Molecular identification of fungi isolated from Dracocephalum moldavica L. seeds

Magdalena Frąc\textsuperscript{a*}, Karolina Oszusta\textsuperscript{a}, Anna Kocira\textsuperscript{b}, Sławomir Kocira\textsuperscript{c}

\textsuperscript{a}Institute of Agrophysics Polish Academy of Sciences, Department of Plant and Soil System, Laboratory of Molecular and Environmental Microbiology, Doświadczalna 4, 20-290 Lublin, Poland

\textsuperscript{b}State School of Higher Education in Chelm, Institute of Agricultural Sciences, Pocztowa 54, 22-100 Chelm, Poland

\textsuperscript{c}University of Life Sciences in Lublin, Department of Machinery Exploitation and Management of Production Processes, Głęboka 28, 20-612 Lublin, Poland

Abstract

Dracocephalum moldavica L. which is known as dragonhead is an annual herbaceous aromatic plant belongs to family Lamiaceae. It is native to central Asia and has been naturalized in eastern and central Europe. Extracts and oil from this plant are used widely in the pharmaceutical, cosmetic, food and flavouring industries. Because mycological quality is important for industrial use of seeds of this plant, the goal of the study was to identify the most dominant fungi occurring on Dracocephalum moldavica L. seeds using molecular techniques. The method of molecular identification was based on rDNA-extraction and the subsequent amplification of the D2 large subunit. Two fungal strains (G435/14 and G443/14) were isolated from Dracocephalum moldavica L. seeds. The strains were identified as \textit{Fusarium sporotrichioides} (G435/14) and \textit{Alternaria alternata} (G443/14). Most seeds were settling by \textit{A. alternata} and just few by \textit{F. sporotrichioides}.

Keywords: Dracocephalum moldavica L.; fungi indentification; Fusarium, Alternaria, MicroSEQ®

* Corresponding author. Tel.: +48-81-744-50-61; fax: +48-81-744-50-67. E-mail address: mfrac@ipan.lublin.pl
1. Introduction

Dracocephalum moldavica L. which is known as Moldavian balm or Moldavian dragonhead is an annual herbaceous aromatic and honey yielding plant belongs to family Lamiaceae. It is native to central Asia and has been naturalized in eastern and central Europe. Moldavian dragonhead has an attractive blue or white flowers and aromatic lemon scented foliage because the main components of the essential oil are citral and geranyl acetate. Its seeds contain high quality oil, protein and mucilage with health-promoting properties. Extracts and oil from this plant are used widely in the pharmaceutical, cosmetic, food and flavouring industries as indicated by Alaei et al. (2013), Maham et al. (2013) and Yousefzadeh et al. (2013). Recently it has been shown that essential oils from Dracocephalum moldavica flowering aerial parts to act as efficient and biologically safe insect repellent for storage products (Chu et al., 2011). In cultivation of D. moldavica rarely occur diseases and pests that affect the quantity and quality of herb raw materials and seeds. At the beginning of the growing season plants may be occasionally infected by soil borne pathogens. Therefore, because of the possibility of infection by pathogens causing seedling blight it is advisable to seed treatment before sowing, as described by Kwiatkowski et al. (2011). Because mycological quality is important for industrial use of seeds this plant, the goal of this work was to identify the most dominant fungi occurring on Dracocephalum moldavica L. seeds using molecular techniques.

2. Materials and methods

The experimental material in the study was seeds of Dracocephalum moldavica collected from the field after harvest. 50 infected by fungal pathogens and 50 uninfected seeds were selected to the study. The seeds were placed on Bengal rose medium (Biocorp) to enable fungal growth (Fig. 1). In prior to lining seeds (visibly infected and uninfected) on part of seeds were soaked in methylated spirit for 15 min. The other part was not soaked. After strains isolation they were grown on PDA medium (Biocorp) for 4 days and were preliminary identified basing on micro- and macro-morphological observations by microscope and in microcultures, respectively. Two selected strains were identified using molecular methods.

The method of molecular identification was based on rDNA-extraction and the subsequent amplification of the D2 large subunit. Extraction of the fungal DNA was performed with the PrepMan Ultra reagent (Applied Biosystems). A fast MicroSeq D2 LSU rDNA fungal PCR kit (Applied Biosystems) was used to amplify the D2 LSU rDNA region. The next step after PCR purification (using ExoSAP-IT® PCR Products Purification Kit for ABI) was to prepare the sequencing cycle using MicroSeq D2 Fungal ID Sequencing Kit (ABI). Next, the Performa Purification System (EdgeBio-Performa Gel Filtration Cartridges) was performed. Capillary electrophoresis was run through an ABI 3130×l sequencer (Applied Biosystems) with a 50 cm capillary array and polymer POP6_1. The MicroSEQ® ID software was used to assess the raw sequence files and to perform sequence matching to the MicroSEQ® ID (Applied Biosystems) to validated the reference database according to Frąc et al. (2014).

3. Results and discussion

The studies on pathogens infected Moldavian dragonhead are few and ambiguous. So far, it has been found that in the cultivation of Moldavian dragonhead are pathogens causing seedling blight (Kwiatkowski et al., 2011). Two fungal strains (G435/14 and G443/14) were isolated from Dracocephalum moldavica L. seeds and identified using a molecular method. G435/14 was genetically identified as Fusarium sporotrichioides (specimen score was 43; top match for this strain was recorded with DSM 62423), while G433/14 as Alternaria alternata (specimen score 43; top match was found with DAOM 208486). The length of consensus sequences was 278 and 212 bp for F. sporotrichioides and A. alternata, respectively.
The study showed, that the surface of *Dracocephalum moldavica* seeds was settled by numerous fungi, but belonging to the same genus – monoculture (Fig. 2). The populations of microbial species detected on the seeds surface depended on the level of infection. *A. alternata* dominated on the surface of studied seeds whereas *F. sporotrichioides* occurred occasionally (Fig. 3).
Although *Dracocephalum moldavica* is resistant to fungal infection, identified fungi, especially *Alternaria alternata*, naturally occurs on the surface of different seeds (Frąc et al., 2010). Traditionally, morphology-based approach is mainly used for fungal identification. But, such type of identification is long –lasting, needs huge experience and sometimes in very difficult, because some microscopic fungi don’t have a sexual state (Ding et al., 2011). Generally, fungi with similar morphology possess a high level of genetic variation that is the way that molecular identification of these organisms is useful tool in their classification.

Figure 4 shows the distance tree based on the D2 large subunit rDNA gene sequence. The species were extracted as species with highly similar sequences to G435_14 and G443_14 by MicorSeq research. The results (Fig. 4) indicated that G435_14 was closely related to the *Fusarium* group composed by *F. verticillioides*, *F. proliferatum*, *F. poae* and *F. sporotrichioides*. However, similarity 100% identity (278 bp) was obtained for *F. sporotrichioides*.

![Fig. 4. Neighbor-joining phylogenetic tree based on rDNA-D2 LSU sequences of G435_14 and G443_14 fungal strains.](image)

Based on the distance tree G443_14 strain grouped into the following species: *Cochliobolus carbonum*, *Alternaria alternata*, *Ulocladium botrytis*, *Alternaria geophila* and *Stemophyllum vesicarium*. However, low bootstrap values were found with some species, including *Ulocladium botrytis*, *Alternaria geophila* and *Stemophyllum vesicarium*. The sequence of G443_14 agrees with that of *Cochliobolus carbonum* and *Alternaria alternata* (100% identity for 212 bp). The sequences analyses, but also micro- and macroscopic observations, allowed to identified the G443_14 as *A. alternata*. These studies show that the phylogenetic relationships established from molecular data can be correlated with morphological data. The electropherogram of sequencing presented at Figures 5 and 6 shows the part of sequences as particular nucleotides.
Fig. 5. Elektroforegram of sequencing of G435_14 fungal strain (*F. sporotrichioides*).

Fig. 6. Elektroforegram of sequencing of G443_14 fungal strain (*A. alternata*).
Summary and Conclusions

In this study the fungal isolates were classified based on sequencing a region with the D2 LSU rDNA gene which was considered to fungi identification. Comparison of sequences of the fungal genotypes to those available in the MicroSeq database revealed that they were closely related to particular species. The strains were identified as *Fusarium sporotrichioides* (G435/14) and *Alternaria alternata* (G443/14).

The study also indicated that most seeds were settling by *A. alternata* and just few by *F. sporotrichioides*.

Acknowledgements

The analyses were performed using equipment bought with European Union funds – The Eastern Poland Development Programme 2007-2013, Regional Laboratory of Renewable Energy, Institute of Agrophysics PAS.

References


