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# Molecular Taxonomy Among *Mentha spicata*, *Mentha longifolia* and *Ziziphora tenuior* Populations using the RAPD Technique

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Abstract

The Random Amplified Polymorphic DNA (RAPD) technique was used to study the molecular taxonomy and genetic relationship between two *Mentha* species namely, *Mentha spicata* and *Mentha longifolia*, and *Ziziphora tenuior*. Sixteen RAPD primers showing polymorphic bands were used for the construction of the dendrogram and a similarity matrix. A total of 2001 bands were obtained; 419 of them were polymorphic. Similarity values among the studied samples ranged from 0.68 to 0.03. High similarity values were obtained between two samples of *Mentha spicata* (0.68) collected from local markets and between three samples of *Mentha longifolia* (0.64) as well. RAPD analysis confirmed that *Mentha* species are genetically different from *Ziziphora tenuior* and a genetic variation was found between and within the species tested for this study. The cluster analysis clearly differentiated *Mentha spicata and Mentha longifolia* from *Ziziphora tenuior*. Molecular analysis with RAPD markers stressed their ability for differentiation between families, genus, and species of living organisms particularly *Mentha* species and *Ziziphora tenuior*.



Keywords: *Mentha, Ziziphora*, RAPD, Taxonomy, Jordan.

## 1. Introduction

Jordan has a rich flora of medicinal plants with diverse biological properties. *Mentha* L. species are one of the most important medicinal and aromatic plant species used in Jordan and worldwide. These are sources of essential oils that are widely used in food, flavour, cosmetic and for pharmaceutical purposes. *Mentha* (M.), is the most important genus of aromatic perennial herbs belonging to the Labiatae (Lamiaceae) family and distributed mostly in temperate and sub-temperate regions of the world. It contains a number of taxa with high economic essential oils and within this section *Mentha*, five basic Eurasian and African species (*M. arvensis* L., *M. aquatica* L., *M. spicata* L., *M. longifolia* (L.) Huds., and *M. suaveolens* Ehrh.) have been identified, with eleven naturally occurring named hybrids (Lawrence, 2007; Bhat *et al*., 2002). The species of section *Mentha* typically have chromosome number 2n=2x=12, but the other species vary widely, with *M. spicata* L. and *M. longifolia* have 2n=2x=48 and 2n=2x=24, respectively (Lawrence, 2007; Murray, 1960).

The spearmint, *M. spicata,* is a hybrid of *M. longifolia* and *M. rotundifolia*, morphological, cytological and biochemical data have shown that the tetraploid species of *M. spicata* (2n=48; Lawrence, 2007) originated by chromosomal doubling of hybrids between the two closely related and inter-fertile diploids, *M. longifolia* and *M. suaveolens* (Harley and Brighten, 1977). The ketone constituent of the oil is important in three ways; with one of them is to give the oil and herbage its characteristic odor (Murray, 1960). Oil from an individual of the polymorphic species *M. spicata* may have any (but only one) of the three ketone groups (Murray, 1960). The chemical constituents in the oil of *M. spicata* were 58% carvone, 8% limonene, 10% dipentene, 7% dihydrocarveol, and it can be used in foods, beverages, tooth paste mouth wash, soaps, detergents and perfumes and medicinally as stimulant carminative, anti-spasmodic and in bronchitis and fever (Bhat *et al.,* 2002). The essential oil of *M. spicata* showed good activity against larvae of fourth instar of Anophel stephensi (Hadjiakhoond *et al.*, 2000) also it has radical scavenging activities (Souri *et al.,* 2008). At the level of folk medicine, the leave decoction of *M. spicata* can be taken twice a day for a week to cure throat infection and indigestion (Mahato and Chaudhary, 2005). Mint is usually taken after a meal for its ability to reduce indigestion and colonic spasms by reducing gastrocholic reflux (Bhat *et al.,* 2002). In Egypt, *M spicata* is cultivated for its volatile oils; it is also used in food flavoring (Bader *et al.*, 2003), as a culinary herb, and in toothpaste and chewing gum industry (Naghibi *et al.,* 2005).

The very musty odor of *M. longifolia* (L.) Huds. (2n=2x= 24) is that of pure piperitone oxide, its principal ketone. This species has smaller amounts of the related

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ketone, piperitenone oxide (Murray, 1960). *M. longifolia* has 56% piperitone oxide, 20% pipertenone, disophenol, disohenolene (in traces) and is mainly used for treatment of nausea, gastralgia, neuralgia rheumatism, bladder stone, gall stone, rheumatism, jaundice, diarrhoea, toothache, stomachache, anti-infection, dyspnea, flatulence, gastrodynia, dyspepsia, sedative, stomach tonic, insect repellent and headache as well as its being used as a vegetable in most parts of Iran, especially in the Northern region (Bhat *et al*., 2002; Naghibi *et al.,* 2005). The essential oil of *M. longifolia* has important compounds (menthol, menthone, pulegone,) having interesting antimicrobial activities, after 24 h of bacteria treatment with *M. longifolia* essential oil, they noted a big damage in *S. typhimurium* and *E. coli* (rod bacteria), whereas damage is less important in coccoid bacteria (*M. luteus* and *S. aureus*) (Hafedh *et al.,* 2010).

The genus *Ziziphora* (Z.) belongs to the family Labiatae and consists of four species (*Z. clinopodioides* Lam., *Z. captitata* L., *Z. persica* Bunge. and *Z. tenuior* L.) that are widespread all over Iran. *Z. clinopodioides*, with the common Persian name "kakuti-e kuhi" is an endemic species and grows wild in Iran, Afghanistan, Iraq, and Talish (Verdian-Rivi, 2008). *Z. tenuior* is distributed in a defined area particularly at southern part of Jordan. It has an attractive odor and the local communities use it to make tea. *Z. tenuior* is a common teapot herb and used for treatment of fever, dysentery, coughing, diarrhea, painful menstruation, bladder stone, abortifacient and stomach tonic (Naghibi *et al.,* 2005).

In Jordan, Al-Quran (2005) reported that the largest genera was *Mentha* including: *M. aquatica, M. graveolens* L., *M. longifolia, M. piperita* L., *M. pulegium* L. and *M. spicata.* In the past, seed protein analysis and morphology were used for taxonomy and evolutionary studies between and within species and subspecies levels. Šarić-Kundalić *et al.*,(2009) conducted a taxonomic study on the anatomical, morphological and photochemical differentiation of the genus *Mentha* L. (Lamiaceae) in Bosnia & Hercegovina and Slovakia. Nowadays, molecular markers have been used to define the species relatives and their taxonomy. Among them, RAPD and AFLP have the utility of being used as a means of studying taxonomy and genetic diversity among different *Mentha* species (Gobert, *et al.,* 2002; Khanuja, *et al.,* 2009 and Shasany, *et al.,* 2005). This study aims at studying the molecular taxonomy and the genetic relationships among two species of *Mentha* namely M. *spicata* and *M. longifolia* and *Z. tenuior* , using RAPD molecular analysis.

## 2. Materials and Methods

### *2.1. Plant material*

This study includes a total of 30 samples of *Mentha* species composed of 10 samples of *M. spicata* collected from local markets, 10 samples of a wild *Z. tenuior* collected from Al-Shoubak district, and 10 samples of a wild *M. longifolia* collected from the flow of the Hussban stream in Jordan during 2009/2010 to be used for molecular taxonomy based on RAPD analysis. DNA analysis was conducted at the National Center for Agricultural Research and Extension (NCARE).

### *2.2. DNA isolation*

Total cellular DNA was extracted following the procedure as described by Doyle and Doyle (1987), with minor modifications. Approximately 20 mg of fresh leaves of *Mentha* samples were ground in liquid nitrogen and mixed with 600 µl of freshly preheated 2x CTAB solution with 0.8g PVPP in 2ml tubes then placed at 65ºC for 30 min. The mixture was added to 600 µl of chlorophorm/ isoamyl alcohol (24:1), vortexed for few seconds, and then centrifuged at 13,000g for 10 min. The supernatant was placed in 2ml tubes with 600 ml isopropanol, and then shaken until the threads of DNA appeared, then centrifuged for 10 min at 13000g. The solution was poured from the tubes, and the pellet was left to dry. 600 µl of cooled 70% ethanol was added to the pellet and was placed in the refrigerator (–20ºC) overnight. Next day, ethanol was poured from the tubes, the pellet was allowed to dry and 150µl of TE was added and the whole mixture was placed at 65ºC for 30min. Four micolitrers of RNAase (10mg/ml) were added per tube and incubated for 60 min at 37ºC. DNA quantity was measured using a S2100 UV/VIS DIODE-Array-Spectrophotometer, machine Version 1.7.

### *2.3. PCR amplification*

The PCR reaction was performed as described by Williams *et al*. (1990) with 10-mer ologonucleotides synthesized by Operon technologies (Almeda, Calif.). The final PCR volume of 25 µl contained 10 x buffer with MgCl2, 20ng of total genomic DNA, 0.25 mM dNTPs (Promega), 100 µM of primers, 1.5mM MgCl2 and 1U of Taq polymerase. Amplification was carried out in thermocycler (MJ Research, USA, Model PCT-200), one cycle of 1 min at 94°C followed by 44 cycles, each consisting of a denaturation step for 1min at 94°C, followed by an annealing step for 1min at 36°C and an extension step for 2 min at 72°C, followed by a final extension step for 5 min at 72°C. After the final cycle the samples were cooled to 4°C. Samples of 10 µl RAPD-PCR product were analyzed by electrophoresis on 1.4% agarose gel and the amplified products were detected under UV light after staining by ethidium bromide. Forty 10-mer primers (Table 1), corresponding to kit A, B, C, D, T, W and Z, were used to study the taxonomy of *Mentha* species.

**Table 1**. Primers names and their sequences used for *M. spicata*, *Z. tenuior* and *M. longifolia* species in this study.

| Primer name | Sequence 5'-3' | Primer name | Sequence 5'-3' |
|---|---|---|---|
| OPA16 | AGCCAGCGAA | OPD10 | GGTTCACACC |
| OPA18 | AGGTGACCGT | OPD11 | AGCGCCATTG |
| OPA20 | GTTGCGATCC | OPD12 | CACCGTATCC |
| OPB01 | GTTTCGCTCC | OPD14 | CTTCCCCAAG |
| OPB04 | GGACTGGAGT | OPD16 | AGGGCGTAAG |
| OPB05 | TGCGCCCTTC | OPD18 | GAGAGCCAAC |
| OPB08 | GTCCACACGG | OPD20 | ACCCGGTCAC |
| OPB09 | TGGGGGACTC | OPT03 | TCCACTCCTG |
| OPB10 | CTGCTGGGAC | OPT05 | GGGTTTGGCA |
| OPB12 | CCTTGACGCA | OPT10 | CCTTCGGAAG |
| OPB13 | TTCCCCCGCT | OPT13 | AGGACTGCCA |
| OPB14 | TCCGCTCTGG | OPT15 | GGATGCCACT |
| OPB17 | AGGGAACGAG | OPT16 | GGTGAACGCT |
| OPB19 | ACCCCCGAAG | OPT19 | GTCCGTATGG |
| OPC09 | CTCACCGTCC | OPT20 | GACCAATGCC |
| OPC10 | TGTCTGGGTG | OPW04 | CAGAAGCGGA |
| OPC12 | TGTCATCCCC | OPW17 | CTCCTGGGTT |
| OPC20 | ACTTCGCCAC | OPZ12 | TCAACGGGAC |
| OPD04 | TCTGGTGAGG | OPZ15 | CAGGGCTTTC |
| OPD06 | ACCTGAACGG | OPZ16 | TCCCCATCAC |

*2.4. Data analysis*

RAPD bands were manually scored as present (1) or absent (0) for the estimation of the similarity among all the tested samples. A matrix of similarity (Jaccard) and similarity of coefficients (Nei and Li, 1979) were calculated and a dendrogram was obtained by clustering according to the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) using SPSS (V., 11.0) software. Polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

## 3. Results

From 40 initially applied primers, only 16 showed reproducible fragments with easily recordable bands. The total number of bands, the number of polymorphic bands along with the percentage of polymorphism are shown in Table 2. A total of 2001 RAPD fragments were consistently recognized, of which 419 were polymorphic in all the tested samples (Table 2). High percentages of polymorphism (26%) showed by OPB01 and OPT16, 26% by OPD06 and OPT15 and 25% for OPT20 (Table 2).

The number of bands varied in different samples with levels of similarity between the samples ranging between 0.68 to 0.03 (Table 3). The highest average similarity index value of 0.68 was observed between two samples (11 and 12) of *M. spicata*. The dendrogram was produced for *Mentha* species and samples showed three main clusters (Figure 1). The first cluster consisted of individuals numbered from 1-10 of *Z. tenuior*. The second cluster consisted of individuals numbered from 11 to 20 samples of *M. spicata*. The third cluster included the samples of *M. longifolia* from 21 to 30. The level of similarity between *Mentha* species and *Z. tenuior* species ranged from 0.68 to 0.02 (Table 3). *Z. tenuior* showing a range of similarity with *M. spicata* and *M. longifolia* (0.21 to 0.06) and (0.13 to 0.03), respectively. On the other hand, genetic variability within each species was found, which is obvious through the presence of sub-clusters within each cluster (Figure 1).

**Table 2**. Total bands, number of polymorphic bands, percent polymorphism and maximum and minimum number of bands per primer of most polymorphic RAPD primers used among *M. spicata*, *Z. tenuior* and *M. longifolia* in this study.

| Primer name | Total bands/primer | Number of polymorphic bands | % of polymorphism | Max./ Min. band per primer |
|---|---|---|---|---|
| OPB01 | 99 | 29 | 29 | 6/2 |
| OPB06 | 140 | 22 | 16 | 6/1 |
| OPB08 | 99 | 20 | 20 | 5/1 |
| OPB09 | 124 | 26 | 21 | 10/1 |
| OPB10 | 113 | 19 | 17 | 6/2 |
| OPB19 | 145 | 27 | 19 | 7/2 |
| OPD06 | 109 | 28 | 26 | 7/1 |
| OPD10 | 171 | 25 | 15 | 10/2 |
| OPD14 | 186 | 37 | 20 | 9/2 |
| OPD16 | 166 | 32 | 19 | 8/4 |
| OPT03 | 133 | 29 | 22 | 8/2 |
| OPT05 | 116 | 22 | 19 | 6/2 |
| OPT10 | 122 | 28 | 23 | 8/3 |
| OPT15 | 113 | 29 | 26 | 9/1 |
| OPT16 | 88 | 26 | 29 | 5/1 |
| OPT20 | 77 | 19 | 25 | 5/1 |
| **Total bands** | **2001** | **419** | **Mean: 22.6** | |

## 4. Discussion

Due to their medical benefits, a very high percentage of the world's population relies on medicinal and aromatic plants (Lawrence, 2007). *Mentha* species are resources for essential oils enriched in certain monoterpenes and are widely used in food, flavor, cosmetic, and pharmaceutical industries (Bhat *et al*., 2002). The following primers OPB01 and OPT16, OPD06 , OPT15, OPT16 and OPT20 showed the highest levels of polymorphism 29%, 29%, 26%, 26% and 25%, respectively, and can be used for further testing of the rest of *Mentha* species 'in Jordan' with molecular and biochemical association.

In this study, *M. spicata, M. longifolia* and *Z. tenuior* formed the three different clusters indicating that each species has a unique DNA sequence, and that a genetic variability exist among them. This result is in agreement with the findings of Mustafa and Bader (2005) who reported that the difference among species could be related to the variants in the alleles numbers between *Mentha* species, and it may be more obvious in the asexual plants *M. longifolia*. The genetic variability, found among the species, could be due to out-breeding and the wide dispersal of seeds and pollen grains. The genetic variation between *Mentha* species can also be explained by the differences in chromosomes numbers (2n=2x= 24) and (2n=2x= 48) in *M. longifolia* and *M. spicata*, respectively (Lawrence, 2007; Murray, 1960). Divergence between *M. longifolia* and *M. spicata* could be a reflection of the impact of environmental variation among the samples of *Mentha* species. This result was in accordance with the results obtained by (Mustafa and Bader, 2005).

In addition to genetic variations, the results of this study indicate that each species has different morphological and biochemical characteristics. Molecular analysis is considered one of the best methods of studying molecular taxonomy to identify and differentiate between species. The findings of the present investigation will be helpful for traditional healers, the local community and all those involved in the study of ethnomedicine, and for scientists to further test these systems. Cultivation should be oriented in the future for essential oil production of *Mentha* species and *Z. tenuior*. Further studies, including the morphological traits, cellular biochemical, molecular data, isozyme polymorphism and karyotyping, should be taken into consideration in the future.

Table 3: RAPD similarity matrix based on similarity coefficient of the amplified bands for *Ziziphora tenuior*, *Mentha spicata* and *Mentha longifolia* collected from different regions in Jordan.

| | *Ziziphora tenuior* | | | | | | | | | | *Mentha spicata* | | | | | | | | | | *Mentha longifolia* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 1 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.41 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.46 | 0.44 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.35 | 0.40 | 0.53 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 0.30 | 0.27 | 0.36 | 0.35 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 0.30 | 0.30 | 0.38 | 0.40 | 0.46 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 0.36 | 0.30 | 0.41 | 0.40 | 0.52 | 0.47 | 1.0 | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 0.24 | 0.24 | 0.31 | .33 | 0.39 | 0.35 | 0.49 | 1.0 | | | | | | | | | | | | | | | | | | | | | | |
| 9 | 0.21 | 0.21 | 0.026 | 0.29 | 0.41 | 0.35 | 0.43 | 0..51 | 1.0 | | | | | | | | | | | | | | | | | | | | | |
| 10 | 0.22 | 0.17 | 0.28 | 0.30 | 0.32 | 0.30 | 0.37 | 0.39 | 0.52 | 1.0 | | | | | | | | | | | | | | | | | | | | |
| 11 | 0.13 | 0.13 | 0.17 | 0.16 | 0.16 | 0.15 | 0.17 | 0.17 | 0.20 | 0.21 | 1.0 | | | | | | | | | | | | | | | | | | | |
| 12 | 0.11 | 0.12 | 0.14 | 0.14 | 0.14 | 0.14 | 0.16 | 0.15 | 0.20 | 0.21 | 0.68 | 1.0 | | | | | | | | | | | | | | | | | | |
| 13 | 0.16 | 0.12 | 0.16 | 0.15 | 0.16 | 0.15 | 0.14 | 0.14 | 0.18 | 0.20 | 0.64 | 0.53 | 1.0 | | | | | | | | | | | | | | | | | |
| 14 | 0.10 | 0.10 | 0.13 | 0.12 | 0.14 | 0.15 | 0.12 | 1.14 | 0.15 | 0.14 | 0.64 | 0.53 | 0.59 | 1.0 | | | | | | | | | | | | | | | | |
| 15 | 0.10 | 0.11 | 0.14 | 0.16 | 0.12 | 0.11 | 0.11 | 0.13 | 0.15 | 0.14 | 0.43 | 0.39 | 0.44 | 0.42 | 1.0 | | | | | | | | | | | | | | | |
| 16 | 0.10 | 0.09 | 0.10 | 0.11 | 0.10 | 0.08 | 0.09 | 0.12 | 0.13 | 0.10 | 0.43 | 0.43 | 0.48 | 0.45 | 0.53 | 1.0 | | | | | | | | | | | | | | |
| 17 | 0.11 | 0.12 | 0.14 | 0.14 | 0.15 | 0.15 | 0.13 | 0.17 | 0.18 | 0.18 | 0.42 | 0.42 | 0.47 | 0.42 | 0.49 | 0.47 | 1.0 | | | | | | | | | | | | | |
| 18 | 0.09 | 0.06 | 0.09 | 0.09 | 0.11 | 0.10 | 0.09 | 0.12 | 0.15 | 0.15 | 0.44 | 0.39 | 0.46 | 0.46 | 0.44 | 0.47 | 0.60 | 1.0 | | | | | | | | | | | | |
| 19 | 0.09 | 0.12 | 0.11 | 0.09 | 0.08 | 0.07 | 0.11 | 0.09 | 0.10 | 0.08 | 0.18 | 0.15 | 0.21 | 0.16 | 0.22 | 0.21 | 0.19 | 0.16 | 1.0 | | | | | | | | | | | |
| 20 | 0.06 | 0.08 | 0.10 | 0.09 | 0.08 | 0.08 | 0.07 | 0.09 | 0.10 | 0.09 | 0.14 | 0.12 | 0.16 | 0.14 | 0.16 | 0.16 | 0.15 | 0.11 | 0.37 | 1.0 | | | | | | | | | | |
| 21 | 0.08 | 0.09 | 0.11 | 0.08 | 0.05 | 0.08 | 0.07 | 0.08 | 0.07 | 0.06 | 0.08 | 0.08 | 0.12 | 0.09 | 0.17 | 0.14 | 0.13 | 0.07 | 0.23 | 0.16 | 1.0 | | | | | | | | | |
| 22 | 0.10 | 0.12 | 0.13 | 0.08 | 0.11 | 0.11 | 0.08 | 0.08 | 0.10 | 0.11 | 0.13 | 0.11 | 0.14 | 0.12 | 0.15 | 0.13 | 0.13 | 0.10 | 0.17 | 0.10 | 0.31 | 1.0 | | | | | | | | |
| 23 | 0.09 | 0.10 | 0.11 | 0.10 | 0.08 | 0.07 | 0.08 | 0.07 | 0.08 | 0.08 | 0.09 | 0.09 | 0.07 | 0.08 | 0.13 | 0.13 | 0.12 | 0.08 | 0.13 | 0.10 | 0.20 | 0.25 | 1.0 | | | | | | | |
| 24 | 0.09 | 0.08 | 0.10 | 0.07 | 0.09 | 0.08 | 0.07 | 0.06 | 0.07 | 0.07 | 0.11 | 0.10 | 0.14 | 0.09 | 0.11 | 0.10 | 0.09 | 0.10 | 0.12 | 0.08 | 0.17 | 0.16 | 0.25 | 1.0 | | | | | | |
| 25 | 0.07 | 0.05 | 0.07 | 0.05 | 0.04 | 0.05 | 0.05 | 0.07 | 0.06 | 0.07 | 0.09 | 0.06 | 0.10 | 0.07 | 0.08 | 0.11 | 0.10 | 0.10 | 0.07 | 0.06 | 0.23 | 0.16 | 0.21 | 0.36 | 1.0 | | | | | |
| 26 | 0.07 | 0.07 | 0.10 | 0.07 | 0.04 | 0.08 | 0.08 | 0.08 | 0.03 | 0.09 | 0.08 | 0.07 | 0.08 | 0.06 | 0.07 | 0.07 | 0.07 | 0.05 | 0.09 | 0.09 | 0.16 | 0.15 | 0.20 | 0.19 | 0.23 | 1.0 | | | | |
| 27 | 0.07 | 0.07 | 0.11 | 0.12 | 0.10 | 0.09 | 0.08 | 0.14 | 0.07 | 0.09 | 0.09 | 0.08 | 0.09 | 0.07 | 0.12 | 0.010 | 0.09 | 0.08 | 0.09 | 0.06 | 0.16 | 0.16 | 0.17 | 0.28 | 0.23 | 0.25 | 1.0 | | | |
| 28 | 0.10 | 0.09 | 0.10 | 0.09 | 0.12 | 0.09 | 0.09 | 0.08 | 0.08 | 0.08 | 0.10 | 0.11 | 0.08 | 0.09 | 0.10 | 0.12 | 0.09 | 0.08 | 0.11 | 0.08 | 0.15 | 0.12 | 0.23 | 0.19 | 0.16 | 0.23 | 0.19 | 1.0 | | |
| 29 | 0.09 | 0.09 | 0.08 | 0.08 | 0.11 | 0.09 | 0.08 | 0.05 | 0.06 | 0.06 | 0.09 | 0.12 | 0.12 | 0.10 | 0.14 | 0.12 | 0.11 | 0.10 | 0.09 | 0.05 | 0.17 | 0.19 | 0.21 | 0.24 | 0.16 | 0.22 | 0.23 | 0.39 | 1.0 | |
| 30 | 0.09 | 0.05 | 0.12 | 0.10 | 0.10 | 0.07 | 0.06 | 0.08 | 0.08 | 0.11 | 0.08 | 0.10 | 0.09 | 0.08 | 0.11 | 0.09 | 0.10 | 0.08 | 0.09 | 0.07 | 0.14 | 0.21 | 0.24 | 0.26 | 0.21 | 0.24 | 0.28 | 0.23 | 0.29 | 1.0 |

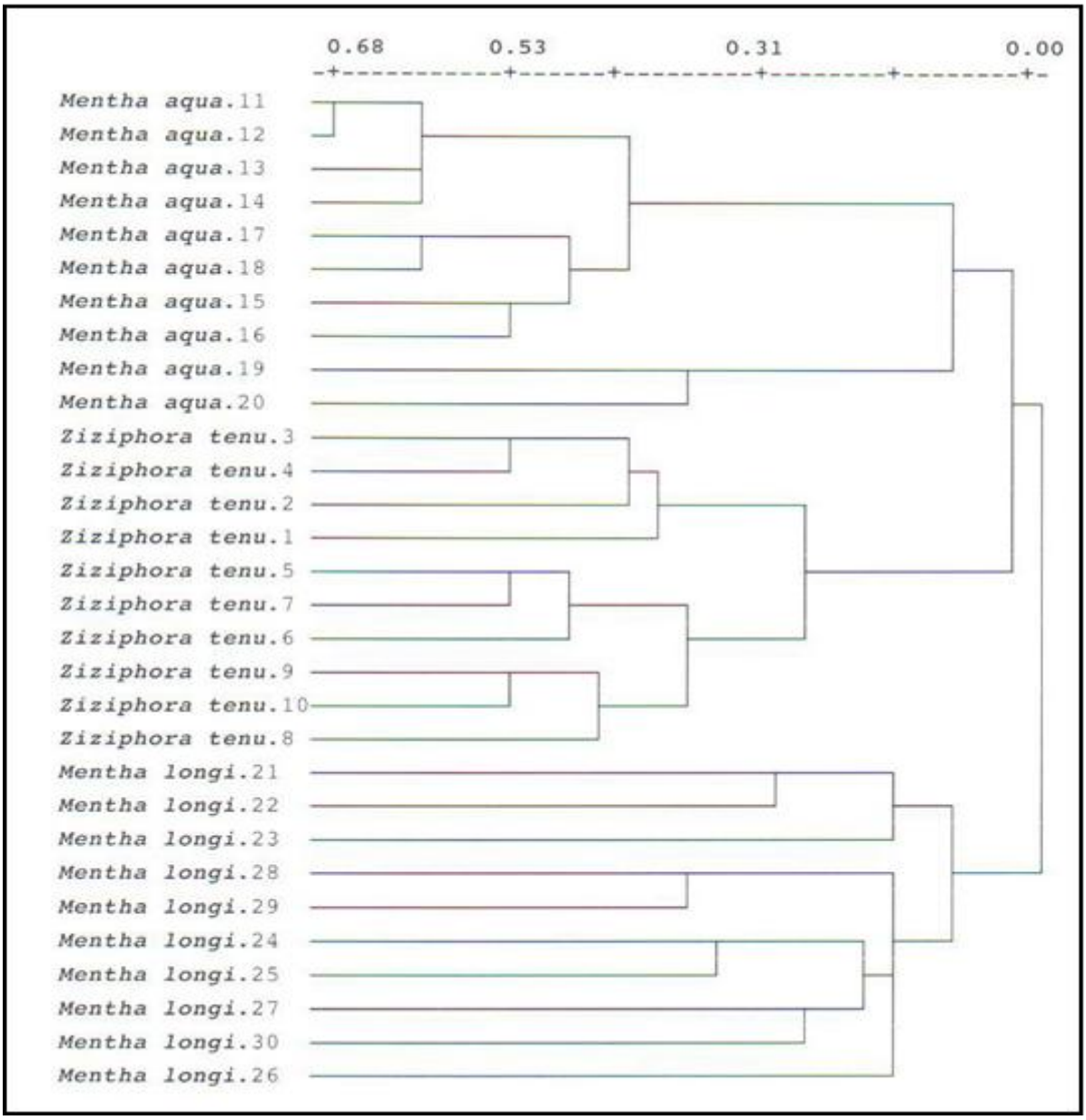


**Figure 1**. A dendrogram of M. spicata, Z. tenuior and M. longifolia genotypes using sixteen polymorphic RAPD primers, based on Jaccards´ coefficient of similarity.

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