Evaluation of resistance of winter oilseed rape seedlings to Phoma lingam (Tode ex Fr. Desm) using mycelium test

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The dry rot and stem canker of cruciferous plants (Leptosphaeria maculans (Desm), conidial stage: Phoma lingam (Tode ex Fr. Desm) is one of the most severe diseases of winter oilseed rape (Brassica napus). The main criterion of pathogenity of Phoma lingam is aggressitivity of chosen pathotypes (E. Koch, H Badawy 1989)

Pathogenity of dry rot disease to cruciferous was described by many authors (Delwiche, Williams 1979; Badaway H.M.A., Hoppe 1989; Barbetti 1975 i1976; Bonman J.M. Delwiche, Gabrielson 1984; Hammond-Kim, Lewis B.G. 1987; Hanacziwskyi B., Drysdalale R.B. 1984; H.M. Curtis, Xu Xiao Hua, Willtam, 1984).

The targed of research conducted in Plant Breeding and Acclimatization Institute (IHAR) in Poznań, is to find the best conditions for the performing of mycelium test on the most effective way. Elaborated method allowes to select seedlings more tolerant to Phoma lingam from a large number (ca. 40 000) of seedlings of winter rape (plant growth stage "A").

Material and methods

Seeds of double low materials of winter rape bred by IHAR were taken for testing. There were breeding lines and four Polish varieties Mar, Polo, Leo and BOH 1491. Six aggressive pathotypes of Phoma lingam from IHAR collection were used for inoculation (Starzycki, Starzycka 1992).

The mycelium test

The test was described by Starzycki and Brun (1991). Some modification were introduced to this method what allowed to increase the efficiency of selection for more resistant plants in stage "A".

Following nutrient media were used for this test:
-agar medium B5 - for seeds germination
-agar medium V8- for growth of pathogen mycelium.

Medium B5 prepared according to Gamborg (1968) contained only micro- and macro- nutriens (iron in chelat form) solidified by 1.1 percent of agar (Agar Agar SERVA high gel-strength). Medium V8 had included 80 ml of tomato juice per 1 l of distilled water and 1.5 percent of agar. The media were sterilized in micro-wave oven for 10 minutes. The media B5 and V8 were poured into 90 sterile Petri dishes for each medium.

Seeds sterilization

The seeds were sieved to obtain fraction of equal size. Chosen seeds were sterilized in 96% ethanol for 3 minutes. Next they were washed three times in distilled water and dried on absorbent-paper. In each Petri dish with B5 medium were cut out 50 holes. The seeds were placed in the holes. After 4 days seedlings achieved stage "A".

Preparation of pathotypes of Phoma lingam

Six aggressive pathotypes chosen from collection of Phoma lingam phathotypes were proliferated on V8 medium. After a few days, when hyphae of fungus had grown the medium in, the stripes of about 5 cm^2 were cut out from the medium. The stripes were homogenized and mixed. So prepared inoculum was spread on the surface of V8 medium in Petri dishes. After 4 days medium V8 on test dishes was grown up by mycelium.

Seedling inoculation

B5 medium with seedlings was taken from Petri dishes and put down on fourth-day old culture of P.lingam. After 10 days estimation of resistance of plants has been made. The less injured seedlings were cut off and placed in hydroponic culture for secondary rooting.

Hydroponic Test With Mycotoxin of Phoma lingam

Agar disc with fifth day old aggressive phathotype of Phoma lingam was put in 1l bulb with liquid V8 medium. The culture was conducted for 30 days in room conditions. The bulb was shaken 1 hour a day. After the incubation period, when toxins stopped growth of mycelium, limpid liquid free of hyphae was taken by sterile syringe and poured on Petri dishes. Two kinds of hypocotyls selected by mycelium test were placed in toxin solution. The one set was taken from resistant seedlings, the second set from susceptible to Phoma lingam. The growth of the plants was observed after 7 days. The hypocotyls of resistant plants grown normaly but hypocotyls of susceptible plants and their seed leaves were decolored and decayed.

To confirm obtained result also Williams test was made on plants selected by mycelium test.

Significant differences were obserwed in the process of infection among individual aggresive pathotype and mixture of them. The mixture made much stronger attack of disease (table no 1). Hydroponic test with toxins and Williams test confirmed higher resistance of plants selected by mycelium test in development stage "A" (tables 2 and tables 3).

Results

Table 1.

Comparison of number of resistant plants in three winter rape varieties after inoculation by aggressive pathotype "17" and by mixture of six aggressive pathotypes of Phoma lingam (Tode ex Fr. Desm) mycelium test.

Variety	LEO		POLO		MAR	
	a	b	a	b	a	b
Number of tested plants	1500	1500	1500	1500	1500	1500
Number of resistant plants	24	74	33	91	41	157

a - mixture of pathotypes
b - pathotype no. "17"

Table 2.

Results of hydroponic test with mycotoxins – confirmation of resistance to Phoma lingam of plants selected by mycelium test.

	Resistant plants	Susceptible plants
Number of tested plants	15	30
Number of resistant plants	15	0
Number of susceptible plants	0	30

Table 3.

Results of Williams test on the set of plants selected by mycelium test.

Resistant plants		Susceptible plants	
Plant	Degree of Phoma attack*	Plant	Degree of Phoma attack*
1	3333	1	4455
2	3333	2	6465
3	3343	3	6666
4	3232	4	6677
5	4423	5	9999
6	3344	6	4464
7	5543	7	6646
8	2352	8	7777
9	43	9	6644
10	2222	10	2344
11	2222	11	7743
12	44	12	7722
13	4444	13	3353
14	5533	14	5544
15	3322	15	2233

* scale 1–9 ; 1 - healthy plant; 9 -plant attacked very strong

Conclusions

- Presented method can be used to test for resistance to Phoma lingam a large number of winter rape plants in stage "A"(seedlings).
- This test due to direct contact and interaction beetween plant and pathogen allows to avoid uncontrolled influence of biothic agents.
- The test is conformable to Williams test and hydroponic test with mycotoxins

- The mixture of six pathotypes causes much stronger infection of plants than single pathotype.

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