Scientia Horticulturae 153 (2013) 136–142

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Mycorrhizal infection, essential oil content and morpho-phenological characteristics variability in three mint species

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ARTICLE INFO

Article history:
Received 24 September 2012
Received in revised form 14 January 2013
Accepted 22 January 2013

Keywords:
Essential oil
Mentha
Mint
Mycorrhiza
Variation

ABSTRACT

This study investigated the natural variation of arbuscular mycorrhizal associations and variation in morpho-phenological characteristics and essential oil content of 40 accessions of *Mentha* species including *Mentha spicata* L., *Mentha piperita* L. and *Mentha longifolia* (L.) Hudson collected from 13 provinces of Iran. The colonization by Arbuscular Mycorrhizal Fungi (AMF) was naturally found in 38 accessions ranged from 1.4 to 71.8% of infection. There was a significant variation between and within species in terms of essential oil content differing from 0.30 to 3.33% beyond the previously reported range. Accessions collected from colder climate conditions exhibited significantly higher oil content than those from warmer conditions. *M. longifolia* had significantly higher oil content than the other two species. A high variation in fresh weight and leaf water content was also observed and higher mean values were obtained in accessions of *M. piperita* and *M. longifolia*, respectively. A dendrogram generated using the UPGMA algorithm classified the 40 accessions into four distinctive groups based on the species and discriminating characteristics. The high variability in naturally mycorrhizal infection, essential oil content and morpho-phenological characteristics suggests the possibility of improving mint accessions for horticultural and medicinal uses through selection in breeding programs.

1. Introduction

Mint has been exploited for essential oil production and herbage yield in a variety of applications including pharmaceutical, ornamental, food and vegetable uses and for confectionery and cosmetics industries (Zeinali et al., 2004). For each purpose, a great number of clones and species should be evaluated before selection for high levels of essential oil and vegetable yield, based on a well-developed method to choose between and within the species (Mirzaie-Nodoushan et al., 2001). To date, little attention has been given to the evaluation of diverse mint collections and the factors affecting oil content and production of edible tissues.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants forming the largest group symbiotically associated with agricultural crops (Smith and Read, 1997). They help plant species to uptake water and nutrients and make physiological changes to increase growth and productivity of host plants (Gupta and Janardhanan, 1991; Bethlenfalvay and Linderman, 1992). Today, AMF are important components of rhizosphere microbial communities in natural ecosystems as well as they are extensively used as biofertilizers in agroecosystems (Smith and Read, 1997). Although mycorrhizal detection and investigations of their impacts on medicinal plants have rarely been conducted, they have been observed to be associated with medicinal and aromatic plants (Gupta et al., 1995). The symbiotic AMF can also induce changes in the accumulation of secondary metabolites, including phenolics in roots and aerial parts and also essential oil of host plants (Devi and Reddy, 2002; Rojas-Andrade et al., 2003; Yao et al., 2003; Copetta et al., 2006; Toussaint et al., 2007). The recognition of the status of mycorrhizal association and its variation in medicinal and aromatic plants is, therefore, of particular concern to improve the quantity of pharmaceutical substances.

In *Mentha* species, little is known about the distribution and naturally infection of AMF and their effects on either the production of essential oil or plant secondary metabolic pathways; though, there are reports that AMF could affect genetic variation of mint (Van der Heijden et al., 1998). In *Mentha arvensis* L. Gupta et al. (2002) reported that mycorrhizal inoculation significantly increased oil content and yield compared to non-mycorrhizal plants. Freitas et al. (2004) also observed that inoculation with AMF led to an increase of 89% in the essential oil and menthol contents of *M. arvensis* plants. In *Mentha piperita*, Mucciarelli et al. (2003) observed that colonization by a non-mycorrhizal fungus increased essential oil content and altered the oil composition. Yet, no attempts have been made

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http://dx.doi.org/10.1016/j.scienta.2013.01.014

No.	Province	Temperature (°C)	Rainfall (mm)	Climate
1	Western Azerbaijan	11.5 ±1.86	404	
2	Hamadan	11.3±1.83	328	
3	Kermanshah	14.3±1.54	427	
4	Lorestan	17.2±1.55	409	
5	Alborz	14.9±1.62	248	
6	Ghazvin	14.0±1.83	351	
7	Northern Khorasan	16.1±1.24	253	
8	Khozestan	25.3±1.37	161	
9	Markazi	21.9±1.97	192	
10	Esfahan	20.3±1.43	120	
11	Yazd	19.1±1.57	125	
12	Fars	23.5±1.32	195	
13	Razavi Korasan	19.1±1.92	174	

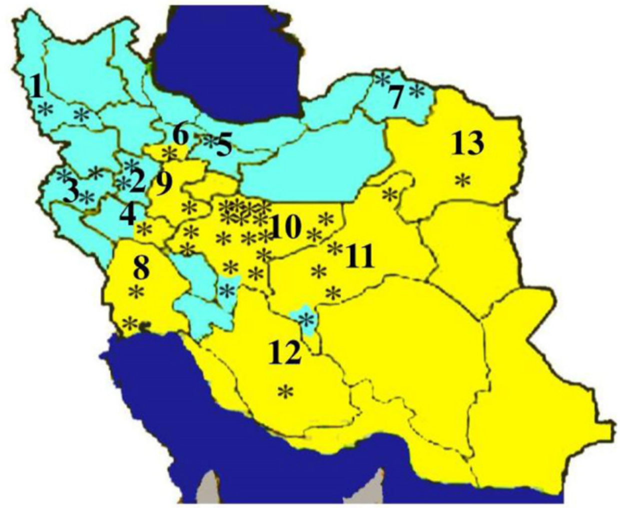

Fig. 1. Collection sites map of 40 mint accessions (*). [cyan box] and [yellow box], colder and warmer region, respectively.

to investigate the variability of AMF colonization among *Mentha* species and to reveal the adaptation of plant-fungus accessions to different climatic conditions in a diverse genetic population.

Three economically important *Mentha* species including *Mentha spicata*, *Mentha longifolia* and *M. piperita* have an extensive geographical range in Iran which in turn may bring about a significant genetic variation in these species for breeding mint in terms of important characteristics. For this purpose, collection and evaluation of diversity in plant germplasm is a prerequisite. On the other hand, environmental factors including the relationship between mint plant and mycorrhizal colonization may also affect the morpho-phenological and physiological characteristics. This means that any report on genetic variation of mint should include the infection status of plant with mycorrhiza. The objectives of this study were, therefore, to survey the variation of morpho-phenological, and essential oil content and their relations in a broad mint germplasm collected from 13 provinces of Iran and investigate the effect of climate conditions of collected samples on different characteristics. The variation of AMF infection was also reported in genotypes of three *Mentha* species collected from various regions.

2. Material and methods

2.1. Plant material

A total of 28 accessions of *M. spicata* (which is the prevalent species of mint in Iran), 6 accessions of *M. longifolia* and 6 accessions of *M. piperita* were collected from their natural habitats in 13 provinces of Iran (Fig. 1). The collection strategy was to survey all environmental conditions that we categorized them into two groups; colder and warmer areas based on annual temperature. It is worth mentioning that colder areas have relatively higher precipitation compared to warmer areas. In each province, the most common accessions of species were collected with whole soil around, bagged and transferred to laboratory. A sample of plant roots including 20 rootlets originating from rhizomes and stolons and from each accession was immediately taken and the soil was then removed from the surface of the roots under running tap water. After washing, root samples were prepared for determination of percent colonization by arbuscular mycorrhizae using the modified staining method of Phillips and Hayman (1970) as described below.

2.2. Morpho-phenological characteristics analysis

To evaluate the variation of morpho-phenological characteristics and essential oil content in the same condition, the accessions were transplanted into a bare field (not planted before) located at Isfahan University of Technology, Isfahan, Iran (latitude 35°44′N, longitude 51°10′E, altitude 1320 m) and allowed to grow during 2009–2010. Field plots were 1.5 m long and 1.5 m wide arranged in a randomized complete block design with three replications. Between and within row spacing was 50 and 20 cm, respectively. In each row, 5 plants with the same size were cultured. The soil was a clay-loam type, well drained, with a pH of 6.8 fertilized with 60 kg N/ha and 30 kg P/ha before planting. Weeds were manually eliminated and plants were irrigated twice a week.

Days to full flowering were calculated since initiation of regrowth on March 20. Then after, plants were cut at crown level; fresh weight per plant was immediately measured. Plants were oven-dried in 70 °C for 48 h and dry weight per plant was also recorded in each plot. The number of branches on the main stem, plant height, the number of flowers per plant, flower length, leaf length and width were also determined based on 10 measurements for each accession in each plot. The average values were used for data analysis. Leaves were removed from 5 plants and their leaf areas were measured using a leaf area meter (Model LI-3 100, LI-COR, Inc., Lincoln, NE). After desiccation, leaf water content was calculated as (leaf fresh weight − leaf dry weight) × 100/leaf fresh weight.

2.3. Mycorrhizal colonization assessments

The washed roots were cut into ∼1-cm pieces, cleared in 10% KOH (24 h) and then rinsed in water. The materials were next acidified in 5% lactic acid (24 h) and the samples were again rinsed in water. The roots were stained with 0.05% aniline blue in 80% lactic acid (24 h) and finally stored in 80% lactic acid (Phillips and Hayman, 1970).

The percentage of root fungal colonization was estimated according to the gridline intersect method (Giovannetti and Mosse, 1980). Twenty root pieces (20 cm) mounted on slides in glycerol:lactic acid (5:1) were examined at 800× and 1000× magnifications using a Nikon BH-2 light microscope containing an ocular crosshair eyepiece. Thirty intersections between roots and crosshairs were observed for presence or absence of AMF and the