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Original scientific article

SUNFLOWER GENOTYPES TOLERANCE TO CHARCOAL ROT (*Macrophomina phaseolina* (TASSI) GOID.) UNDER THE FIELD CONDITIONS

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Sunflower (*Helianthus annuus* L.) is one of the most important crops grown in the world, but it can be attacked by more than 30 different pathogens. The areal of *Macrophomina phaseolina* (Tassi) Goid., one of the sunflower pathogens nowadays often found in temperate regions, has been spreading over the last few years due to climate change. The most effective eco-friendly method for controlling charcoal rot caused by *M. phaseolina* is growing resistant sunflower cultivars. Due to that, 24 commercially available hybrids and 70 inbred lines were tested for *M. phaseolina* tolerance in a two-year trial. Under the field conditions, two different inoculation methods were used – the Unwounded Stem Base Inoculation (USBI) and Toothpick (TP) method. This study identified five highly tolerant hybrids and 12 inbred lines that can be used in breeding programs for improvement of future genotypes. Sunflower genotype screening tests for *Macrophomina* tolerance indicated that both inoculation methods should be applied together to provide reliable results, and that stem lesion length is a reliable trait for disease severity assessments.

Key words: charcoal rot; inoculation methods; *Helianthus annuus, Macrophomina phaseolina*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important industrial crops grown in the world, cultivated in more than 70 countries (RAUF *et al.*, 2012) with a total production of 47.863,077 tons in 2017 (FAO, 2019). In Serbia, sunflower was grown on 239,148 hectares with

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the total yield of 733,706 tons (average 3.1 t/ha) in 2018 (STATISTICAL OFFICE OF RS, 2019). Sunflower growing area has been increasing in Serbia over the last three years. The most limiting factors in sunflower production are weather conditions and plant diseases. In order to obtain high yields, a number of plant pathogens should be controlled. More than 30 different economically important sunflower pathogens have so far been identified worldwide (ŠKORIĆ, 2016).

Humidity and cold prevailing in the temperate regions favour the development of common sunflower diseases such as white rot of root, stem and sunflower heads caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, downy mildew caused by *Plasmopara halstedii* (Farlow) Berlese & de Toni, brown stem canker caused by *Diaporthe* (*Phomopsis*) *helianthi* Munt.-Cvet., and sunflower black stem caused by *Phoma macdonaldii* Boerema.

Due to climate change, some years saw dry and very hot conditions with average max temperatures around 30°C in the growing season, even in temperate regions. These are unfavourable conditions for the development of the above mentioned sunflower diseases, but in such years presence of sunflower charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is more frequent. *M. phaseolina* is a common soil-borne pathogen of more than 500 cultivated and wild plant species that occurs mainly in regions with arid subtropical and tropical climates. It is commonly registered in Pakistan (JALIL *et al.*, 2013), China (ZHANG *et al.*, 2016), and India (SURIACHANDRASELVAN *et al.*, 2005), where reported yield losses were up to 90% in sunflower. In favourable years, the fungus was registered in the European countries as well, including Spain (JIMENÉZ-DIAZ *et al.*, 1983), Italy (MANICI *et al.*, 1992), Hungary (CSÖNDES *et al.*, 2011), Slovakia (BOKOR, 2007), the Czech Republic (VEVERKA *et al.*, 2008), Romania (IONITĂ *et al.*, 1996), Bulgaria (ALEXANDROV, 1999) and Serbia (TANČIĆ *et al.*, 2012). Generally, charcoal rot caused by this pathogen can reduce seed yields by 20-36% throughout the world (JIMENEZ-DIAZ *et al.*, 1983) caused by premature ripening. The first high incidence of disease in the former Yugoslavia was recorded in the early 1960s, negatively affecting head diameter and yield (AĆIMOVIĆ, 1998).

The infection can occur as early as the seedling stage. Fungus grows through plant roots and slowly moves to the above-ground parts, where pathogen microsclerotia obstruct the vessels and eventually cause plant wilting; however, the first visible symptoms cannot usually be detected until the flowering stage. Control of this important pathogen is complicated due to often delayed appearance of visible symptoms, large number of host plants (https://www.plantwise.org/knowledgebank/datasheet/32134#HostPlantsSection), and above all the persistence of *M. phaseolina* in the soil as large amount of microsclerotia that are viable for more than four years (ISLAM *et al.*, 2012).

Beside optimal weather conditions, one of the limiting factors for pathogen development is the genetic background of sunflower genotypes. Although *M. phaseolina* is monotypic and no physiological races have been reported, it has high genetic variability. In combination with the site-specific nature of *M. phaseolina*, this has made studies on genetics of charcoal rot resistance difficult. Therefore, genetics of sunflower resistance against *M. phaseolina* have not clearly been demonstrated and different findings have been reported (KHAN, 2007; KAYA, 2016; SEILER, 2012; ŠKORIĆ, 2016). The sources of the resistance against charcoal rot remain unrevealed due to insufficient number of studies and lack of available data.

Selection for resistance as a preventive method is the most efficient and economically most justified method. Results on NS sunflower hybrids resistance to the most common sunflower pathogens *Phoma macdonaldii* (DEDIĆ *et al.*, 2012), *Diaporthe (Phomopsis) helianthi* (JOCIĆ *et al.*, 2004; DEDIĆ *et al.*, 2009) and *S. sclerotiorum* (DEDIĆ *et al.*, 2011) were published, but this is the first study on NS sunflower hybrids tolerance to charcoal rot (*M. phaseolina*). The aim of this study was to determine the variability of sunflower inbred lines and hybrids in response to *M. phaseolina* infection, to detect tolerance within selected genotypes, and to compare efficacy of inoculation methods for *M. phaseolina* tolerance screening tests.

MATERIAL AND METHODS

Plant and Fungal Material Used

M. phaseolina isolate RŠ-H-15 was obtained from sunflower stem showing charcoal rot symptoms that was collected in Zrenjanin (Serbia) and isolated according to the standard IFVCNS laboratory procedure. The isolate RŠ-H-15 was chosen for artificial inoculation due to its high pathogenicity observed on the sunflower plants in the field in 2009 (data not published), the high growth rate *in vitro* on Potato Dextrose Agar Media (90.0 mm for three days) and its pathogenicity on sunflower seedlings (TANČIĆ *et al.*, 2012a). The morphological identification of the isolate was confirmed by RAPD analyses with OPA 02-20 primers (TANČIĆ ŽIVANOV, 2019).

Table 1. List of sunflower material tested for M. phaseolina tolerance in the 2-year field trial

Sunflower hybrids			E-early; ME- medium early; ML-medium late; L-late	
NS-H-45 (L)	VELJA (ME)	VLADIMIR (ME)	ORION (ML)	NS-ROMEO (ME)
VRANAC (ML)	PERUN (E)	DUŠKO (ME)	OSKAR (ML)	NS-DESPOT (L)
MILAN (ME)	BAĆA (ML)	BRANKO (ME)	ORFEJ (E)	NS-NOVAK (L)
BAČVANIN (ML)	SREMAC (ME)	NOVOSAĐANIN (E-ME)	NS-GLADIJATOR (ME)	NS-FANTAZIJA (ML)
NS-H-111 (ML)	ŠUMADINAC ME)	NS-H-8080 (E)	NS-KONSTANTIN (ME)	
Sunflower inbred lines			E-early; ME- medium early; ML-medium late; L-late	
HA-26 (ME)	CMS1-223 (ME)	UK-25 (L)	OD-2-ST (ML)	AS-52 (ME)
HA-74 (ML)	CMS-3-8 (L)	UK-17 (L)	OD-DI-112 (ML)	AS-92 (ML)
HA-98 (ML)	PR-ST-28 (L)	UK-90 (L)	OD-DI-119 (ML)	BT-VL-20 (ME)
HA-26-OR (ME)	IMI-AB-6 (L)	SU-AB-3 (ML)	OD-DI-111(ML)	BT-VL-24(ME)
HA-26-PR (ME)	PR-ST-3 (ME)	SU-AB-18 (ML)	OD-DI-18 (ML)	BT-VL-17(ME)
PH-BC2-64 (ML)	JM-8 (ME)	UK-58-ST (L)	OD-DI-20 (ML)	BT-VL-18(ME)
PH-BC2-92 (L)	NS-BW-3 (ML)	SU-AB-14 (ML)	OD-DI-98 (ML)	BT-VL-2 (ME)
PH-BC1-53 (ME)	IMI-AB-1 (ML)	SU-AB-10 (ML)	BR-3 (ME)	BT-VL-30 (ME)
PH-BC1-162 (ML)	IMI-AB-18 (ME)	SU-AB-6 (ML)	BR-1 (ME)	DOP-16-08 (ME)
PH-BC1-74 (ML)	IMI-AB-24 (ML)	SU-AB-15 (ML)	BR-2 (ME)	DOP-32-08 (L)
PH-BC1-158 (ME)	IMI-AB-19 (ME)	SU-AB-4 (ML)	VL-A-8 (ME)	DOP-6-08 (ME)
CMS1-30 (ME)	IMI-AB-14 (ML)	OD-ST-10/1 (ML)	DOP-33-08 (L)	DOP-27-08(ME)
CMS1-90 (ME)	IMI-AB-12 (L)	OD-ST-Ž-10 (ML)	AS-95 (ML)	OD-3369 (ML)
CMS1-122 (ME)	UK-87 (L)	OD-4-ST (ML)	AS-87 (ML)	HA-441 (ML)

In total, 24 sunflower commercial hybrids (including one susceptible control Perun) and 70 inbred lines (Table 1) were tested for tolerance to pathogen *M. phaseolina* in the 2-year field trial located at Rimski Šančevi, Novi Sad (Serbia; 45° 19' 33" N 19° 49' 38" E).

Field Experiment Design

Sunflower hybrids and experimental lines were tested for tolerance to pathogen *M. phaseolina* under field conditions at Rimski Šančevi, Novi Sad, Serbia in 2010 and 2011. Crops were sown manually on 28 April 2010 and 26 April 2011 in a randomized complete block design in three replications. Three rows (12 plants per row) of each genotype were sown per replication, with 70 cm distance between rows, and 30 cm distance between plants. Ten plants in each row were tested using different inoculation methods. Rows with maize flour and sand inoculum without pathogen and sterilized toothpicks were used as a control.

Artificial inoculation methods

For artificial inoculation with *M. phaseolina* two methods were used: Unwounded Stem Based Inoculation (USBI) which is close to natural infestation process and Toothpick Method (TP) which considers infestation by microslerotia through the wound in plants (MIHALJČEVIĆ, 1980).

USBI method used maize flour and sand medium (ratio 1:19) to grow pathogen microsclerotia. Sterilized medium was inoculated with 3-5 PDA plugs (Ø 5 mm^2) with actively growing mycelia of *M. phaseolina*, and incubated at 30ºC in the dark for 2 weeks with periodical shaking of the flasks for equal distribution of microslerotia. In the field, 30 days after germination, each plant was inoculated around stem base with 2 g of the previously prepared inoculum (MIHALJČEVIĆ, 1980).

Sunflower inoculation by TP method used sterilized toothpicks in potato dextrose medium (PDA) with actively growing *M. phaseolina* mycelia (MIHALJČEVIĆ, 1980). The plants were inoculated in the flowering stage (R-5.1) with one toothpick inserted 1 cm above the first stem node of each plant.

Disease for both methods was assessed on 26th August 2010 and 24th August 2011 in sunflower maturity stage (R-9) by cutting each plant longitudinally and measuring the length of lesions with microsclerotia formed in stems.

Data analyses

Disease was assessed in sunflower maturity stage on 30 plants per genotype. Estimation was done according to a 7-grade scale: 1 – microslerotia formed up to 5 cm; 2 – microslerotia formed up to 6-10 cm; 3 – microslerotia formed up to 11-20 cm; 4 – microslerotia formed up to 21-30 cm; 5 – microslerotia formed up to 31-40 cm; 6 – microslerotia formed up to 41-50 cm; 7 – microslerotia formed above 50 cm or plant completely wilted. Disease severity (DS) was calculated according to McKinney's Index formula (MCKINNEY, 1923). Furthermore, according to the obtained McKinney's index in both years, scattered plot was constructed for the assessment of genotype tolerance to *M. phaseolina* infection. Due to high variability in genotype responses to *M. phaseolina* infection, genotypes were classified in six ranges: highly tolerant (HT, 0%), tolerant (T, 0.1-10%), moderately tolerant (MT, 11-30%), moderately susceptible

(MS, 31-60%), susceptible (S, 61-80%), and highly susceptible (HS, 81-100%), depending on the highest disease severity obtained in this 2-year trial.

Additionally, sunflower genotypes with successful inoculations in both trial years were presented according to stem lesion lengths by bar plot (mean +/- SE) in R programme. Environment*genotype interactions based on mean disease severity data were also analysed by ANOVA in R programme.

RESULTS

Sunflower genotypes were tested under field conditions at Rimski Šančevi, Novi Sad, Serbia in a two-year trial, characterized by different weather conditions. The year 2010 saw a significantly higher amount of rainfall in the growing season (May-August) than the annual average rainfall for Novi Sad (Fig. 1), which is unfavourable for the development of *M. phaseolina*. However, the year 2011 saw lower amounts of rainfall in the growing season, with the complete absence of rainfall in August (Fig. 1). Additionally, the average temperature in August 2011 was 4°C higher than the annual average temperature, which favoured the development of *M. phaseolina* and led to higher disease severity registered in the field than the previous year.

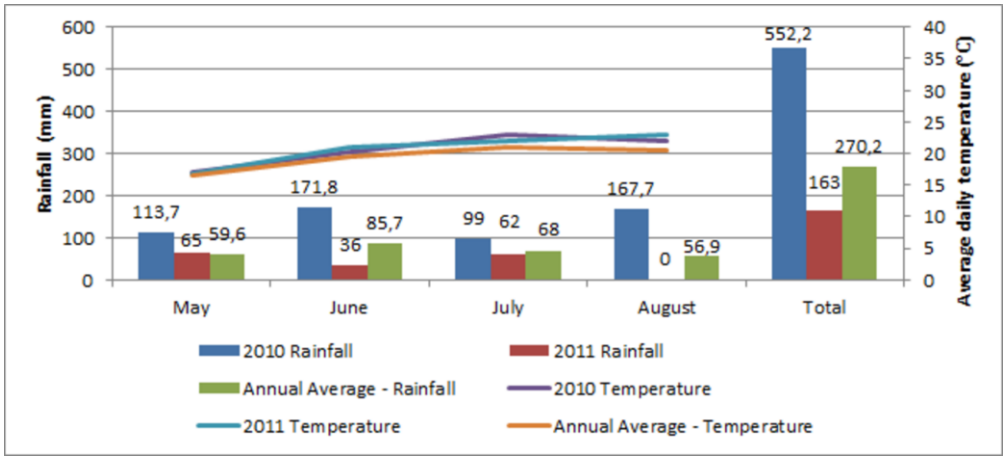

Fig. 1 Annual average temperature and rainfall data for period 1971 - 2000, and data for growing seasons of 2010 and 2011 in Novi Sad (www.hidmet.gov.rs)

Analyses of genotype response variability based on stem lesion lengths are shown in Fig. 2 and 3. Three hybrids and 14 inbred lines expressed some level of susceptibility to charcoal rot tested by USBI method in both trial years (Fig. 2). The highest susceptibility to disease tested by USBI method was found in genotypes CMS1-30 and susceptible control Perun. Moderately tolerant were Orfej and inbred lines NS-BW-3, BR-2, BT-VL-17, PH-BC1-53, and DOP-33-08 shown in the lower left quadrant in Figure 2. Furthermore, 10 hybrids and 29 inbred lines

expressed some level of susceptibility to charcoal rot tested by TP method in both trial years (Fig. 3). Only three hybrids showed higher susceptibility to the disease including positive control and 11 inbred lines, while moderate tolerance was registered in five hybrids (Orfej, Vranac, NS-H-45, NS-Despot, and Šumadinac) and six inbred lines (Fig. 3).

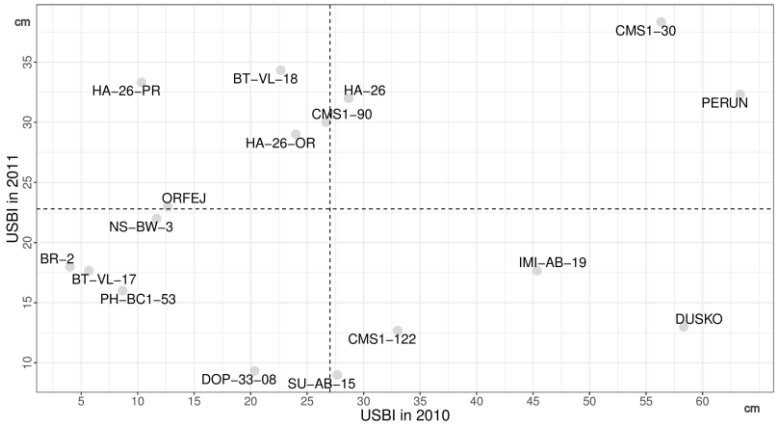

Fig. 2 Variability of stem lesion lengths (cm) of sunflower genotypes susceptible to charcoal rot tested by USBI method in 2010-2011

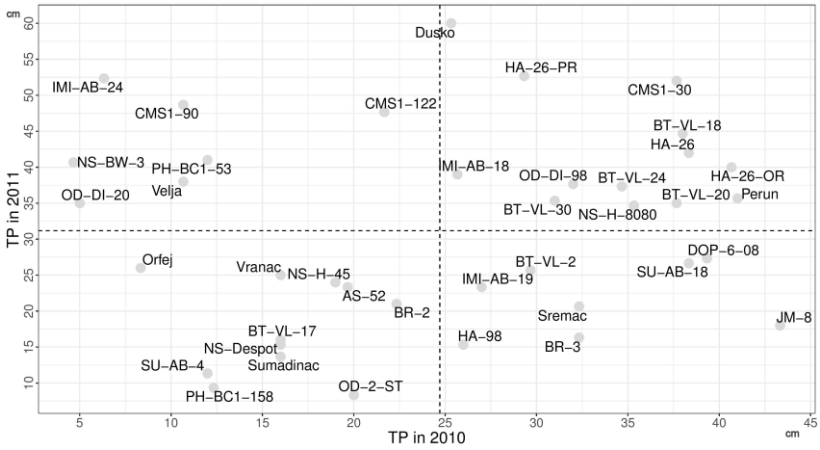

Fig. 3 Variability of stem lesion lengths (cm) of sunflower genotypes susceptible to charcoal rot tested by TP method in 2010-2011

Disease severity was calculated based on stem lesion lengths and microsclerotia presence. ANOVA analyses of disease severity based on mean disease rates indicated significant influence of genotype and year*genotype interactions for both tested methods (Table 2).

Table 2. ANOVA analyses of disease severity

USBI - Hybrids	F value	Df	USBI – Inbreed Lines	F value	Df
rep	2.3571^{ns}	2	rep	2.1429 ^{ns}	4
year	0.0023^{ns}	1	year	0.0138^{ns}	1
gen	3.0470***	23	gen	8.3662***	69
gen x year	6.8876***	23	gen x year	4.5507***	69
Error		94			303
TPM - Hybrids	F value	Df	TPM – Inbreed Lines	F value	Df
rep	2.4122^{ns}	2	rep	0.3965^{ns}	2
year	9.2187**	1	year	13.3554***	1
gen	6.0924***	23	gen	5.8623***	69
gen x year	3.9093***	23	gen x year	5.7092***	69
Error		94			278

According to ANOVA analyses and mean disease severity rates of hybrids tested by USBI method, statistically significant difference was found between tolerant genotypes (Branko, Orion, NS-H-45, NS-Romeo, NS-Novak, NS-Fantazija, NS-H-111, Baća, NS-Despot, and Oskar) and Milan, Duško, Perun, and NS-H-8080. Additionally, inbreed lines tested by USBI method showed that inbreed lines SU-AB-18, DOP-33-08, SU-AB-15, CMS1-122, HA-26-OR, HA-26, BT-VL-18, CMS1-90, and CMS1-30 were significantly susceptible in comparison with the other tested inbreed lines (data not shown).

According to ANOVA analyses and mean disease rates of hybrids tested by TP method, statistically significant difference was found between tolerant genotypes (Orion, NS-Novak, NS-Romeo, NS-H-111, and NS-Fantazija) and Šumadinac, NS-Gladijator, Duško, Velja, Perun and NS-H-8080. Inbreed lines tested by TP method showed statistically significant difference between tolerant genotypes (AS-92, IMI-AB-12, DOP-27-08, IMI-AB-6, UK-58-ST, PR-ST-28, OD-ST-Z-10, PH-BC2-64, SU-AB-6, PH-BC2-92, AS-87, AS-95, and IMI-AB-1) and genotypes with different levels of susceptibility - BR-3, DOP-33-08, IMI-AB-19, BT-VL-30, SU-AB-14, BT-VL-20, BT-VL-24, SU-AB-18, JM-8, DOP-6-08, CMS1-122, HA-26, HA-26-OR, CMS1-90, AS-52, IMI-AB-18, CMS1-30, BT-VL-18, and HA-26-PR (data not shown).

Furthermore, for more strict classification of the genotypes, the highest disease severity obtained in 2010-2011 was considered as the most reliable value for sunflower genotype screening, and those values based on both test methods are depicted in Figures 4 and 5.

The tolerant sunflower hybrids under the conditions of 2010-2011 were NS-H-45, NS-H-111, Orion, NS-Romeo, NS-Novak, NS-Fantazija, Vladimir, Branko, Oskar and NS-Despot (Fig. 4). The most susceptible hybrids were susceptible control Perun, as expected, and NS-H-8080 which showed even higher susceptibility than the susceptible control (Fig. 4).

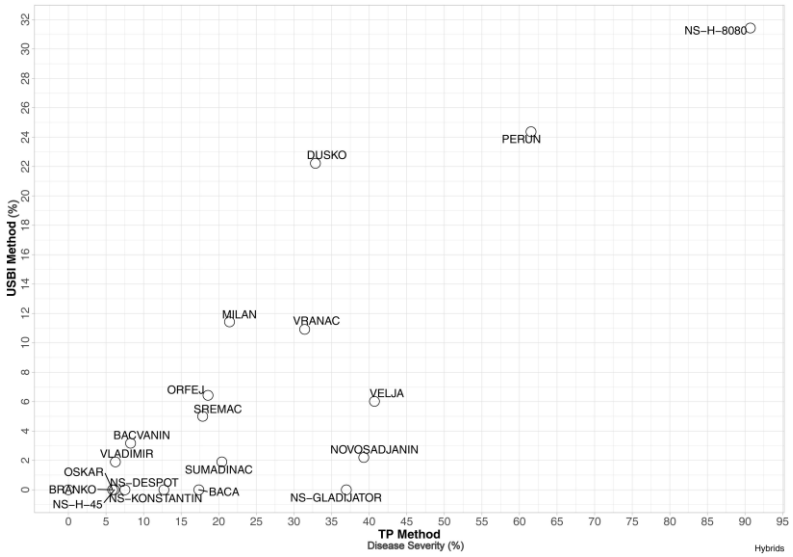

Fig. 4 Disease severity (%) of charcoal rot (*M. phaseolina*) on tested sunflower hybrids obtained by USBI and TP methods in 2010-2011

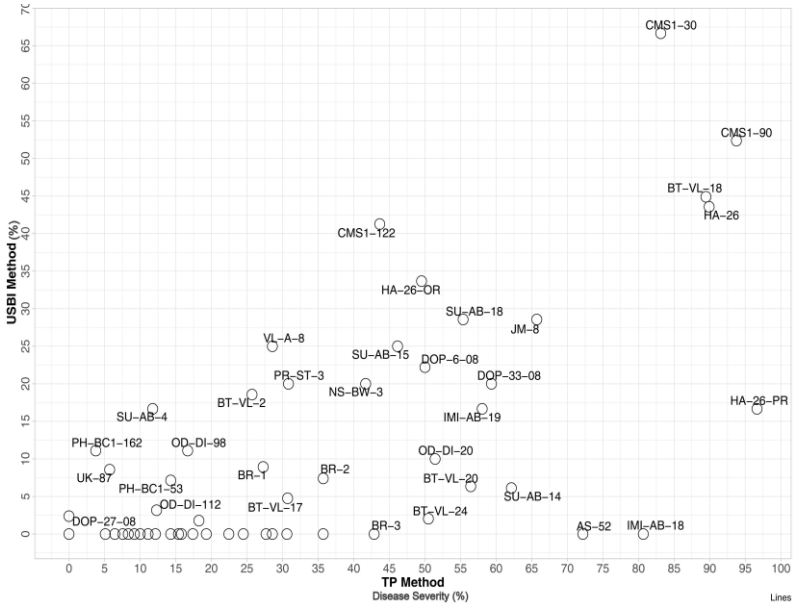

Fig. 5 Disease severity (%) of charcoal rot (*M. phaseolina*) on tested sunflower inbred lines obtained by USBI and TP methods in 2010-2011

Table 3. The list of highly tolerant (HT) and highly susceptible (HS) sunflower genotypes to charcoal rot according to highest disease severity obtained in the period 2010-2011

Sunflower hybrids	
Highly Tolerant (HT)	Highly Susceptible (HS)
NS-H-111 (ML)	NS-H-8080 (E)
ORION (ML)	PERUN (E)
NS- ROMEO (ME)	
NS- NOVAK (L)	
NS-FANTAZIJA (ML)	
Sunflower inbred lines	
Highly tolerant (HT)	Highly Susceptible (HS)
PH-BC2-64 (ML)	HA-26 (ME)
PH-BC2-92 (L)	HA-26-PR (ME)
PR-ST-28 (L)	CMS1-30 (ME)
IMI-AB-6 (L)	CMS1-90 (ME)
IMI-AB-1 (ML)	IMI-AB-18 (ME)
IMI-AB-12 (L)	BT-VL-18 (ME)
UK-58-ST (L)	
SU-AB-6 (ML)	
OD-ST-Ž-10 (ML)	
AS-95 (ML)	
AS-87 (ML)	
AS-92 (ML)	

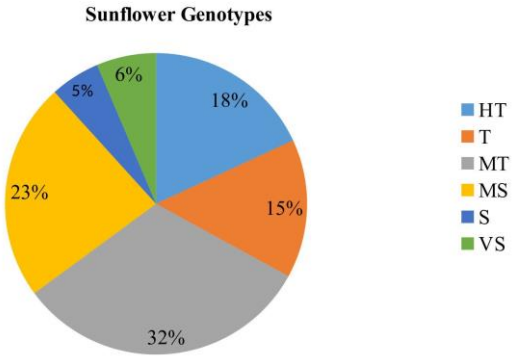

Fig. 6 Sunflower genotypes response to *M. phaseolina* infection: highly tolerant (HT), tolerant (T), moderately tolerant (MT), moderately susceptible (MS), susceptible (S), and highly susceptible (HS)

As shown in Figure 5, highly susceptible inbred lines were CMS1-30, CMS1-90, BT-VL-18, HA-26, HA-26-PR, IMI-AB-18, AS-52, and SU-AB-14 in 2010-2011.

According to the highest disease severity, but regardless of year or testing method, 79% of the tested hybrids and 83% of the inbred lines showed some level of susceptibility (Fig. 6).

The most susceptible genotypes belong to the early or medium early maturity group (Table 3). Complete absence of disease symptoms using both inoculation methods in both trial years was registered in five hybrids and 12 inbred lines (Table 3). Most of these hybrids and lines belong to medium late to late maturity group.

DISCUSSION

When comparing the efficacies of the inoculation methods, it can be concluded that TP method is more certain to obtain high disease incidence even when the environmental conditions are not favourable for the pathogen development, as was the case in 2010. Good artificial inoculation method should be easy to handle, imitate pathogen's natural path of infestation and produce a high infection rate (DEGENER *et al.*, 1998). TP method is easy to handle and produces high infection rate, but does not imitate pathogen's natural infestation path. Direct inoculation of the stem often implies artificial wounding of the plant in which case some levels of tolerance might be skipped leading to increased susceptibility. On the other hand, USBI method mimics the pathogen's natural path of infestation and is easy to handle, but there is always a risk of failure due to unfavourable environmental conditions. Environmental factors are limiting for the disease appearance, and high humidity immediately after infection could completely stop the disease development, while dry and warm period will increase formation of microsclerotia in the stem and finally lead to symptoms appearing at the plant maturity stage. Reliance on the natural infestation and pathogen attack is not recommended for a successful testing of breeding material due to high dependence on the environmental conditions and non-homogeneous inoculum distribution in the soil which can provide unreliable results (SERRE *et al.*, 2004). In order to avoid the inconvenience of the failure due to unfavourable weather conditions or inadequate inoculum distribution in the field, both USBI and TP methods should be applied together in sunflower genotype testing to charcoal rot tolerance. Successful evaluations of sunflower genotypes susceptibility to charcoal rot by aggressive inoculation methods, including toothpick method, were reported by other researchers (DE OLIVIERA *et al.*, 2004; ŠKORIĆ, 2012; TAHA *et al.*, 2018). The current study showed that microsclerotia formation in the stem and stem lesion lengths are reliable traits for screening against *M. phaseolina*. Disease assessments in the field are mostly based on the symptoms visible on the stems which are presented as an infected area percentage (DAY and MACDONALD, 1995; BOKOR, 2007; MAHMOUD, 2010). Although more demanding, measurement of microsclerotia presence and lesion length in the stem is more reliable and can confirm presence of the pathogen even when visible symptoms are absent. Stem colour showed high correlation with disease scoring in the early stage screening tests (SHEHBAZ *et al.*, 2018).

Considering the susceptibility screening test of the sunflower genotypes, the obtained results indicated that the most susceptible genotypes belong to the early or medium early maturity group. Our positive control Perun, an early hybrid, was also used by other researchers and showed average disease severity of 4.88 out of 6 in greenhouse pathogenicity test with *M. phaseolina* isolates originating from Egypt (MAHMOUD, 2010). Inoculation method was similar

to USBI, disease severity was registered 10 weeks after sowing and varied in Perun from 4.58 to 5.08 depending on isolate aggressiveness. This confirms that Perun can be used as a positive control in further screening tests regardless of *M. phaseolina* isolate origin.

Furthermore, complete absence of disease symptoms using both inoculation methods in both trial years was registered in five hybrids and 12 inbred lines. Most of these hybrids and lines belong to medium late to late maturity groups and have good adaptation to different environmental conditions and high tolerance to drought. Due to the fact that sunflower tolerance to drought is related to *Macrophomina* tolerance, breeding of these genotypes showed great achievements. ŠKORIĆ (2016) reported that *Phomopsis* resistance is positively correlated with *Macrophomina* and *Phoma* resistance, as well as with drought tolerance. FICK and MILLER (1997) have concluded that one approach in breeding for drought resistance is to develop high-yielding cultivars that flower and mature before soil water conditions become limiting. Early sunflower hybrids generally have lower leaf area index, lower total evapotranspiration and lower yield potential than later ones. According to ŠKORIĆ (2009), early sunflower hybrids are most often susceptible to *Macrophomina*, so in cases when there is an early occurrence of drought such hybrids may become affected, thus nullifying any positive effect early maturity may bring. Our results are consistent with this statement since the early genotypes showed different levels of susceptibility, while full vegetation genotypes expressed tolerance to *Macrophomina*.

Development of tolerant genotypes is one of the most important methods for managing charcoal rot diseases on sunflower (ABOUTALEBI *et al.*, 2014; TAHA *et al.*, 2018). Sources of resistance are mainly found in wild species of the genus *Helianthus*, as well as in cultivated sunflowers. Wild sunflower species are inexhaustible sources of disease resistance genes and provide a high level of tolerance (field resistance) to *Phomopsis/Diaporthe helianthi, Macrophomina phaseolina, Pustula helianthicola* and *Alternaria* spp. (ŠKORIĆ, 2016). SEILER (2012) concluded that interspecific hybrids based on *H. tuberosus* have high tolerance to charcoal rot, and wild species *H. mollis, H. maximiliani, H. resinosus, H. tuberosus,* and *H. pauciflorus* have also shown tolerance. Additionally, TANČIĆ *et al.* (2012) reported that accessions of *H. annuus, H. tuberosus* and *H. petiolaris* have shown high tolerance to charcoal rot. Some resistant genotypes have been derived from varietal populations (VNIIMK, Krasnodar) developed by interspecific hybridization with *H. tuberosus* (KAYA, 2016). According to SHEHBAZ *et al.* (2018) *H. argophyllus* was identified as immune for charcoal rot and has the potential to diversify the initial source of disease resistance in sunflower elite germplasm.

Certain level of resistance has been found in cultivated sunflowers as well. Inbred lines HAR-1 and HAR-2 used to be tolerant to almost all charcoal rot isolates while USDA public line HA 822 was susceptible to the disease development and two charcoal rot isolates were virulent in affecting the head weight (AHMAD *et al.*, 1991). According to KHAN (2007), resistance in sunflower genotype is a dominant character and presence of two complimentary genes, *MP 1* and *MP 2*, is essential in resistant cultivars. Resistance to *M. phaseolina* is horizontal and controlled by polygenes (KAYA, 2016). After all, studies of wild sunflower species have so far been insufficient to identify resistance genes as the sources of resistance against charcoal rot.

The present study identified highly tolerant cultivars NS-H-111, Orion, NS-Romeo, NS-Novak and NS-Fantazija that could be used widely to control charcoal rot, while inbred lines PH-BC2-64, PH-BC2-92, PR-ST-28, IMI-AB-6, IMI-AB-1, IMI-AB-12, UK-58-ST, SU-AB-6,

OD-ST-Ž-10, AS-95, AS-87, and AS-92 should be used for breeding programs in order to improve future cultivars. Disease control in sunflower eco-friendly production generally means genetic resistance of the host plants, and sunflower resistance to main economically important diseases is one of the most desirable traits. Successful breeding for disease resistance considers monitoring of interactions between the host (sunflower), certain pathogen and the environment, stability of sunflower resistance to certain pathogens, and application of general principles of resistance breeding.

CONCLUSIONS

Due to the global change of climate and spreading of *Macrophomina phaseolina* area, the breeding for charcoal rot resistance is becoming important. The most effective method for disease control is the use of resistant or tolerant sunflower genotypes. The present study identified five highly tolerant hybrids and 12 inbred lines that can be used in breeding programs for improvement of future genotypes. This is the valuable information for future more comprehensive research that will explain the genetic basis of tolerance to *M. phaseolina* in the examined genotypes and type of its inheritance. In sunflower genotype screening tests for *Macrophomina* tolerance, both USBI and TP methods should be applied together to provide reliable test results. Microsclerotia presence and measurement of stem lesion lengths were proved to be reliable traits that can confirm the presence of the pathogen even when visible symptoms are absent.

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REFERENCES

ABOUTALEBI, R., A. DALILI, S. RAYATPANAH, A. ANDARKHOR (2014): Evaluation of sunflower genotypes against charcoal rots disease in vitro and in vivo condition. World App. Sci. J., *31*(4): 649-653.

AĆIMOVIĆ, M. (1998): Sunflower diseases. Feljton, 544-573 (in Serbian).

AHMAD, I., K. BURNEY, S. ASAD (1991): Current status of sunflower diseases in Pakistan. In: Proceedings of National Symposium on Status of Plant Pathology in Pakistan, Karachi, 53.

ALEXANDROV, V. (1999): Incidence of charcoal rot of sunflower caused by *Sclerotium bataticola* Taub. in Bulgaria. Bulg. J. Agric. Sci., *5*: 867-890.

BOKOR, P. (2007): *Macrophomina phaseolina* causing a charcoal rot of sunflower through Slovakia. Biologia Bratislava, *62* (2): 136-138.

CSÖNDES, I., A. CSEH, J, TALLER, P. POCZAI (2011) Genetic Diversity and Effect of Temperature and Ph on the Growth of *Macrophomina phaseolina* Isolates from Sunflower Fields in Hungary. Mol. Biol. Rep., *39*(3): 3259-3269.

DAY, J.P., M.V. MACDONALD (1995): Plant-pathogen interactions of sunflower and *Macrophomina phaseolina in vitro* and *in vivo*. Plant Path., *44*: 261-269.

DE OLIVEIRA, M.E, A.T. NETO, R.M.V.B.C. LEITE, V.B.R. CASTIGLIONI, C.A.A. ARIAS (2004): Mutation breeding in sunflower for resistance to Alternaria leaf spot. Helia, *27*(41): 41–50.

DEDIĆ, B., I. BALALIĆ, I. RADEKA, N. LAČOK, S. TANČIĆ (2009): Otpornost novosadskih hibrida suncokreta prema prouzrokovaču mrko-sive pegavosti stabla u uslovima prirodne zaraze. Pestic. Phytomed., *24*(3): 197-201.

DEDIĆ, B., S. MAŠIREVIĆ, D. MILADINOVIĆ, S, JOCIĆ, S, TERZIĆ, S. TANČIĆ, J. MRĐA (2012): Screening of sunflower inbred lines tolerance to Phoma black stem. Rastenievdni Nauki, *6*:41-44.

DEDIĆ, B., D. MILADINOVIĆ, S. JOCIĆ, S. TERZIĆ, S. TANČIĆ, N. DUŠANIĆ, V. MIKLIČ (2011): Sunflower Inbred Lines Screening for Tolerance to White Rot on Stalk. Field Veg. Crops Res., *48*:167-172.

DEGENER, J., E.A. MELCHINGER, K.R. GUMBER, V. HAHN (1998): Breeding for Sclerotinia tolerance in sunflower: A modified screening test and assessment of genetic variation in current germplasm. Plant Breeding, *117*:367-372.

FAO (2018): Food and Agriculture data. http://www.fao.org/faostat/en/#home

FICK, G.N., J.F. MILLER (1997): Sunflower Breeding. In: Schneiter AA (ed), Sunflower Technology and Production. ASA Inc, CSSA Inc, SSSA Inc Publishers. pp.395-441.

IONITĂ, A., H. ILIESCU, S. KUPFERBERG (1996): *Macrophomina phaseolina* – One of the Main Pathogens of Sunflower Crop in Romania. In: Proceedings of 14th Int. Sunflower Conference. International Sunflower Association, Beijing, China, 718-723.

ISLAM, S., S. HAQUE, M.M. ISLAM, E.M. EMDAD, A. HALIM, Q. HOSSEN, Z. HOSSAIN, B. AHMED, S. RAHIM, S.RAHMAN, M. ALAM, S. HOU, X. WAN, J.A. SAITO, M. ALAM (2012): Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics, *13*:493.

JALIL, S., H.A. SADAQAT N.H. TAHIR A.H. SHAFAULLAH, N. ALI (2013) Response of Sunflower under Charcoal Rot (*Macrophomina phaseolina*) Stress Conditions. Int. J. Sci. Eng. Res. (IJSER) *1*(3):89-92.

JIMENÉZ-DIAZ, R.M., M.A. BLANCO-LÓPEZ, W.E. SACKSTON (1983): Incidence and distribution of charcoal rot of sunflower caused by *Macrophomina phaseolina* in Spain. Plant Dis., *63*:1033-1036.

JOCIĆ, S., N. LAČOK, V. MIKLIČ, D. ŠKORIĆ, Y. GRIVEAU (2004): Testing two isolates of *Diaporthe/Phomopsis helianthi* in a population of sunflower recombinant inbred lines. Helia, *27*(41):129-136.

KAYA, Y. (2016): Sunflower. In: Gupta SK (ed). Breeding Oilseed Crops for Sustainable Production: Opportunities and Constraints. Academic Press, 55-88.

KHAN, S.N. (2007): *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. Mycopath., *5*(2): 111-118.

MAHMOUD, A.F.A. (2010): Molecular and Biological Investigations of Damping-off and Charcoal-Rot Diseases in Sunflower. PhD thesis, Sabanci University, Edirne, Turkey.

MANICI, L.M., C. CERATO, F. CAPUTO (1992): Pathogenic and Biological Variability of *Macrophomina phaseolina* (Tassi) Goid. Isolates in Different Areas of Sunflower Cultivation in Italy. In: Proceedings of 13th International Sunflower Conference. International Sunflower Association, Pise, Italy, 779-784.

MCKINNEY, H.H. (1923): Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J. Agric. Res., *26*:195–217.

MICHEL, J.F. (2000): Seed infection of mungbean by *Macrophomina phaseolina*. CRC for Tropical Plant Pathology and Department of Botany, Queensland University, Australia.

MIHALJČEVIĆ, M. (1980): Sources of resistance to *Sclerotium bataticola* Taub. in sunflower inbred lines and hybrids. Msc thesis, Faculty of Agriculture, Novi Sad (in Serbian).

OLAYA, G., G.S. ABAWI, N.F. WEEDEN (1996): Inheritance of resistance to *Macrophomina phaseolina* and identification of RAPD markers linked with the resistance genes in beans. Phytopath., *86*:674-679.

RAUF, S., M. SHAHZAD, J.A.T. DA SILVA, I.R. NOORKA (2012): Biomass partitioning and genetic analyses of salinity tolerance in sunflower (*Helianthus annuus* L.). J. Crop Sci. Biotech., *15*:53–57.

STATISTICAL OFFICE OF THE REPUBLIC OF SERBIA (2019): http://data.stat.gov.rs/Home/Result/130102?languageCode=sr-Cyrl

SEILER, G. (2012): Utilization of wild *Helianthus* species in sunflower breeding. In: Škorić D, Sakač Z (eds). Sunflower Genetics and Breeding. (International monography). Serbian Academy of Sciences (SASA), Republic of Serbia, 344–430. ISBN: 978-88-81125-82-3

SERRE, F., P. WALSER, D. TOURVIEILLE DE LABROUCHE, F. VEAR (2004): *Sclerotinia sclerotiorum* capitulum tolerance tests using ascospo-res: results over the period 1991-2003. In: Proceedings of 16th International Sunflower Conference, Fargo, North Dakota, USA: 129-133.

SHEHBAZ, M., S. RAUF, A.M. AL-SADI, S. NAZIR, S. BANO, M. SHAHZAD, M.M. HUSSAIN (2018): Introgression and inheritance of charcoal rot (*Macrophomina phaseolina*) resistance from silver sunflower (*Helianthus argophyllus* Torr. & A. Gray) into cultivated sunflower (*Helianthus annuus* L.). Australas Plant Pathol *47*: 413–420.

ŠKORIĆ, D. (2009): Sunflower breeding for resistance to abiotic stresses. Helia, *32*(40):1-16.

ŠKORIĆ, D. (2016): Sunflower Breeding for Resistance to Abiotic and Biotic Stresses (Chapter 25). In: Shanker AK, Shanker C (eds.) Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives. DOI: 10.5772/62159. https://www.intechopen.com/books/abiotic-and-biotic-stress-in-plants-recent-advances-and-future-perspectives/sunflower-breeding-for-resistance-to-abiotic-and-biotic-stresses

ŠKORIĆ, D. (2012): The Genetics of Sunflower. In: Kovačević Z, Škorić D, Sakač Z (eds.) Sunflower Genetics and Breeding-International Monogram. Serbian Academy of Sciences, Serbia, 1–125.

SURIACHANDRASELVAN, M., K.E.A. AIYYANTHAN, R. VIMALA (2005): Host range and cross inoculation studies on *Macrophomina phaseolina* from sunflower. The Madras Agricl. J., *92*(4-6): 238-240.

TAHA, M.M., A.F. MAHMOUD, M.A, HASSAN, A.M. MAHMOUD, M.A. SALLAM (2018): Potential resistance of certain sunflower cultivars and inbred lines against charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid. J. Phytopath. Pest Manag., *5*(3):55-66.

TANČIĆ, S., B., DEDIĆ, A., DIMITRIJEVIĆ, S., TERZIĆ, S., JOCIĆ (2012a): Bio-ecological relations of sunflower pathogens – *Macrophomina phaseolina* and *Fusarium* spp. and sunflower tolerance to these pathogens. Rom. Agric. Res., *29*:349-359.

TANČIĆ, S., S. TERZIĆ, B. DEDIĆ, J. ATLAGIĆ, S. JOCIĆ, V. MIKLIČ (2012b): Resistance of wild sunflower species to *Macrophomina phaseolina*. In: Proceedings of the Forth Joint UNS-PSU International Conference on Bioscience: Biotechnology and Biodiversity, Serbian Seed Association, Novi Sad, Serbia, 34-37.

VEVERKA, K., J. PALICOVÁ, I. KŘÍŽKOVÁ (2008): The Incidence and Spreading of *Macrophomina phaseolina* (Tassi) Goidanovich on Sunflower in the Czech Republic. Plant Protec. Sci., *44*:127-137.

ZHANG, Y.Y., Y. YU, K. WANG, M. LI, D.S. XU, J. ZHAO (2016): First Report of Sunflower Charcoal Rot Caused by *Macrophomina phaseolina* in Jilin and Inner Mongolia, China. Plant Dis Notes, https://doi.org/10.1094/PDIS-10-15-1152-PDN

RAZNOVRSNOST GENOTIPOVA SUNCOKRETA U TOLERANTNOSTI PREMA UGLJENASTOJ TRULEŽI (*Macrophomina phaseolina* (TASSI) GOID.) U USLOVIMA POLJA

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Izvod

Suncokret (*Helianthus annuus* L.) je jedna od najvažnijih ratarskih kultura gajena širom sveta, koju može parazitirati više od 30 različitih patogena. Areal vrste *Macrophomina phaseolina* (Tassi) Goid., koja je jedan od čestih patogena suncokreta u umerenom klimatskom pojasu, se u poslednje vreme širi usled klimatskih promena. Najefikasniji metod za kontrolu ugljenaste truleži suncokreta koju izaziva *M. phaseolina* jeste gajenje otpornih genotipova suncokreta. Stoga je 24 komercijalno dostupnih hibrida i 70 inbred linija testirano na tolerantnost prema *M. phaseolina* u dvogodišnjem ogledu. U uslovima polja, dve različite metode inokulacije su korišćene – Inokulacija bez povrede osnove stabla (USBI) i metoda čačkalica (TP). Istraživanja su identifikovala 5 visoko tolerantnih hibrida i 12 inbred linija koje mogu biti korišćene u oplemenjivačkim programima za unapređenje budućih genotipova suncokreta. Skrining testovi za tolerantnost genotipova suncokreta na *M. phaseolina* su ukazali da je preporučljivo istovremeno primenjivati obe metode inokulacije kako bi se obezbedili pouzdani rezultati, kao i da je dužina lezija na stablu pouzdan karakter za procenu intenziteta oboljenja.

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