IDENTIFICATION OF MOLECULAR MARKERS FOR SOME MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS IN SOME MEDICINAL PLANTS

BY

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2000

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ABSTRACT

Ahmed Hassan Mohammed, Identification of molecular markers for some morphological and biochemical characters in some medicinal plants. Unpublished master of Science Thesis, Genetics Dept., Fac. of Agric., Ain Shams Univ., 2005.

The present study aimed to studying some chemical compositions, morphological characters and determines biochemical and molecular markers to two moringa species (*M. oleifera* and *M. pregrina*) as well as three mentha cultivars (Siwa, Baladi and Felfeli). Seeds and leaves of *M. peregrina* and *M. oleifera* can be considered as rich source for crude proteins and carbohydrates. While, the leaves of it's have a poor of crude proteins and Crude fiber. The oil content of *M. peregrina* was higher than that of *M. oleifera*. Menthone is the main component of essential oil from Felfeli genotype. Carvone is the main ketone component of essential oil from Baladi and Siwa genotypes. Protein electrophoresis was efficient to discriminate two moringa species but it was no efficient in characterization of three mentha genotypes.

Our results suggest that RAPD markers are the best choice for the evaluation of diversity and assessing the genetic relationships between moringa and mentha genotypes with high accuracy. Conservationist may use the information of the present study to make effective decisions regarding the global protection and management of moringa and mentha species in Egypt.

Key words: Medicinal plans, mentha, moringa, morphological studies, SDS-PAGE, RAPD and ISSR-PCR.

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I-INTRODUCTION

The production of herbal remedy drugs became so important, especially with flooding the pharmaceutical Egyptian market with a number of synthetic drugs of questionable efficacy associated with the increasing cost of such drugs. Therefore, the demand of high-yield, high-quality medicinal plants will continue to increase in the future. Also, a growing concern with the awareness of the side effects of the drugs associated with regular exposure to synthetic chemicals has triggered a “back to nature” idea with an appeal of new discovery natural products are necessary to meet primary health care and veterinary needs. Genetically improved and modified medicinal and aromatic plants with a legal standard of purity, uniformity and high levels of the economically important active ingredients could ensure a constant supply of quality medicinal plants and individual natural compound for the processing, marketing industry and biotechnology (**Brevoot, 1997**).

More than two thousand species grown wild in Egypt with no complete inventory of medicinal and aromatic plants in each region and, in general the list of medicinal plants in Egypt and the Arab countries is inexhaustible. There is no complete inventory of medicinal plants of the region (**Batanouny, 1999**).

Genetic diversity refers to the variation of genes within species, that is, the heritable variation within and between populations of organisms. In the end, all variation resides in the sequence of the four base pairs that compose the DNA molecule and, as such, constitute the genetic code. Other kinds of genetic diversity can also be identified at all levels of organization in the nucleus, including the amount of DNA per cell, chromosome number and DNA structure (**Tasrif *et al*., 2004**).

DNA fingerprint is a new technology that has matured and is poised for very widespread practical application, such as the identification of plants in commerce, plant breeding and research. In

addition, commercial applications include the protection of medicinal plant breeder's rights and patents, quality control in plant production, processing, and labeling of plant-derived drugs (**Soltis *et al.,* 1992**).

Traditionally, diversity within and between populations was determined by assessing differences in morphology. These measures have the advantage of being readily available, do not require sophisticated equipment and are the most direct measure of phenotype, thus they are available for immediate use, an important attribute. However, morphological determinations need to be taken by an expert in the species, they are subject to changes due to environmental factors and may vary at different developmental stages and their number is limited (**Tasrif *et al*., 2004**).

The objectives of this study were:

•. Determines the morphological and biochemical properties

by SDS-PAGE, RAPD and ISSR-PCR in two Moringa species and three Mentha cultivars

• Studies of biochemical and molecular characterization

II- REVIEW OF LITERATURE

Moringa species:

The *Moringaceae* is a single genus family with 14 known species. Of these, *Moringa oleifera* is the most widely known and utilised species. It is native to sub-Himalayan regions of India and is now naturalized in many countries in Africa, Arabia, SE Asia, Caribbean Islands and South America (**Ramachandran *et al.,* 1980**). Moringa is a fast-growing tree which can reach 12 m in height at maturity, yielding up to 120 tonnes/ha/yr when planted very densely for use as forage (**Makkar and Becker, 1997**). As *M. oleifera* trees have a loose canopy, which prevents excessive crop shading, they are useful for alley cropping. Foliage can be regularly pruned and left in the field to improve soil fertility or fed to livestock in a cut-and-carry system. The leaves are highly nutritious containing significant quantities of Vitamins A, B and C, Ca, Fe, P and protein. Laboratory analysis (**Makkar and Becker, 1997**) showed that the protein concentration in leaves is about 27% with negligible amounts of tannins (1 to 23 g/kg) in all fractions of the *M. oleifera* plant and high levels of sulphur-containing amino acids. Young leaves are used by farmers in India as cattle fodder to improve milk yields (**Bostock-Wood, 1992**) and in Zimbabwe as animal feed (**Clarke, 1994**). Both large and small-scale farmers in Tanzania grow *M. oleifera* for extraction of seed oil, so there is potential to use the foliage for feeding livestock (**Sarwatt *et al.,* 2002**).

Moringaceae also include M. peregrina, this native of the Red Sea region and it spread in Halayb and Shalatein. When Moringa peregrina seedlings start out, they have broad leaflets and a large tuber. Through many dry seasons, the shoot dies back below ground to the tuber. As the plant gets older, the leaves get longer and longer, but the leaflets get smaller and smaller and more widely spaced. Adult trees produce leaves with a full complement of tiny leaflets, only to drop them as the leaf matures.

Mentha species:

Mints belong to the genus Mentha, in the family Labiatae (Lamiaceae) which includes for about 3000 species of plants spread in the warm and temperate regions all over the world. They are mainly grasses and shrubs, very fragrant and rich in medicinal properties, of great worth in natural medicine and pharmacopoeia, and include also other commonly grown oil-yielding plants such as basil, sage, rosemary, marjoram, lavender, pennyroyal and thyme. Within the genus Mentha there are several different species, varying in their appearance, aroma and end use. The most common ones are spearmint (*M. spicata*), peppermint (*M.* × *piperita*), mint (*M.* × *piperita* var. citrata) and apple mint (*M. rotundifolia*). All are low-growing plants, readily sending out runners, or stolons, which develop new roots and shoots at the nodes.

1. Morphological characters studies of medicinal plants:

Babos *et al.,* (1981) studied The morphology, ecological characteristics, habitat and wood anatomy for *Capparis sessilis*, a shrub 0.5-2.5 m tall, *C. pachaca*, a tree 3-6 m tall, *C.* odoratissima, a tree or shrub 2-7 m tall and *C. stenosepala*, a shrub or tree 1.5-3 m tall.

Paul and Sen (1987) studied seeds morphology type in *Capparis* revealed the presence of 2 distinct seed types. Small (S) seeds had a 10-seed weight of 375 mg, were 4.67 X 3.67 mm in size and were pale violet with white markings, while large (L) seeds had a 10-seed weight of 471 mg, were 6.17 X 4.67 mm in size and were cream colored. Germination percentage was 6.67% in S seeds and 40% in L seeds. When pretreated (scarified) with concentrated sulfuric acid, S seeds, with harder seed coats, required a 60 min pretreatment to give a 33.33% germination percentage (45 min for L seeds).

Maffei (1990) studied plant morphological characteristics of 17 *Mentha X verticillata* hybrids were analysed during the growing

seasons of 1988 and 1989. The data obtained were used to study the phenotypic plasticity, the genotypic variation and the genetic variation for phenotypic plasticity. All plants showed high levels of phenotypic plasticity for both oil chemical and morphometric parameters. Higher degrees of genotypic variation were found among the plants for oil components while a higher phenotypic plasticity was observed for morphological parameters. Temperature and rainfall data were collected during the growing seasons and correlated with data obtained for plant oil and morphology. Low levels of phenotypic plasticity and high degrees of genotypic variation were found to form outliers in the population of *M. X verticillata* hybrids. The results obtained confirm a significant environmental effect on the physiology and morphology of the genus Mentha.

Safrazbekyan *et al.,* (1990) studied Morphophological characteristics of caper (*Capparis spinosa L*.) shoots during clonal micropropagation. : Together with normal shoots, vitrified and fasciated shoots formed during the micropropagation of caper. The number of anomolous shoots and their degree of abnormality increased with the duration of culture.

Ozguven *et al.,* (1998) found In Turkey, species of Mentha often occur in mixed populations in which hybrids may be found. The occurrence of about seven species of *Mentha* has been recorded in the flora of Turkey. In this work, the botanical and agronomic characters of *Mentha species* planted in the Botanical Garden were examined and The morphological properties of these *Mentha species* have been found to be lower than reference values

Sobrado *et al.,* (1986) studied Leaf area to leaf wt. ratios of fully mature leaves in (*Capparis verrucosa* and *C. aristiquetae*) was compared with 2 deciduous trees (*Humboldtiella arborea* and *Lonchocarpus dipteroneurus*) and a deciduous vine (*Mansoa verrucifera*) in Venezuela. Seasonal fluctuations of leaf water content per unit dry wt., water potential and pressure were smaller in evergreen than in deciduous species. Evergreen species could