Physiological and Molecular Plant Pathology 102 (2018) 185–192

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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

Mycelial compatibility group and genetic variation of sunflower *Sclerotinia sclerotiorum* in Northeast China

Jia Liu[a,b], Qinglin Meng[b,*], Yunhua Zhang[b,**], Hongtao Xiang[a,c], Yichu Li[b], Fengmei Shi[b], Ligong Ma[b], Chunlai Liu[b], Yu Liu[b], Baohua Su[b], Zhiyong Li[b]

[a] Heilongjiang Academy of Agricultural Sciences Postdoctoral Programme, Harbin 150086, China
[b] Institute of Plant Protection, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China
[c] Institute of Farming and Cultivation, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China

ARTICLE INFO

Keywords:
Genetic variation
Mycelial compatibility group
Sclerotinia sclerotiorum
Simple sequence repeats
Sunflower

ABSTRACT

Sunflower is an economically important crop, particularly for oil production in Northeast China. *Sclerotinia sclerotiorum*, a fungal pathogen can cause severe yield loss of several crop species, including sunflower. In the present study, mycelial compatibility groups (MCGs) were investigated among a total of 115 isolates of *Sclerotinia sclerotiorum* from sunflower fields in different regions of Northeast China. A total of 35 different MCGs were identified, with 1–12 isolates within each group. There were nine MCGs with only one representative isolate. Additionally, simple sequence repeats (SSRs, i.e., microsatellite loci) method was employed to assess the genetic variation among the 115 isolates. The clustering based on SSRs suggested that most isolates that were geographically close were grouped in the same cluster or a close cluster. The clustering results of all 115 isolates suggested that the clusters based on microsatellite loci were highly consistent with the MCGs results. Our findings add value to the current knowledge base on the population structure and genetic diversity of *S. sclerotiorum*, specifically in the context of sunflower cultivation in Northeast China, and may provide valuable information for breeding of sunflower stocks with enhanced resistance towards *S. sclerotiorum*.

1. Introduction

Sunflower (*Helianthus annuus*) is a native North American plant that belongs to the *Asteraceae* family. Sunflower seeds are one of the major sources of edible oil; therefore, sunflower is now cultivated worldwide as an oilseed crop with economic importance. Additionally, sunflower is also cultivated for other uses such as confectionary products and bird food. Sunflower growth area and the seed yield have greatly increased in recent years (https://www.fas.usda.gov/data). China is among the largest sunflower-producing countries in the world, with major growth regions distributed in Northern China [1]. Occurrence of Sclerotinia sunflower diseases causing severe yield loss have been often reported in regions of Northeast China, including Heilongjiang Province, Inner Mongolia, and Shanxi Province [2].

The majority of sunflower diseases reported so far are caused by pathogenic fungi [1]. Sclerotinia stalk and head rot is a major and severe disease in sunflower caused by *Sclerotinia sclerotiorum* infection [3]. *S. sclerotiorum* infection causing sunflower yield losses have been documented worldwide, especially in temperate regions. For example, yield loss of sunflower production caused by *S. sclerotiorum* infection was approximately 5–8% in the United States [4,5]. Complete yield loss (100%) of sunflower due to Sclerotinia head rot has been reported from Buenos Aires Province, Argentina [6–8]. *S. sclerotiorum* (Lib.) de Bary is a necrotrophic fungal pathogen which can infect more than 400 plant species, including the majority of dicotyledonous as well as several monocotyledonous crops [9]. *S. sclerotiorum* can survive as sclerotia in the soil for a few years [3]. Different organs of sunflower plant can be infected by *S. sclerotiorum*, leading to multiple symptoms. Mycelia, developed upon sclerotia germination can directly infect stems and roots of sunflower causing stem rot and plant wilting. Additionally, ascospores produced from apothecia can infect inflorescence of sunflower, causing head rot which results into severe, and sometimes complete yield loss [4].

Genetic diversity is commonly observed among fungal pathogens. Sources of such diversity include but are not limited to mutation and recombination [10]. Identification of mycelial compatibility groups (MCGs) is a commonly used approach to characterize clonal lineages and to assess genetic diversity of fungal pathogens, including *S.*

* Corresponding author.
** Corresponding author. Institute of Plant Protection, Heilongjiang Academy of Agricultural Sciences, No. 368 Xuefu Road, Nangang District, Harbin 150086, China.
E-mail addresses: mqlhlcn@126.com (Q. Meng), yhzhang9603@126.com (Y. Zhang).

https://doi.org/10.1016/j.pmpp.2018.03.006
Received 22 August 2017; Received in revised form 21 March 2018; Accepted 21 March 2018
Available online 22 March 2018
0885-5765/

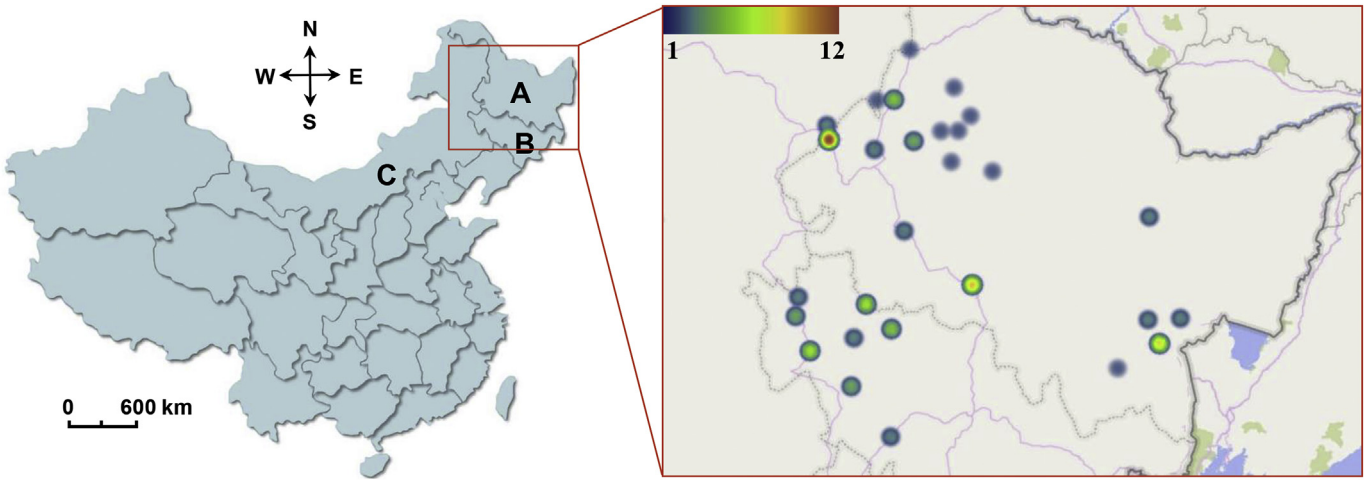

Fig. 1. *S. sclerotiorum* isolates were collected from sunflower fields of three provinces in Northeast China. Letters A, B, and C represent Heilongjiang, Jilin, and Inner Mongolia, respectively. Sampling locations and the number of samples from each location are indicated on the enlarged map (right panel). The map was produced using Power Map in Excel.

sclerotiorum. MCGs are phenotypes determined by a multilocus controlled self-recognition system and can be observed macroscopically [11]. When paired in culture, members belonging to the same MCGs show fused growth into one cohesive colony (anastomose), with no reaction line. In contrast, colonies with mycelial incompatibility show an obvious contact zone characterized by reduced growth, dead cells, or sparse mycelia [12]. MCGs have been determined within *S. sclerotiorum* population isolated from certain geographic regions and/or hosts [13–16]. For example, a total of 205 *S. sclerotiorum* isolates collected from sunflowers in China, England, and Canada were grouped into 39 MCGs [13]. Aldrich-Wolfe et al. (2015) identified 49 MCGs out of the 145 *S. sclerotiorum* isolates from four different crops (i.e., sunflower, canola, dry bean, and soybean) in the north central region of the United States [16]. It is not uncommon to identify MCGs with only one isolate among the isolate population [13,16]. Incompatibility between isolates commonly occurs with isolates from different geographic areas but may also occur within the same field [13,16]. Such phenomena indicate significant genetic diversity of *S. sclerotiorum* as well as the importance of collecting multiple *S. sclerotiorum* isolates in a particular region to assess the genetic diversity of the population.

In addition to identification of MCGs, another standard approach to assess the genetic diversity of *S. sclerotiorum* population is DNA profiling. There are a variety of methods for characterization of genetic structure, such as microsatellite loci, random amplification of polymorphic DNA (RAPD) and sequence-related amplified polymorphism (SRAP) [13,17–20]. Microsatellite loci, also known as simple sequence repeats (SSRs), are tandem repeats of short DNA sequence, with lengths ranging from 1 to 6 nucleotides, distributed in both coding and non-coding regions of prokaryotic and eukaryotic genomes [21,22]. SSRs are locus-specific and multi-allelic, and can be easily detected using polymerase chain reaction (PCR). Therefore, SSRs are molecular markers that are usually unique to a genome and thus are widely applied in evolution, genetics, and crop breeding [23,24]. SSRs have been employed to characterize *S. sclerotiorum* population from a spectrum of hosts in different geographic areas [16,17,25–27]. For example, Sexton and Howlett (2004) and Mert-Türk et al. (2007) employed microsatellite markers to investigate genetic variation among *S. sclerotiorum* population from canola fields in Australia and Turkey, respectively [25,27]. Similarly, the genetic structure of *S. sclerotiorum* isolates from sunflower in the North central United States was analyzed using SSRs markers [16].

Considering that the known genetic diversity of *S. sclerotiorum* population, specifically from sunflower, is still far from complete, we focused on *S. sclerotiorum* population from this crop host in the present study. A total of 115 isolates of *S. sclerotiorum* were collected from sunflower fields in different regions of Northeast China. We employed both MCGs test and SSRs as molecular markers to characterize genetic diversity in this *S. sclerotiorum* population. A total of 35 different MCGs were identified, with 1–12 isolates within each group. Additionally, the clustering based on SSRs suggested that most isolates which were geographically close were grouped in the same cluster or a closely related cluster. The clustering results of all 115 isolates suggested that the clusters based on microsatellite loci were highly consistent with the MCGs results. The findings of this study will improve the knowledge about the population, structure and genetic diversity of *S. sclerotiorum*, specifically in sunflowers from Northeast China, and may provide valuable information for sunflower breeding with enhanced resistance towards *S. sclerotiorum*.

2. Materials and methods

2.1. S. sclerotiorum isolates

Sampling was conducted in 208 sunflower fields across 27 cities in Northeast China (Middle West and West of Jilin Province, East and Northwest of Heilongjiang Province, and East of Inner Mongolia, near the border of Heilongjiang) during 2012. Concurrent disease information pertaining to the geographic areas is provided in Suppl. Table 1. The number of samples drawn from each field was proportional to the field area, i.e., 1 sample for every ten mu (1 mu = 0.0667 ha). Sclerotia (~10) were collected from the rotted sunflower head of each infected plant. Collected sclerotia were first surface sterilized using 75% ethanol for 1–2 min and then rinsed three times with sterile distilled water. Sclerotia were then placed on potato dextrose agar (PDA) media at 22 °C until the formation of mycelia (~3–5 days). Single spore isolation was performed for all the collected sclerotia. A total of 115 *S. sclerotiorum* isolates were collected from these samples (Fig. 1 and Suppl. Table 2).

2.2. Determination of mycelial compatibility groups (MCGs)

For the MCGs assay, mycelial plug (5 mm in diameter) was obtained from the colony edge of each *S. sclerotiorum* isolate after 2–3 days of growth. Two different plugs were placed in one petri dish with PDA medium as a pairing, and then cultured in dark at 22 °C for 7–10 days. Isolates from the same region were tested first to obtain the MCG information for the specific region. For isolates from different regions, one isolate from one MCG of the selected region was randomly chosen and then tested against one randomly selected isolate from another MCG of the other region under comparison. The hyphae intermingling