Characterization of genetic diversity in *Colletotrichum acutatum* strains isolated from sour and sweet cherries in Poland

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**INTRODUCTION**

Anthracnose, manifested on cherries as a bitter rot disease, has been reported in sour cherry orchards in several European countries, as Lithuania, Hungary, Serbia, Montenegro, Russia, Czech Republic and Norway, while in sweet cherries only from Hungary, Switzerland and Norway (Borve and Stensvand and references therein, 2006). Cherry fruits might be infected during early spring, on various fruit developmental stages, but the disease symptoms usually occur when the fruits getting ripe or after harvest. Diseased fruits show in the lesion collapsed but intact peel and salmon-colored spore masses in concentric rings. The causal agent of bitter rot affecting sour and sweet cherry was firstly identified on the basis of morphological traits as *C. gloeosporioides* (Penz.) Penz. and Sacc. (Olszak and Piotrowski, 1985; Ivanovic and Ivanovic, 1992; Kajati et al., 2002; Borve et al., 2010), however, in most cases it was further re-classified by PCR analysis as *Colletotrichum acutatum* Simmonds ex. Simmonds (Borve et al., 2010; Streenivasaprasad and Talhinhas, 2005).

In Poland, severe occurrence of sour cherry bitter rot was firstly reported in middle 70s in the Southeastern regions (Olszak and Piotrowski, 1985). Recent screening conducted on fungal isolates derived from anthracnose lesions occurring on various fruit and berry crops, including few cases of sour cherries, revealed wide dissemination of pathogenic *C. acutatum* on diseased plants also in Poland (Bryk et al., 2012). The species affiliation of fungi responsible for bitter rot on sour cherries in our country has not been examined yet. On the other hand, the bitter rot on sweet cherries has not been reported yet in Poland. However, there have been some records suggesting occurrence of this disease in local orchards recently (unpublished data).

The purpose of this study was to identify the causal agents of anthracnose on sour and sweet cherries in Poland and to determine their genetic diversity using molecular tools.

**MATERIALS AND METHODS**

**Fungal isolates**

In Summer 2012 and 2013 the fruits of sour and sweet cherries with bitter rot symptoms were collected from 29 various cherry orchards, located in central and in north and south west of Poland. In total, 111 isolates of fungi from bitter rot symptoms: 93 from sour and 18 from sweet cherries were obtained. On the basis of morphological traits, examined during the fungal growth on PDA medium in pure cultures, all isolates were classified to the genus *Colletotrichum* spp.

**Analyses of genetic diversity**

To determine genetic diversity of all *C. acutatum* isolates, ISSR and RAPD PCR assays were applied. In RAPD assay the OP-U19 (5’-GTGATGCGC-3’) primer was used. In ISSR PCR 2 different primers were used: 5’-GACACAGCAAGACAGA-3’ (referred as (GACA), Weising et al., 1989) and 5’-CACCGACAGCGACGACAG-3’ (referred as (CAG), Rodriguez and Yoder, 1991).

**RESULTS**

All isolated fungi were identified as *C. acutatum* based on PCR with species specific primers.

**Fig 1.** Products of PCR of DNA of *Colletotrichum* spp. fungi isolated from sour and sweet cherries amplified with primers CaInt2 and ITS4, visible on agarose gel. In the reaction with CgInt and ITS4 primers no products were observed.

Analysis of genetic diversity of isolated fungi showed that they don’t create homogenic group.

**Fig 2.** Agarose gels with exemplary amplification patterns obtained in PCR with:

- a) ISSR (GACA) primer
- b) ISSR (CAG) primer
- c) RAPD OP-U19 primer

**Fig 3.** Dendrograms showing the genetic diversity of analysed fungal isolates, based on PCR with:

- a) joined results of ISSR PCR (GACA), analyses and (CAG) analyses and a) RAPD analysis.

**CONCLUSIONS**

1. All fungi, isolated from symptomatic sour and sweet cherries, were identified as *Colletotrichum acutatum*.
2. Both analyses showed diversity of analysed isolates, but in most cases the obtained bootstrap values were lower than 50. In joined ISSR PCR analyses all isolates were divided into 2 heterogenic clusters, containing isolates from both sour and sweet cherries (Fig. 3 a), while in RAPD assay almost all isolates were clustered in one heterogenic group, but one isolate BiW2 was clearly outgrouped (Fig. 3 b).
3. No relationship was found between obtained ISSR and RAPD PCR patterns and localisation or host plant.
4. Further studies on the genetic diversity and characteristics of *Colletotrichum* isolates will be conducted.

**REFERENCES**