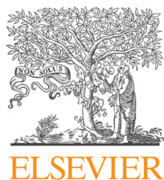
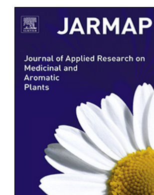
Journal of Applied Research on Medicinal and Aromatic Plants xxx (xxxx) xxx–xxx



Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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



Phylogentic relationship of cold tolerant *Mentha arvensis* variety 'CIM Kranti' with some released varieties as assessed through physiological and molecular analysis

Sunita S. Dhawan[a,*], Anand Mishra[a], Pankhuri Gupta[a], J.R. Bahl[b], R.P. Bansal[c]

[a] *Biotechnology Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, UP, 226015, India*
[b] *Plant Breeding Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India*
[c] *Technology Transfer Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India*

A R T I C L E I N F O

Keywords:
Cold tolerant genotype
Glutathione reductase
Menthol mint
Molecular polymorphism
RWC
SOD

A B S T R A C T

An elite genotype of *Mentha arvensis* was developed through half sib progeny selection with cold tolerance and higher yields of essential oil during winters. Molecular variations were assessed among nine released commercial varieties and the new variety 'CIM-Kranti' by RAPD and ISSR. In total, 20 RAPD primers and 16 microsatellite primers were used to detect the polymorphism among ten varieties of *Mentha arvensis* including new genotype. Phylogenetic analysis revealed a close relationship of the variety 'CIM-Kranti' to 'Gomti' and 'Shivalik' genotype of *Mentha arvensis*. RAPD and ISSR analysis resulted in two different phylogenetic relationships of the 10 genotypes. Comparative physiological variations were assessed by analyzing antioxidants, glutathione reductase, lipid peroxidation assay and relative water content of the new variety CIM Kranti, which clearly demonstrated tolerance towards cold therefore having potential of giving higher additional essential oil yields during winters.

1. Introduction

Mentha arvensis species are one of the important medicinal and aromatic plant species widely distributed and cultivated in the different regions of the world. These are sources of economically important essential oils that are widely used in food, flavor, cosmetics and pharmaceutical purposes. *Mentha arvensis* is a perennial herb belonging to the Labiatae (Lamiaceae) family and distributed mostly in temperate and sub-temperate regions of the world (Ibrahim Mohammad, 2011). *Mentha* has specific ability to produce menthol consisting seven enzymatic steps which start from five carbon compound isopentnyl diphosphate takes place in glandular trichomes (Croteau et al., 2005). Genus *Mentha* consist of different species shows considerable chemical diversity in the essential oil composition and are considered as industrial crops producing series of monoterpenes which are economically and commercially valuable (Aksit et al., 2013). In *M. arvensis* menthol constitute 70–80 % of total essential oil. Genetic improvement(s) is an important aspect in *Mentha* species leading to wider adaptation, higher herbage and essential oil yield and better quality of oil which will permit economical production of mint related commodities (Khanuja et al., 2000). A newly developed *M. arvensis* genotype CIM-Kranti was compared to other varieties of (Gupta et al., 2017) Saksham, Kosi, Himalaya, Kushal, Damroo, Saryu, Gomti, Shivalik and Sambhav (Mishra et al., 2016) which are widely cultivated in northern plain of India by RAPD and ISSR analysis to carry out genetic identity and molecular profiling of new genotype. The improved variety of menthol mint has been developed through half-sib progeny selection from parent variety Gomti. This variety is claiming to be a cold tolerant (Bahl et al, 2013). This variety is suitable for commercial cultivation to generate additional income without any additional input for cultivation during both winter as well as summer seasons. Genetic variation is required for improvement and management (Heywood, 2002). RAPD and ISSR are two basic marker systems having 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., 2000; Rodrigues et al., 2013; Shasany et al., 2005). RAPD analysis should lead to the saturation of genome without a requirement of previous genetic information (Williams et al., 1990). RAPD is the most widely used molecular marker for analysis of DNA fingerprinting. The RAPD technique has become an increasingly popular tool in genetic studies (Emadpour et al., 2009).The

* Corresponding author at: Principal Scientist, Biotechnology Division CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, UP, 226015, India. Phone: +91+5222718523; Fax: +91+522234366.

E-mail addresses: sunita.dhawan@cimap.res.in (S.S. Dhawan), mishra.anand653@gmail.com (A. Mishra), pankhurigupta188@gmail.com (P. Gupta), jr.bahl@cimap.res.in (J.R. Bahl), rp.bansal@cimap.res.in (R.P. Bansal).

https://doi.org/10.1016/j.jarmap.2018.06.004
Received 30 November 2017; Received in revised form 12 June 2018; Accepted 20 June 2018
2214-7861/ © 2018 Elsevier GmbH. All rights reserved.

Please cite this article as: Dhawan, S.S., Journal of Applied Research on Medicinal and Aromatic Plants (2018), https://doi.org/10.1016/j.jarmap.2018.06.004

technique being successfully used in various crops and medicinal plant species as *Medicago* (Yu and Pauls, 1993), *Rice* (Mackill, 1995), *Banana* (Howell et al., 1994), *Brassica* (Jain et al., 1994), *Bacopa* (Tripathi et al., 2012), *Podophyllum* (Naik et al., 2010), *Butea* (Vashishtha et al., 2013), *Zingiber* (Mohanty et al., 2014) and *Curcuma* (Das et al., 2011) for the estimation of genetic variability as well as for the cultivar identification. ISSR markers, which involve PCR amplifications of DNA using a primer, composed of a microsatellite sequence of short repeats anchored at 3′or 5′end by 2–4 arbitrary, could be used to find genetic diversity. ISSRs marker had already been used for cultivar identification for *Bean* (Muthusamy et al., 2008), *Wheat* (Deshmukh et al., 2012), *Barley* (Guasmi et al., 2012), *Rheum* (Wang et al., 2012) and *Houttuynia* (Wei and Wu, 2012). In this study, we compared a newly developed genotype, analyzed and evaluated for genetic diversity using RAPD and ISSR markers, in order to establish a baseline to assist future breeding programs of this species. Also, we aimed to report molecular differentiation by RAPD and ISSR for the assessment of genetic diversity. The physiological analysis was done to assess the tolerant nature of genotype compared to cultivated commercial variety Saksham by antioxidant assay, lipid peroxidation and mechanical damage measurement.

2. Materials and methods

2.1. Plant material

This study includes a total of 10 commercial varieties of *M. arvensis* for comparative profiling and analysis including new varieties/genotype. *Mentha arvensis* samples were grown and collected from experimental farm CSIR-CIMAP. The released varieties are Saksham, Kosi, Himalaya, Kushal, Shivalik, Saryu, Gomti, Sambhav, Damroo and new variety CIM Kranti (Padalia et al., 2013) A detailed description of the varieties was provided in Table 1.(Lal, 2013; Mishra et al., 2017).

2.2. Isolation of genomic DNA from leaf tissues of menthol mint varieties

Total genomic DNA was extracted following the procedure as described by Khanuja et al., (1999) with slight modifications. 100 mg of fresh leaves of *Mentha* accessions were ground in liquid nitrogen and mixed with 3 ml of freshly prepared preheated extraction buffer containing 100 mM Tris buffer, 0.5 M EDTA 20% cetyl trimethyl ammonium bromide 5 M NaCl 1% polyvinylpyrrolidone (PVP) and β mercaptoethanol (All from Sigma Aldrich, St. Louis, MO). An equal amount of chloroform / isoamyl alcohol (Merck, Darmstadt, Germany) (24:1) was added and inverted to mix for few seconds, then centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to new tubes with 1.5 ml of NaCl with 0.6% of isopropanol (Merck, Darmstadt, Germany) and mix, incubated at room temperature for 1 h and centrifuged for 10 min at 13000 rpm and the pellet was dried and 400 μl of high salt TE buffer: 1 M NaCl, 10 mM Tris-Cl (pH 8.0) and 1 mM EDTA was added and the whole mixture was placed at 42 °C for 30 min. An equal amount of chloroform /isoamyl alcohol (24:1) added and centrifuged for 10 min for 13000 rpm and the supernatant was transferred to the new centrifuge tube and double volume of absolute ethanol was mixed for precipitation (incubate for 2 h in −20 °C. Then centrifuged at 12000 rpm for 10 min and the pellet was washed with 70% ethanol (Merck, Darmstadt, Germany) and dissolved in deionized water.

2.3. RAPD- PCR reaction

The RAPD-PCR for all genotypes of *M. arvensis* reaction were performed (Williams et al., 1990) with decamer oligonucleotides synthesized by Operon technologies (Almeda, California). The final PCR volume of 25 μl contained 10 x buffers with $MgCl_2$ (25 mM), 15–25 ng of total genomic DNA, 25 mM dNTPs (Bangalore Genei, Bangalore, India), and 10 pmol Primers and 0.6 units of Taq polymerase (Bangalore Genei, Bangalore, India). Amplification was carried out in thermal cycler (Veriti, Applied Biosciences, California, USA.) one cycle of 1 min at 94 °C followed by 44 cycles, each consisting of a denaturation step of 1 min at 94 °C, followed by an annealing step of 1 min 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 25 μl amplified products were analyzed by electrophoresis on 1.2% agarose gel and the amplicon were detected under UV light after staining with Ethidium bromide (Sigma Aldrich, St. Louis, MO).

2.4. PCR reaction for ISSR

ISSR amplification was carried out in 25 μl contained, 10 x buffers with $MgCl_2$ 15–25 ng of total genomic DNA, 25 mM dNTPs, and 10 pM of primers, and 0.6U of Taq polymerase. Amplification was carried out in thermal cycler one cycle of 2 min at 94 °C followed by 40 cycles, each one consisting of a denaturation step for 30 s at 94 °C, followed by an annealing step for 30–45 sec at 52–57 °C (as per primer) 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. ISSR product 25 μl were analyzed by electrophoresis on 1.5% agarose gel in 1xTAE buffer and the amplified products were detected under UV light after staining with ethidium bromide. λ EcoRI/Hind III (Bangalore Genei, Bangalore, India) double digest,10 kb was used as a molecular standard according to size.

2.5. Data analysis

RAPD and ISSR bands were scored as present (1) or absent (0) for the estimation of the similarity among all the analyzed samples and total amplified bands, a number of polymorphic bands and percentage of polymorphic bands (PPB) were recorded. Polymorphism information content (PIC), the effective multiplex ratio (EMR), marker index (MI) and resolving power (RP) was calculated. PIC was calculated according

Table 1
A detailed description of *M. arvensis* varieties used in genetic analysis (Lal, 2013; Mishra et al., 2017).

S.No	Genotypes	Origin/ Development	Morphological Characteristics
1	Shivalik	Introduction from China	Long, broad leaves, thick suckers.
2	Himalaya	Hybrid of Gomti and Kalka	Dark green, broad leaves erect, thick suckers.
3	Damroo	Selection in open pollinated seed Pr progenies of Shivalik variety	Dark green, leathery broad leaves, erect, thick suckers, thick stem.
4	Sambhav	Single somaclonal variant of cultivar 'Himalaya'	Light green leaves, thin suckers.
5	Gomti	Seedling variant of Shivalik	Dark green, broad leaves erect, thick sucker.
6	Kushal	Somaclonal selection	Dark green, broad leaves erect, thick suckers, tolerate high moisture in soil.
7	Saksham	Clonal variant of Himalaya	Dark green, broad leaves erect, thick suckers.
8	Kosi	Half-sib progeny selection	Light green leaves, bushy, thin suckers.
9	Saryu	Variety was developed through intensive selection.	Light green, leathery broad eaves, erect, thick
10	CIM-Kranti	Half sib progeny selection from several seed raised progenies of variety Gomti.	Dark green, tall, broad leaves grow in multiple seasons, soft fibrous white suckers.

Download English Version:

https://daneshyari.com/en/article/10215765

Download Persian Version:

https://daneshyari.com/article/10215765

Daneshyari.com