	M19	8	37	45	82.22
20	PAUS-19	M20	6	40	46	86.96
21	PAUS-27	M21	8	38	46	82.61
22	SM-2014-7	M22	10	43	53	81.13
23	IC-267375	M23	8	45	53	84.91
24	Green flesh	M24	9	36	45	80.00
25	Khasta Kharbooza	M25	9	33	42	78.57
26	MM-4305	M26	9	41	50	82.00
27	MM-4279	M27	9	38	47	80.85
28	Kajri	M28	11	38	49	77.55
29	MM-2014-2-1	M29	11	31	42	73.81
30	PAUS-12	M30	11	33	44	75.00
31	MM-2014-11	M31	11	35	46	76.09
32	MM-105	M32	9	47	56	83.93
33	IC-274034	M33	11	47	58	81.03
34	MM var-4	M34	11	41	52	78.85
35	MS-3	M35	11	39	50	78.00
36	PAUS-9-1	M36	11	34	45	75.56

S. No.	Genotypes	Code	Number of amplified alleles		Total	Poly-morphism (%)
			Monomorphic markers	Polymorphic markers		
37	MM var-3	M37	11	35	46	76.09
38	PAUS-17-1	M38	11	35	46	76.09
39	PAUS-16	M39	11	33	44	75.00
40	PAUS-30	M40	11	36	47	76.60
41	MM-4226	M41	11	38	49	77.55
42	MM-3864	M42	11	45	56	80.36
43	MM-3965	M43	11	33	44	75.00
44	Canary yellow-1	M44	7	36	43	83.72
45	Canary yellow-2	M45	11	36	47	76.60
46	MM-105-1	M46	11	36	47	76.60
47	Carabeangold	M47	9	31	40	77.50
48	Durasol	M48	11	29	40	72.50
49	Durgapura madhu	M49	11	32	43	74.42
50	PAUS-27-1	M50	11	33	44	75.00
51	PAUS-13	M51	11	31	42	73.81
52	MM-2014-2	M52	11	36	47	76.60
53	Honey Dew	M53	11	37	48	77.08
54	Kajri Doctor	M54	11	36	47	76.60
55	Natal	M55	11	36	47	76.60
56	Kajri-1	M56	11	32	43	74.42
57	MC-2014-2	M57	11	39	50	78.00
58	PAUS-28	M58	11	35	46	76.09
59	Punhehri sunhehri	M59	9	33	42	78.57
60	MC-2012-6	M60	9	37	46	80.43
61	MM selection-103	M61	9	33	42	78.57
62	MM-2012-4	M62	9	38	47	80.85
63	WM-2013-2	M63	11	43	54	79.63
64	WM-11	M64	9	37	46	80.43
65	SM-2013-5	M65	9	36	45	80.00
66	PAUS-4	M66	9	40	49	81.63
67	SM-2013-18	M67	9	36	45	80.00
68	SM-2013-2	M68	9	38	47	80.85
69	Hara Madhu	M69	9	38	47	80.85
70	PAUS-31	M70	9	38	47	80.85
	Total		682	2548	3230	-----
	Average		22.73	84.93	107.67	78.81

g) Clustering pattern based on principal component analysis and SSR marker analysis

The comparison of clusters based on principal component analysis and SSR marker analysis indicated that clustering pattern was different for the two approaches. The genotypes clustered in one group based on biplot analysis were found to be scattered in different clusters when compared with SSR marker analysis. This happening may be due to reason that molecular markers analysis may reveal genetic diversity based on overall genetic constitution of the test material. While, diversity based on morphological is for few traits of agronomic interest for which germplasm is being subjected to intensive selection for long period of time. The expression of these traits may also be effected by environmental effect. Assessment of diversity at molecular level represents a more realistic picture of test material. Thus, the SSR markers have shown good potential in analyzing the genetic diversity among melon groups.

CHAPTER V

SUMMARY

The present investigation entitled “Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits” was conducted at Department of Vegetable Science and School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, during the spring-summer seasons of 2015 and 2016.

For this investigation seventy melon accessions collected from diverse geographical locations such as, USA, India, Israel and Afghanistan consisting of various horticultural melon groups, viz. *cantalupensis*, *reticulatus*, *inodorus*, *momordica* and *callosus* were used for diversity analysis. Eight plants of each genotype were transplanted in the field with 3 meter row to row and 0.45 meter plant to plant spacing in a randomized block design. Four plants from each accession were randomly selected for recording data. In this study, diversity among melon accessions was characterized for morphological characters, fruit quality traits and at molecular level using SSR markers.

For morphological characterization, genotypes were evaluated for thirty-three traits viz., days to first female flower appearance, days to last fruit harvest, days to 1st fruit harvest, number of fruits per plant, fruit weight (g), yield per plant (kg/plant), length of fruit (cm), fruit width (cm), seed cavity area (length × breadth cm²), rind thickness (mm), flesh thickness (cm), vine length (cm), firmness, fruit shape index, seed cavity length (cm), seed cavity breadth (cm), seed cavity index, flesh color, shape of leaf, fruit rind color, fruit patches, fruit peduncle at maturity, fruit shape at peduncle end, fruit shape at blossom end, fruit surface, fruit sutures, fruit surface netting, flesh texture, total soluble solids (%), pH, titrable acidity (%), β-carotene (mg per 100 g fresh fruit weight) and ascorbic acid content (mg per 100 g of fresh fruit weight). The average of four plants was used for statistical analysis. Analysis of variance showed highly significant genotypic effects for all the characters. Vine length showed significant variations among melon genotypes ranged from 61.2 cm (PAUS-15) to 162.2 cm (Hara Madhu). For shape of leaf, forty-two (60.0%) were pentalobate shape, twenty-four (34.3%) possessed entire leaf shape, three (4.2%) genotypes were found 5-palmately lobed and one genotype (1.4%) possessed trilobate leaf shape. Significant differences were observed for days to 1st female flower appearance. Minimum no. of days to appearance of 1st female flower were recorded in PAUS-13 (56.5 days), while maximum were exhibited in SM-2015-2 (71.0 days) these were statistically at par with genotype MM-105-1 (71.0 days) and PAUS-30 (71.0 days). Earliest fruits were harvested from genotype MM-4279 (80.5 days after sowing) however, genotype MM-2012-4 took maximum days (105 days) to first fruits harvest. Minimum number of days to last harvest was recorded in genotype SM-2013-9 (98.5 days), but last fruits of genotype IIVM-3 were harvested after 115.5 of sowing. Fifty-nine genotypes were found slipable (84%), while eleven genotypes

(16%) were observed to be non-slipable. For fruit shape at peduncle end, sixty four (91%) were rounded at peduncle base, while six genotypes (8.5%) possessed pointed shape at peduncle end. Similarly, for fruit shape at blossom end, sixty four (91%) were intermediate at peduncle base, but six genotypes (8.5%) possessed pointed shape at peduncle end. Out of total seventy genotypes, sixty-five (93%) were found to have smooth surface, while the remaining five (7%) genotypes were identified, as grooved. Sixty-two (89%) were non-sutured, while sutures were present in eight (11%) genotypes. Forty-six genotypes (66%) had no-netting, one (1.4%) had moderate netting and twenty-three (33%) were found to be densely netted.

Fruit rind color varied among genotypes. Majority (58.6%) of the accessions had orange color, (18.6%) possessed creamy white, four genotypes (5.7%) had yellow, eleven genotypes (15.7%) had yellow green, and one genotype had other color of rind. Fruit patches were observed only on three genotypes, Afghanistan collection-1, Afghanistan collection-2 and Khasta Kharbooza. Considerable variation was observed in fruit weight parameter ranging from 92.5 (WM-2013-2) to 2000 g (Honey Dew) with overall mean of 821.6 g. Number of fruits per plant varied from 1.3 (Canary Yellow-2) to 8.5 (MM-4226). Maximum yield (Kg/ plant) was exhibited in genotype Honey Dew (6.5 kg per plant), while minimum in wild melon cultivars WM-2013-2 (0.72 kg/ plant). All the melon accessions shows significant variation for fruit length, ranging from 6.8 cm (MC-2012-6) to 21.0 cm (Afghanistan collection-1). Similarly, fruit breadth ranged from 4.8 cm to 14.9 cm. In the present investigation, fruit shape index value varied from 0.80 to 1.96. Mostly, genotypes were round, except Durpapura Madhu, Afghanistan collection-1, Afghanistan collection-2, Canary yellow group and snapmelon genotypes which were observed to be oval or elongated. Length of seed cavity varied from 4.3 cm (MC-2012-6) to 15.3 cm (Afghanistan collection-1). Similarly, seed cavity breadth ranged from 3.4 cm (WM-11) to 7.7 cm (MM-2014-3). Seed cavity area ranged from 19.3 cm² (WM-11) to 108.8 cm² (Afghanistan collection-1). In the present investigation, seed cavity index value varied from 0.69 to 2.31 with mean value of 1.4. It was observed that 11% genotypes had round seed cavity. Significant variations were found among all the genotypes for flesh thickness. Maximum flesh thickness (3.4 cm) was observed in genotype, Green flesh while minimum flesh thickness (0.8 cm) was recorded in WM-2013-2. Thinnest rind (1 mm) was recorded in snap melon genotypes. However, maximum rind thickness (7.13 mm) was reported in *reticulates* genotype, PAUS-30.

The highest total soluble solid content (11.3) was recorded in genotype, MM var-4. However, snapmelon genotype (SM-2013-2) reported the lowest total soluble solid content (1.63). Similarly, for β -carotene content significant variability was observed among melon genotypes for ranging from 0.02 mg/100 g (Natal) to 3.32 mg/100 g (MS-3). There existed a significant variability for ascorbic acid content ranged from 14.5 (MM-2014-2) to 46.3 mg/100 g (Punjab Sunhehri). Snapmelon genotypes possessed maximum titrable acidity 0.63

(SM-2013-2) however, minimum 0.10 was reported in *cantalupensis* genotype, MM-4279. Maximum pH value was observed in genotype PAUS-8 (7.0). However, minimum was reported in MC-2012-6 (3.9). For improving shelf life of melon fruits firmness is an important trait for maintaining the quality of fruits. Genotype Hara Madhu (2.4 lb/ inch²) possessed minimum firmness, but PAUS-9-1 (10.5 lb/ inch²) genotype showed maximum firmness. Sixteen genotypes (22.9%) were of creamish white flesh color, four genotypes (5.7%) of yellowish green color, eleven (15.7%) genotypes recorded green flesh color and thirty-nine (55.7%) genotypes possessed orange flesh color. Similarly, fifty-three (61%) possessed intermediate flesh texture, eleven genotypes (16%) were found to be mealy and six (9%) were crispy.

To determine variability in muskmelon genotypes principal component analysis was used and results indicates that great genetic variation was present in muskmelon genotypes with regards to β -carotene content, titrable acidity, number of fruits per vine, fruit weight, yield, firmness. Percent variability reporteded by the 1st seven components was 29.2%, 17.3%, 12.4%, 9.3%, 6.2%, 4.4% and 3.9%, respectively. Biplot analysis clustered 70 melon genotypes into four clusters with one un-clustered melon genotype. Clusters formed mainly on the basis of variability existed in fruit weight, number of fruits / vine, rind and flesh thickness of fruit, TSS, Titrable acidity, pH, firmness. Cluster I, consisted snapmelon (*C. melo* var. *momordica*) genotypes. Cluster II consists two wild melon genotypes (*C. melo* var. *momordica*). Cluster III had genotypes with highest fruit weight, length of fruit, breadth of fruit, seed cavity area, flesh thickness, rind thickness. Cluster IV consisted those genotypes which had high β -carotene, TSS, pH, firmness and single un-clustered accession MC-2012-6 was observed. In correlation analysis, Fruit weight showed positive correlation with fruit yield, seed cavity area, flesh thickness, rind thickness and firmness. However, fruit weight had significant and -ve correlation with no.of fruits / plant, ascorbic acid and tirtable acidity. Number of fruits / plant had significant and negative correlation with seed cavity area, flesh thickness, rind thickness, TSS, β - carotene and firmness. Fruit yield gives positive and significant correlation with seed cavity area, flesh thickness, days to 1st female flower appearance and pH. Fruit shape index had positive and significant correlation with seed cavity area and titrable acid. However, fruit shape index shows negative correlation with flesh thickness, TSS, β -carotene, ascorbic acid and pH. Further, seed cavity area had positive and significant correlation with flesh thickness, rind thickness and firmness. Flesh thickness shows positive and significant correlation with rind thickness, TSS, β -carotene, pH and firmness. However, flesh thickness shows negative correlation with titrable acidity. Days to 1st female flower appearance shows positive and significant correlation with days to 1st fruit harvest and days to last fruit harvest. Similarly, days to 1st fruit harvest shows positive and significant correlation days to last fruit harvest, TSS and β -carotene content. However, days

to 1st fruit harvest significantly negative correlation with titrable acidity. β -carotene shows positive and significant correlation with ascorbic acid and negative correlation with titrable acidity. However, titrable acidity gives negative and significant correlation pH. While, total soluble solid content depicted positive and significant relationship with β -carotene, ascorbic acid and pH.

Examination of genetic variability and association among genotypes is adept to facilitate selection of parents in progression of F₁ and inbred melon cultivars. Therefore, in the present investigation, genetic divergence among seventy melon genotypes was estimated with thirty microsatellites. All the markers got amplified where twenty four markers were polymorphic and amplified total 67 alleles. Four markers amplified 4 alleles, eleven displayed 3 alleles and 9 markers amplified two alleles. Maximum number of amplified were 4 with a mean value of 2.8 alleles per locus. Seven unique/specific alleles were amplified in twenty-three genotypes by seven markers. Therefore, using these markers 25 genotypes were identified. Most of the accessions identified by specific alleles were of *reticulatus* and *cantalupensis* group. While, no unique allele was amplified in any accession of *inodorous* group. It has been observed that most of the markers with maximum number of alleles amplified, differentiate accessions through specific alleles.

In a total of seventy accessions, thirty SSR primers amplified total 3230 alleles with an average of 107.67 alleles per genotype. Average number of amplified fragments for polymorphic markers was 84.93, while monomorphic markers it was 22.73. The maximum number of alleles was amplified in accession IC-274034 while minimum in Riogold. However, percentage of polymorphic marker was highest in accession PAUS-19. Out of 24 primers, three exhibited 100 percent polymorphism, four was having 75 percent, ten with 66.7 percent and the remaining seven exhibited 50 percent polymorphism. Average polymorphism (%) of all the polymorphic primer pairs across all the genotypes was 67.4.

The range of polymorphic information content was from 0.090 to 0.614 with a mean value of 0.43 across all genotypes. Ten SSR primers revealed PIC value more than 0.50. PIC values and no. of amplified alleles by primer exhibited no correlation. The SSR markers used were having a lot of genetic variability as the heterozygosity value was very high (0.99). The average observed heterozygosity (53%) within a melon germplasm was higher as compared to the average expected heterozygosity (48%). As the observed heterozygosity is higher than expected, therefore it is suspect of an isolate breaking effect i.e. the mixing of two isolated population.

The cluster analysis (DARwin 6.0) classify accession into three major groups where each group was further grouped into three sub clusters. Maximum no. of thirty six accessions was grouped in Cluster II. The distribution of accessions into clusters by diversity analysis is

independent to the horticulture group and geographic origin of the accessions. The similarity coefficient estimated by dice similarity coefficient was ranged from 0.34 to 0.91. Based on this molecular data, it was concluded that genetic diversity among these melon accessions is relatively high. Thus, estimation of genetic similarity and classification of the accessions would provide a basis for parental selection in future breeding strategies to be used for improvement of melon.

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ANNEXURE I

Meteorological data for the month of February to May, 2016, PAU Ludhiana

Month	Air Temperature			Soil Temperature									Relative Humidity			Vapour Pressure			Wind		Sun-Shine	Rain-fall	Evapo-ration	
				5 Cm Depth			10 Cm Depth			30 Cm Depth									Speed	Direction				
	(°C)			(°C)									%			mm						(Km/h)	(hrs)	(mm)
	Max.	Min.	Mean	M	E	Mean	M	E	Mean	M	E	Mean	M	E	Mean	M	E	Mean	M	E				
February	23.0	9.0	16.0	11.6	25.3	18.5	12.9	21.2	17.0	16.0	16.4	16.2	91.6	45.6	68.6	8.5	9.4	9.0	3.1	2.4	23.3	7.7	8.8	72.5
March	28.0	14.5	21.3	17.4	31.0	24.2	18.3	27.0	22.8	21.3	21.8	21.5	88	43	66	12.0	11.5	11.8	3.7	3	23	8.2	41.1	124.2
April	36.6	19.6	28.1	24.3	41.8	33.1	26.0	35.4	30.7	29.1	30.1	29.6	62.0	21.7	41.8	12.5	9.3	10.9	3.8	4	25	9.7	3.0	250.4
May	39.6	24.7	32.1	28.5	45.7	37.1	30.4	38.8	34.6	33.1	34.4	33.7	57	27	42	15.5	13.4	14.5	6.0	7	17	9.1	25.2	306.6

VITA

Name of the Student : Dildar Singh
Father's name : S. Jagtar Singh
Mother's name : Smt. Ajit Kaur
Nationality : Indian
Date of birth : 14th October, 1992
Permanent home address : VPO Kattu, Teh. & Distt. Barnala
Punjab - 148105

EDUCATIONAL QUALIFICATION

Bachelor degree : B.Sc. Agriculture (Hons.)
University : Punjab Agricultural University, Ludhiana
Year of award : 2014
OGPA : 7.52/10.00
Master's degree : M.Sc. (Vegetable Science)
University : Punjab Agricultural University, Ludhiana,
Punjab, India
OCPA : 8.00/10.00
Title of Master's Thesis : Genetic divergence among melon (*Cucumis melo* L.) groups for growth, yield and quality traits

Awards/Distinctions/Fellowships/Scholarships:

- University Merit Scholarship during Bachelor's Degree Programme
- Sikh Minority Scholarship during Master's Degree Programme