MORPHOLOGICAL, HISTOLOGICAL AND MOLECULAR IDENTIFICATION OF NUCELLAR EMBRYOS AND THEIR COMPATIBILITY WITH SOME MANGO CULTIVARS

By

HAMED HOSNY HAMED

B.Sc. Agric. Sci. (Pomolgy), Fac. Agric., Cairo Univ., Egypt, 2005

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Approval Committee

Dr. Mohamed Abd El Wahab Khamis .....
Professor of Pomology, Fac. Agric., Banha University

Dr. Mohamed Ahmed Fayek .....
Professor of Pomology, Fac. Agric., Cairo University

Dr. Mohamed Helmy Abd El-Zaher .....
Assistant Professor of Pomology, Fac. Agric., Cairo University

Dr. Omaima Ahmed Kilany .....
Professor of Pomology, Fac. Agric., Cairo University

Date: / / 2009

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. Omaima Ahmed Kilany
Professor of Pomology, Fac. Agric., Cairo University

Dr. Mohamed Helmy Abd El-Zaher
Assistant Professor of Pomology, Fac. Agric., Cairo University

Name of Candidate: Hamed Hosny Hamed **Degree:** M.Sc.
Title of Thesis: Morphological, Histological and Molecular Identification of Nucellar Embryos and Their Compatibility with Some Mango Cultivars
Supervisors: Dr. Omaima Ahmed Kilany
Dr. Mohamed Helmy Abd El-Zaher
Department: Pomology **Approval: / / 2009**

ABSTRACT

This investigation was carried out in the Pomology department, Faculty of Agriculture, Cairo University, during the period from 2006 to 2008 on seeds and seedlings of three polyembryonic mango cultivars as rootstocks (i.e. Sukkary, Ewaise and Zebda) and two mango cvs. as scions (i.e. Keit and Sedik). The investigation was conducted to study the effect of different treatments on mango seeds germination and the morphological characteristics of arising seedlings. Distinguishing between sexual and nucellar embryos in polyembryonic mango seeds and their arising seedlings were also studied. In addition, to study the compatibility between nucellar seedlings of the tested cultivars and two commercial cvs., the relationship between grafting success and leaf mineral, phenols, indoles, amino acids and sugars of both rootstocks and scions was also studied. The important results were:- Treating mango seeds by removing the hard coat just before sowing resulted in the highest germination percentages and the least average days required for seed germination. There were many anatomical variations between the zygotic and nucellar embryos of the cultivars under study. All of the tested three mango cultivars had specific markers. Detectable differences were found between the DNA fingerprints of each analyzed DNA samples. We could recommend using Sukkary rootstock for the scions of Keit and Sedik by using top-cleft grafting method at September month, this grafting type exhibited the best histological connection at the grafts union region and these scions contained the higher leaf content of N, P, K and Ca, total indoles, amino acids and sugars, and the least leaf content of total soluble phenols. Histological details were varied according to the degrees of compatibility and incompatibility between the scions and the rootstocks.

Key Words: Mango- Grafting- Histology- Fingerprint- Leaf endogenous content

DEDICATION

I dedicate this work to whom my heart felt thanks; to my departed Father ***Dr. Hosny****, my mother* ***Mona*** *and my brothers* ***Mohamed & Abd El-Rahman*** *for their patience and for all the support they lovely offered along the period of my post graduation.*

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CONTENTS

INTRODUCTION

Mango, *Mangifera indica* L., belongs to family Anacardiaceae. Its trees are evergreen, native to south Eastern Asia from India to the Philippine. It is one of the most popular and favorite fruit because of its rich flavor, aroma, pleasant appearance, attractive fragrance and delicious taste. It has been considered to be "the king of fruits" (Purseglove, 1972).

Mango trees have been cultivated in India from 4000 years ago and Portuguese carried the mango to various parts of the world. Now, it is an important fruit crop in India, Ceylon, Philippines, south East Africa, Australia and Brazil.

Egypt is considered one of the major mango producing countries in Africa. It produces a huge tonnage of mango fruits. However, a small percentage of the production is primally exported to regional markets (Litz, 1997).

Mango trees were introduced to Egypt around year 1825 and even since, it has gradually expanded through out the country and become one of the main fruits witch ranks the third after Citrus and Grape. The total acreage of mango in Egypt reached 139,433 feddans in (2005) producing about 416,951 tons with an average of 4,190 tons/fed., these area is concentrated in Ismailia (62,942 feddans), Sharkia (208,62 feddans) and Giza (7,755 feddans) Governorates.

The mango, like other tropical and subtropical fruit trees, is propagated by several methods which can be grouped under two main headings, sexual and asexual. In the sexual method, the propagation is

brought about by seeds, which are either monoembryonic or polyembryonic. Whereas the asexual method, is done by grafting.

Since long, mango is being propagated commercially by grafting using random rootstocks of unknown origin. However, these random rootstocks influence the various desirable characters. These rootstocks are grown from sexually developed mango seeds, which being highly heterozygous, great variations and differences can be noticed in vegetative growth between the trees of the same variety and at the same age grown in one orchard, this variation could be due to the rootstock. Use of vegetative developed seedlings from desired plants as rootstock may help to avoid such problems. During the last couple of years, demand for good quality mango varieties has increased several folds both in domestic market and also international trade.

Polyembryony phenomenon is common in mango cultivars grown in the moist tropics. Trees from nucellar seedlings are identical to the mother plant. In polyembryonic seed, only one embryo is zygotic in origin. It usually degenerates or produces weak and stunted seedlings. About three to eight seedlings usually are observed that originate from a single polyembryonic seed. For commercial plantations, nucellar seedlings can be distinguished from the zygotic seedling on the basis of their greater vigour. (Litz, 1997).

The present investigation aimed to:

1. Study the effect of different treatments on seed germination of three polyembryonic mango cultivars (Sukkary, Ewaise and Zebda cvs.). The morphological characteristics of arising seedlings were also determined.

2. Seed anatomy for distinguishing the sexual and nucellar embryos in the polyembryonic seeds under investigation.
3. Detection of genetic variation between sexual and nucellar seedlings comparing with the donor mother trees for using these nucellar seedlings as identical to the favorite characters of the mother plant.
4. Study the compatibility between vegetative seedlings produced of the tested polyembryonic cultivars and two commercial cvs. (Keit and Sedik), evaluate methods and time of grafting as well as examination the graft union zone histologically for explaining the morphological measurements.
5. Study the relationship between grafting success and leaf mineral content, total soluble phenols, indoles, amino acids and sugars.

REVIEW OF LITERATURE

Ram (1997) mentioned that mango can be propagated sexually and asexually. The sexual method of propagation consists of growing plants from seeds of monoembryonic mangoes. However seed propagation does not ensure true-to-type plant reproduction of monoembryonic mango, but it considered as the only method of rootstock production in mango.

In addition, Cordeiro *et al.* (2006) indicated that mango can be propagated by polyembryonic seeds or by grafting. For commercial purposes, grafting is the most appropriate method because it maintains the genetic characters of the propagated cultivar. To obtain grafted mango, it is important to use polyembryonic cultivars as rootstocks, since they produce zygotic and many nucellar seedlings. The nucellar seedlings maintain the genetics of the mother plant. They are preferred for grafting, since they supposedly give more uniformity to the orchard. In general, nurserymen use the most vigorous seedling to graft, believing that they are nucellar. But, the orchard disuniformities of height and yield are very common among mango trees of commercial orchards.

1. Effect of different treatments on germination characters of mango seeds

a. Germination percentage

Abdel-Galil (1992) concluded that, the husked Zebda mango seeds gave germination percentage of 96.22% compared with 45.56% for the unhusked seeds. Similarly, Padma and Reddy (1997) indicated that germination percentage of mango seeds improved by coat removal,

which gave germination percentage 78.57%, compared with 45.71% in the intact seeds. In addition, Perez *et al*. (1998) in study carried out on mango cv. Pico de Loro seedlings. The number of germinated seeds was counted daily in order to calculate percentage of germination (PG) and rate of germination (RG) and number of seedlings per seed (NSS). They found that, seed germination began at the 7th day and extended to the 11th day. PG was 90% and RG 8.2 days and NSS was 4.1 seedlings.

In addition, Abdel-Galil (2002), on studying the effect of some treatments on mango seeds, found that germination percentage differ according to cultivars and treatments. It ranged from 13.33% in Hindi Bisinnara cv. to 90.67% in Mabrouka cv. The germination percentage, generally in husked seeds was 84.78% compared with 19.78 % in unhusked seeds, regardless of cultivars. The highest germination percentage, however, was in the husked seeds of the polyembryonic seeds (Hindi Bisinnara and Goleck cv.). He also found that, freshly extracted mango seeds of ripe fruits germinate with higher frequency (76 – 91 %); also seed germination of mango can be improved by husking of the seeds.

Abd El-Zaher (2004) found that removal of the seed coat gave the highest germination percentage, followed by cracked coat then coated seed which came in the last rank (87.6, 90.6, 81.3 and 74.3%, respectively). Considering the rootstock, Zebda gave the best germination percentage, followed by Hindi Bisinnara then White Sukkary (92.6, 93.3; 89.6, 91.6 and 60.6, 62.6% for two studied seasons, respectively).

b. Number of days required for seed germination

Abdel-Galil (1992) found that the husked seeds gave germination percentage of 39.78, 80.67 and 96.22% after 10, 18 and 24 days from sowing, respectively. Meanwhile the unhusked seeds gave 0, 11.11 and 45.56% after the same period, respectively.

Padma and Reedy (1997) recorded that, the intact mango seeds took 23 days for the first emergence compared with only 10 days in case of seed coat removal, while it took 58.21 days for attaining 50% of final germination compared with 29.46 days in case of seed coat removal. In addition, Perez *et al*. (1998) observed that, germination of mango seeds began at 7^{th} day after sowing and extended to the 11^{th} day with the average of 8.2 days.

Abdel-Galil (2002) found that the germination rate of the husked mango seeds were 25.91, 26.59, 25.53 and 25.43 days in Hindi Bisinnara, Goleck, Pairi and Mabrouka cvs., respectively, with a mean of 25.87 days. These values were lower than that of unhusked ones which were 31.85, 30.73, 31.07 and 31.18 days, respectively with a mean of 31.21 days.

Moreover, Abd El-Zaher (2004) found that, the period required for germination of Zebda mango seeds took the lowest period of germination, while Hindi Bisinnara took the longest period. Considering the seed treatments, seed coat removal resulted in the lowest period of germination, while cracking seed coat was significantly equal to the coated seeds in this respect. Zebda with seed coat removal took the lowest period of germination and Hindi Bisinnara coated seed took the longest one.

c. Number of seedlings per seed

Garner and Chaudhri (1976) indicated that about three to eights seedlings usually are observed that originate from a single polyembryonic mango seed. In addition, Singh *et al.* (1983) mentioned that, polyembryonic is common in mango cultivars like White Sukkary, Hindi Bisinnara and Zebda. These cultivars produce nucellar embryos ranged from 2-10 seedlings.

Furthermore, El-Tomy (1995) found that the percentage of the seedlings produce from a polyembryonic mango seeds, which are true to type of the mother tree, were 90 and 91 % for Hindy Be-Sinnara and Zebda cultivar, respectively.

Ram (1997) mentioned that there are two types of mango seeds, viz. the monoembryonic seeds, which contain only one sexual embryo and only one seedling grows from this seed, and the polyembryonic seeds, in which only one embryo is zygotic in origin and three to eight seedling usually arise from the polyembryonic seeds.

In addition, Perez *et al*. (1998) in study carried out on mango cv. Pico de Loro observed that, at 30 days after seed germination the number of seedlings per seed (NSS) was 4.1seedlings.

Abd El-Zaher (2004) indicated that number of germinated embryos per polyembryonic seed was significantly affected by seed coat treatments. Husked seed and coat removal gave the highest number of germinated embryos per seed, followed by that with cracked coat then coated seeds. He added that, from the observation on germination of sex embryo suggested it located and emerged near the upper part of the seed which called the neck of the seed.