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A Method for Evaluation of Sunflower Resistance to *Diaporthe/Phomopsis helianthi* Munt. Cvet. et al.

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Abstract

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The use of a fast and easy to apply method for inoculation is of primary importance in breeding for resistance of sunflower cultivars and hybrids. A fast and easy to apply method for testing the breeding materials resistance to *Diaporthe/Phomopsis helianthi* is suggested to include in the breeding program. Sunflower plants at buttoning stage were inoculated with the help of the straw method. Results were read 10 days after inoculation according to a five degree scale: 0 – no symptoms or traces of pathogen invasion; 1 – spot covering the leaf petiole up to the stem; 2 – spots up to 5 cm long; 3 – spots covering the next internodule; 4 – stem breaking at the place of inoculation.

A high positive correlation (r = 0.86; P = 0.033) was established between the results obtained from the application of the straw test and the natural infection. The results obtained allow as to recommend the straw method for preliminary testing of sunflower germplasm for resistance to *Ph. helianthi.*

Key words: Diaporthe/Phomopsis helianthi, sunflower, method for inoculation

Introduction

Grey spots caused by *Diaporthe/Phomopsis helianthi* Munt. Cvet. et al. are a serious problem for sunflower production in a number of countries worldwide (Scoric, 1994; Gulya, 1997; Carre, 1993), including Bulgaria (Encheva and Shindrova, 1990). The disease has epiphytotic outbreaks in years with hot and moist weather during sunflower vegetation. Breeding of resistant cultivars and hybrids is the most reliable method for disease control. The success of any breeding program for resistance is related to the use of a fast and easy to apply method for inoculation providing higher significance of the results obtained. Presently, there are several methods described in literature for inoculation of sunflower with *Ph. helianthi*. Two French authors, Peres and Regnault (1986) have suggested spreading of infected plant residues in field at the beginning of vegetation accompanied by regular spraying of sunflower during the susceptible stage. In their opinion this method gives good results, especially in regions with occurrence of high percent of natural infection.

Tourvieille et al. (1988) have suggested a method in which small agar disc with developed mycelium of the fungus are placed on the top of the leaf blade and are covered with wet cotton tampon and aluminum foil for longer preservation of moisture. The same authors have developed a method in which

agar disc with developed mycelium of the fungus is placed on a preliminary cut leaf petiole. The inoculum is then covered with wet cotton tampon and aluminum foil.

Recently, Petzoldt and Dickson (1996) have developed a method for testing the physiological resistance of beans (*Phaseolus vulgaris* L.) to white mold (*Sclerotinia sclerotiorum*) known as the straw method. The results from the application of this method correlate to a large extend with those from the natural outbreak of the disease (Hall and Phillips, 1998; Madariga and Hall, 2000).

The present study aimed to investigate the possibilities for the use of the straw method for testing sunflower response to *Ph. helianthi*.

Material and Method

The researches were carried out at DAI-General Toshevo during 2000-2001 and included the hybrids Diamant, Albena, Santafe and NS-H-712, each with different degree of response to the pathogen. The hybrids were sown in two replications, each replication including 25 plants.

Natural infection (Peres and Regnault, 1986). At the end of September the plant residues (stems) showing symptoms of grey spots were collected from field. At stage 5–6th leaf of sunflower 3–4 stems per m^2 were scattered between the rows. Each week the plants were sprinkled from the beginning of buttoning till mass flowering. The number of sprinklings was determined by the meteorological conditions and varied from 2 to 3 per week. The reaction of the hybrids was registered at full flowering according to the following scale: 0 – no symptoms; 1 – small single spot around the leaf petiole; 2 – spots up to 5 cm long; 3 – spots covering two or more internodules; 4 – stem breaking at the place of damage.

Straw test. A modified method of Petzoldt and Dickson (1996) was applied. Plants were inoculated at buttoning stage by cutting the leaf petiole of a leaf from the middle of the plant by a scalpel 3 cm from the stem. A plastic straw containing an agar disc of 7-day old culture of DAI isolate was inserted into the core of the leaf petiole (Figure 1). The *Ph. helianthi* isolate used for inoculation was isolated in June the previous year from infected sunflower crops in the region of DAI. The culture was grown on PDA nutrition medium at 23 °C in dark. To prepare the inoculum plastic drink straws were used with one end closed by heating, their size being 30 x 6 mm. A wet sterile cotton tampon was inserted inside the straw. With the straws thus prepared agar discs from fungus culture were cut, taking care to keep the disc at the very end of the straw. The inoculum was prepared under laboratory conditions 24 hours before inoculation and kept at 4 °C until the moment of use. The reaction of the hybrids was read 10 days after inoculation according to the following five-degree scale: 0 – no symptoms or traces of pathogen invasion; 1 – spot covering the leaf petiole up to the stem; 2 – spots up to 5 cm long; 3 – spots covering the next internodule; 4 – stem breaking at the place of inoculation.

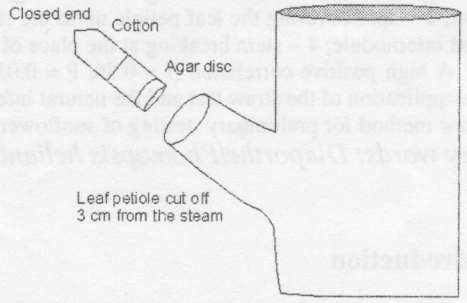

Fig. 1. Straw test procedure including cutting off the leaf petiole 3 cm from the steam and sticking of the straw containing agar disc and wet cotton into the place of cutting

Processing of data: To determine the correlation between the results obtained by the two methods of inoculation, the software Statistics for Windows 95 was used.

Results

Among the studied hybrids averaged for two years hybrid Diamant showed susceptibility to natural infection (Figures 2 and 3). Moderate susceptibility was demonstrated by

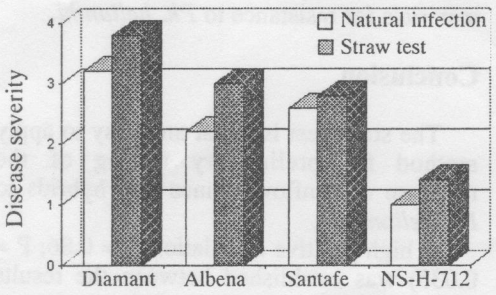

Fig. 2. Reaction of four sunflower hybrids to *Ph. helianthi* in natural infection and inoculation by the straw method average for the period 2000-2001

Fig. 3. Symptoms of *Ph. helianthi* at natural infection

hybrids Albena and Santafe, and hybrid NS-H-712 was resistant.

After inoculating the hybrids by the straw method, the first symptoms of phomopsis were observed 5–6 days after inoculation (Figure 4). The maximum development of symptoms in this method was observed 10 days after inoculation. Among the investigated hybrids, hybrid Diamant showed susceptibility to isolate DAI, and hybrids Albena and Santafe were moderately susceptible. Hybrid NS-H-712 demonstrated moderately resistant reaction.

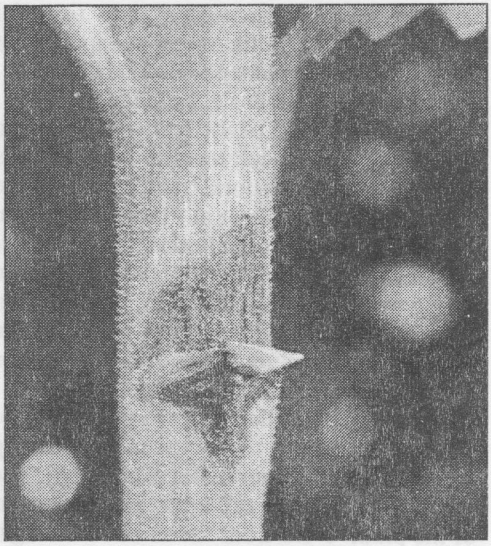

Fig. 4. Symptoms of *Ph. helianthi* after using the straw method

The spots on the host after inoculation were of the same size, which allowed to evaluate the materials as resistant at degrees 0 and 1 from the scale. The materials evaluated with 1.1 to 2 were considered moderately resistant, and those with 2.1 to 3 – moderately susceptible. The breeding materials evaluated from 3.1 to 4 were considered susceptible.

A positive correlation ($r = 0.86$; $P = 0.033$) was established between the results obtained from natural infection and from the straw test.

Discussion

The use of a fast and easy to apply method for inoculation is of primary importance in breeding for resistance of sunflower cultivars and hybrids. Spreading of infected plant residues at the beginning of the vegetation period is considered one of the most suitable methods imitating the natural occurrence of the disease (Peres and Regnault, 1986). However, this method has a number of disadvantages related mainly with the effect of meteorological conditions on the infection process. The regular sprinkling of the crop increases to a large extend the efficiency of this method, but it

requires much organizational and financial efforts. Therefore in the recent years different methods were developed for artificial inoculation of sunflower with *Ph. helianthi*.

The inoculation of the leaf with agar disc containing fungus culture approximates to a large degree the natural realization of the infection (Tourvieille et al., 1988). The main disadvantage of this method is that it is difficult to carry out. Furthermore, when there are strong winds during infection, which often happens in the region of South Dobroudja, a part of the inoculum drops from the leaves and a second inoculation is needed.

Inoculation of the leaf petioles is one of the most commonly used techniques during the recent years (Tourvieille et al.,1988). The leaf petioles are cut off from the middle part of the plant 3 cm from the stem and an agar disc from the fungus is placed on the place of cutting, which is covered by wet cotton and aluminum foil. A shortcoming of the method is that it takes much efforts concerning both the inoculation itself and the preparation of the inoculum. This imposed the working out of a faster and easier to apply method. The method we suggest is similar to that of leaf petiole infection, but in it the preparation of the inoculum is carried out in advance under laboratory conditions, and the inoculation itself is done fast and easy in the field. The preliminary preparation of the inoculum allows to infect a large number of breeding materials for a short time. Our observations showed that one worker can inoculate 500 lines for 3–4 hours. Besides that, this method allows to inoculate the same plant with 2 or more isolates of the fungus. The established positive correlation ($r = 0.86$; $P = 0.033$) between the results from the application of the straw test and the natural infection allowed us to think that this method can be used successfully for testing of the breeding materials for resistance to *Ph. helianthi*.

Conclusion

The straw test is a fast and easy to apply method for preliminary testing of the response of sunflower lines and hybrids to *Ph. helianthi*.

A high positive correlation ($r = 0.86$; $P = 0.033$) was established between the results obtained after application of the straw test and the natural infection.

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