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Scientific paper

Testing Sunflower Inbred Lines for Tolerance to Phoma Black Stem

Boško Dedić

Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia (bosko.dedic@ifvcns.ns.ac.rs)

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SUMMARY

Phoma black stem is caused by a widespread pathogen *Phoma macdonaldii* Boerema. The disease occurs regularly, causing damage by early defoliation and premature ripening. Complete resistance of sunflower to this disease has never been found, but there are reports of differences in response by different genotypes. Fifty-four new inbred lines were tested in our trials conducted at the Rimski Šančevi experimental field. Plants in one trial were non-irrigated, and irrigated in another. Sunflower plants were artificially inoculated with mycelial plugs in the bud stage. The length of stem lesions was measured and compared using an analysis of variance. Disease intensity was generally more severe in the non-irrigated field. There were significant differences in tolerance to Phoma black stem among the tested lines in both trials. The percentage of tolerant genotypes was 1.8%.

Keywords: Sunflower; *Phoma macdonaldii*; Tolerance; Inbred lines

INTRODUCTION

Phoma macdonaldii Boerema is a widespread pathogen of sunflower. The fungus has been confirmed in sunflower crops in Asia, North and South America, Africa, Europe and recently in Australia (Gulya et al., 1997; Mirić et al., 1999). Disease symptoms can appear as black necrotic spots and lesions in all phenological stages and on all organs. Furthermore, the fungus can be found in sunflower seeds although it was not detected in the most recent studies of sunflower seed mycobiota (Lević, 2012). Stem lesions are very distinctive and usually restricted to cortical tissues. The infection of collar and root system may lead to symptoms frequently referred to as premature ripening. Damage from these two types of symptoms measured as yield loss may reach 0.7 and 1.3 t/ha, respectively (Peres et al., 2000).

Research efforts to find genotypes less susceptible to black stem disease have not been as intensive as those focusing on genotypes resistant or tolerant to some other sunflower diseases, such as stem canker (Škorić, 1980; Gulya et al., 1997). However, different levels of resistance have been detected among various cultivated and wild sunflowers (Encheva et al., 2004). Darvishzadeh et al. (2010) found the inbred lines F1250/03, M5-54-1, M6-862-1, two wild accessions and two lines developed by mutagenesis to be tolerant. Although tolerant genotypes were identified, full resistance was not found (Roustee et al., 2000a; Bert et al., 2004).

Besides differences in sunflower genotype response, certain variability has also been proved to exist among different isolates of the fungus (Roustaee et al., 2000a; Larfeil et al., 2002). Therefore, accurate knowledge of the pathogen variability is also required for identification of tolerant genotypes.

The aim of this study was to test inbred lines for tolerance to Phoma black stem in newly developed sunflower inbred lines and to explore the effect of water regime on disease severity.

MATERIAL AND METHODS

Trials were conducted on the experimental field of the Institute of Field and Vegetable Crops at Rimski Šančevi. A total of 54 inbred lines was tested without any previous information about their tolerance to Phoma black stem. Inbreds were part of a breeding programme of finding tolerance to sunflower diseases other than Phoma black stem. Two identical experiments were set in an irrigated and non-irrigated field. The experimental design was a completely randomised block system with three replications. Seeds were sown manually in 3.6 m-long rows. Row spacing was 0.7 m and distance between plants in a row was 0.3 m. Sowing was done in the first decade of April by placing three seeds per spot. Plants were thinned when first true leaves were formed.

The fungus was isolated from stems with disease symptoms found near the trial site in the previous year and the isolate was refined to monosporous for artificial inoculation. Inoculation of five plants per replication of each line was done in the sunflower budding stage (R3) (Schneiter and Miller, 1981), and following a method described by Sessau et al. (2008). Mycelial plugs, half a centimetre in diameter, of a two-weeks-old colony on PDA medium were used for inoculation. The plugs were placed at the intersection of the leaf petiole and stem, and covered with moistened cotton wool and aluminium foil to prevent drying. Cotton and tinfoil were removed after two weeks. In the irrigated field, watering was done using sprinklers that added 20 mm of water three times per week. Irrigation was omitted only when the amount of natural rainfall exceeded the amount planned for irrigation.

The intensity of disease was evaluated by measuring the length of stem lesions 7 weeks after inoculation. Plants without symptoms were not included in statistical analysis. The experiment was designed as a two-factorial trial with irrigation as the first factor and genotype as the second. Genotypes were categorised using a modified scale of Larfeil et al. (2010) (Table 1). Analysis of variance was done using the software Statistica 9.0. Duncan test was used for comparing the means of lengths of necrotic area and based on these results the test lines were marked as tolerant (T), moderately susceptible (MS) or susceptible (S) (Table 1).

Table 1. Assessment of sunflower genotypes based on modified scale by Larfeil et al. (2010) with categories tolerant (T), moderately susceptible (MS) and susceptible (S)

Susceptibility groups	Necrotic stem lenght
T	< 5 cm
MS	5 – 7 cm
S	> 7 cm

RESULTS AND DISCUSSION

First symptoms of disease were noticed approximately two weeks after inoculation as small necrosis of petioles. Necrotic area expanded to stems forming characteristic black spots. The spots elongated and some reached 9 cm in length but only a few fully encircled the stem. According to hydrometeorological data for the summer period when we performed the testing, rainfall was highly above the average, as well as temperatures (Republic Hydrometerological Service of Serbia, 2010).

A total of 98.4% and 94.2% of the plants in the non-irrigated and irrigated trials were successfully inoculated, respectively. F-test detected highly significant differences in disease intensity for irrigation, tested genotypes, and interaction between these two factors (Table 2).

Table 2. Analysis of variance, means and standard errors (SE) of lesion lengths of phoma black stem on sunflower in irrigated and non-irrigated field

Source of variation	F value	p
Irrigation	259.90	< 0.01
Genotype	2.04	< 0.01
Interaction	2.13	< 0.01

The mean lesion length was 5.0 cm in the irrigated field and 6.4 cm in non-irrigated. This difference was highly significant (Table 2). As a result of slower disease development in the irrigated field we compared the means of necrotic lesions for lines grown in non-irrigated conditions. There were 1.8%, 77.8% and 20.4% of tolerant, moderately susceptible and susceptible lines in the non-irrigated field, respectively (Figure 1). In the non-irrigated experimental field, the genotype that had the lowest average necrotic lesion mean was SC2L32 (4.9 cm) and the most susceptible was line SC2L41 (8.7 cm)

(Table 3). The average length of lesions in a group of five genotypes, SC2L42, SC2L1, SC2L52, SC2L5 and SC2L20, was not significantly different than the average necrotic lesion of SC2L32. Furthermore, they were over 1 cm longer on the average than the necrotic lesion of SC2L32 and were also ranked in the homogenous group „b". Therefore they were placed in the category of moderately susceptible genotypes. The reaction of most other genotypes to disease varied and they were found to be moderately susceptible, while 11 genotypes were susceptible with an average necrotic lesion length of over 7 cm, which is equivalent to score 4 on the scale of Larfiel et al. (2010).

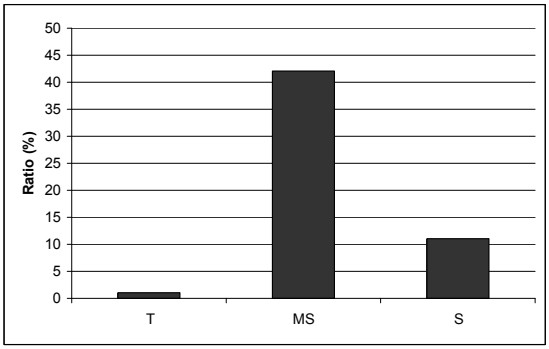

Figure 1. Ratio of tolerant (T), moderately tolerant (MT), moderately susceptible (MS) and susceptible genotypes in total number of tested genotypes calculated using length of stem lesions and expressed as percentage

Table 3. Average necrotic lesion length, standard error and homogeneous groups based on Duncan post-hoc test of some tested genotypes

Sunflower genotype	Mean (cm)	SE
SC2L32	4.92 a	0.15
SC2L 42	6.10 ab	0.43
SC2L 1	6.14 ab	0.66
SC2L 52	6.16 abc	0.32
SC2L 5	6.16 ab	0.21
SC2L 20	6.22 abc	0.43
SC2L 2	6.76 bc	0.22
SC2L 9	6.78 bc	0.13
SC2L 12	6.80 bc	0.43
SC2L 7	6.80 bc	0.45
SC2L 16	6.82 bc	0.37
SC2L 14	6.82 bc	0.39
SC2L 27	6.84 bc	0.54
SC2L 43	7.64 cd	0.38
SC2L 40	8.74 d	0.70

A reliable evaluation of genotype response to a plant disease often requires several successive assessments. Darvishzandeh et al. (2010), examining a case of Phoma black stem, found a strong correlation between disease severity and disease progress rate and came to a conclusion that one disease assessment is adequate for making conclusion about genotype tolerance as we did in our research. Somewhat similar conclusions were made by Larfeil et al. (2010) for inoculation of plants in different growth stages. In addition, they showed that the behaviour of sunflower genotypes in controlled conditions remained unchanged irrespective of the phenological stage reached at the time of inoculation. Most of field testing is usually done at the stage R3-R5 (Carson, 1991; Sessau et al., 2008), as we did as well, but a vast majority of studies have been done in controlled conditions (Mirić et al., 1999; Roustaee et al., 2000a). Furthermore, our results from the non-irrigated field, compared to the irrigated one, are in concordance with the results of Sessau et al. (2010), who found a positive correlation between water stress and *P. macdonaldii* attack severity. Similar to these results, Fayzalla and Marić (1981) found in their four-year research that disease severity was greater in years with drought periods and less precipitation during vegetation period. It was similar to the conditions that prevailed during our research, the exception being short periods of abundant rainfall.

Compared to other studies (Larfeil et al., 2010), we had slightly more intensive disease symptoms on the average. The difference might be either the result of using a more aggressive fungal isolate (Darvishzadeh and Saraffi, 2007), or the environmental factors.

CONCLUSIONS

In conclusion, the reaction of genotypes used in this research showed a great variability after inoculation with *P. macdonaldii*, ranging from susceptible to tolerant. This research confirms the existence of tolerant genotypes among the tested lines. However, only 1.8% of all tested lines were tolerant. The method of artificial inoculation proved to be successful with an average of 98.4% and 94.2% plants developing symptoms of disease in the non-irrigated and irrigated trials, respectively. The disease was more severe in the non-irrigated than in irrigated field, which means that non-irrigated conditions are more suitable for testing. For further research, tolerance of the selected lines should be proved by using different fungal isolates.

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Iznalaženje izvora tolerantnosti za prouzrokovača crne pegavosti stabla suncokreta

REZIME

Crnu pegavost stabla suncokreta prouzrokuje široko rasprostranjen patogen *Phoma macdonaldii* Boerema. Pojava bolesti je stalna na skoro svim područjima gajenja suncokreta, a štete nastaju usled defolijacije i ranog sazrevanja suncokreta. Potpuna otpornost na ovu bolesti nije poznata, a dokazan je različit nivo osetljivosti ispitivanih genotipova. Istraživanje sprovedeno na eksperimentalnom polju Rimski Šančevi je podrazumevalo testiranje 54 nove inbred linije. Ogled se sastojao iz dva odvojena dela. Prvi deo se nalazio u sistemu za navodnjavanje, a drugi u uslovima suvog ratarenja. Biljke suncokreta su veštački inokulisane micelijom gljive u fazi butonizacije. Nakon inokulacije merena je dužina lezija na stablu, a podaci su upoređeni putem analize varijanse. Značajno veći intenzitet bolesti je zabeležen u uslovima suvog ratarenja. U oba dela ogleda zabeležene su značajne razlike u tolerantnosti na crnu pegavost između linija stabla suncokreta. Od ukupnog broja testiranih linija 1,8% je pokazalo zadovoljavajući nivo tolerantnosti.

Ključne reči: Suncokret; *Phoma macdonaldii*; otpornost; inbred linije