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RESPONSE TO BLACK ROOT ROT (*THIELAVIOPSIS TABACINA* FERR.) OF SEVERAL FLU–CURED TOBACCO (*NICOTIANA TABACUM* L.) GENOTYPES IN DIFFERENT TESTING ENVIRONMENTS

ABSTRACT

Several breeding lines and tobacco varieties were tested for degree of resistance to black root root caused by the fungus *Thielaviopsis basicola*. The entries in the study represented an array of forms from fully resistant to very susceptible. Artificial black root rot testing included „chlamydospore test" performed at cotyledonary stage and testing at post–transplant stage by either planting 5–leaf plants in black–root rot inoculated soil or immersing their roots in inoculum suspension prior to transplanting. The results were compared with those from a naturally infested field with two levels of the disease. The most inconsistent results were obtained from the chlamydospore test which failed to differentiate between different levels of tolerance of black root rot. It could only be used to make distinction between fully resistant and the remaining entries. Testing at the post–transplant stage showed, with one exception a rather good fit with the results from the field study, especially when transplants were transferred to pre–inoculated soil. Although the general order of response to black root under field conditions was similar regardless of disease severity, some varieties were more sensitive than others to an increased level of black root rot in the soil.

INTRODUCTION

Black root rot caused by the fungus *Thielaviopsis basicola* (*Chalara elegans*) is one of the principal diseases of tobacco in Poland. Both cli–matic conditions of what is probably the northern–most country pro–ducing tobacco and generally a small size of tobacco farms that prevents the use of long or any crop rotations add to the seriousness of the problem. The situation is particularly acute with flue–cured tobacco. An intensive breeding effort is required to develop a flue–cured cultivar/s with improved resistance to black root rot. In order to make the work faster and more effective it is essential that by screening in an artificial environment (greenhouse, laboratory) a response could be selected that would perform correspondingly well under natural conditions. This re–port compares the results of testing for resistance to black root of 8 se–

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lected cultivars and breeding lines performed under natural field infestation with the results from two artificial inoculation–based tests: a chlamydospore test done on newly–germinated seedlings and a test performed on pot–growing plants at an early post–transplant stage.

MATERIAL AND METHODS

Material

Eight flue–cured tobacco (*Nicotiana tabacum* L.) cultivars and breeding lines were included in the tests:

1. Virginia SCR (VD) – of German origin, previously widely grown
2. TB–570N – breeding line developed at IUNG
3. Wilia – registered cultivar developed at IUNG, Puławy
4. Wiślica – flue–cured standard, developed at Skroniów Tobacco Breeding Station
5. Hevesi 9 –male sterile variety from Hungary
6. PTU – 1098 – breeding line developed at IUNG, Puławy
7. VJ 1 – male sterile variety from Hungary
8. VJ 17 – male sterile variety from Hungary

Field test for black root rot susceptibility

Field tests for black root rot resistance were performed on a farmer's field in a major tobacco growing area. The field was taken out of tobacco production and part of it was set aside for black root rot testing. The plants were grown under regular agronomic regime for flue–cured tobacco in that area. Eight entries were included in a randomized block design with four replications. A clear difference in black root rot intensity across the experiment field offered an additional opportunity to analyse the results separately for the two blocks (2 reps) with an apparently lower intensity of the disease and for the other two with a higher black root rot level.

Disease severity assessment included measurements of plant height and determinations of root weight. For root weight determinations 5 plants were pulled out at full flowering. Prior to weighing, roots were washed and dried. The mean weight of 5 root systems was taken as a plot value for statistical computation. The mean height of 10 plants on each plot, 1st row, plants 3–12, was used as a plot value in statistical analysis.

Test for black root rot susceptibility in pot culture

Seeds of the entries were sown in polystyrene trays (640 cells × m^{-2}) filled with commercial peat (Berbeć – submitted for publication). The trays were overhead–irrigated with a solution of commercial liquid fertilizer diluted to 100 ppm N. At the 5th leaf stage (transplant stage) two methods of inoculation with *Th. basicola* spores were applied:

1. roots of the transplants were immersed in a suspension (1000000 spores × ml^{-1}) of *Th. basicola* chlamydospores and endoconidia and transferred to pots (1 plant per pot) filled with sand.
2. transplants were transferred to pots filled with sand to which *Th. basicola* spores were added at ca. 20.000 per 1g sand.

Pot cultures were maintained in the greenhouse. Fertilization was provided in the form of Olsen nutrient solution. After 4 weeks of growth the plants were removed from pots, their roots washed with water and evaluated for black root rot symptoms. The symptoms were rated on a 11–point scale (score 0 – no disease symptoms; score 10 – total destruction of the root system). After visual evaluation the roots were dried and weighed.

Test for black root rot susceptibility at the cotyledon stage (chlamydospore test)

The test generally followed the procedure as described by Corbaz (1971). Surface sterilized seeds were germinated in on blotting paper in Petri dishes at 20°C. Each entry was sown in 3 Petri dishes. After 10 days suspension of *Th. basicola* endoconidia at the strength of 150.000 per 1 ml obtained from 6 day–old colonies of the fungus was added to the dishes (2 ml per dish). The counts of chlamydospores on the roots of the seedlings were made following 7 and 10 days of incubation at 18°C. The observations were made on 20 seedlings in each Petri dish and the mean results from 60 observations (20 seedlings × 3 petri dishes) were given in the results section.

The results from the greenhouse test and the chlamydospore test based resistance evaluations were graphically compared with the results from the field by plotting on the respective graphs a line representing "relative values from the field". These values, based on mean root weights from the field trial, were scaled down or up by an appropriate factor to match the scale of the y axis of a graph. In order to compare the root weights (ascending as measure of resistance) with visual score–based appraisals (descending as measure of resistance) the inversions of the former were used for comparison.

RESULTS

Response of cultivars and breeding lines to natural field infestation with black root rot

The average plant height of the varieties included in the replicated trial was from 40 cm (susceptible check Virginia VSCR to ca. 150 cm (VJ 17), (Fig. 1a). The latter variety showed the best average growth in the black root rot infested environment. Its height was little affected by level of black root rot infestation (Fig. 1 b, c). The plants of the second and the third best entry (PTU and VJ 1) were not much smaller than VJ 17 at the lower black root rot level but the difference in plant height was

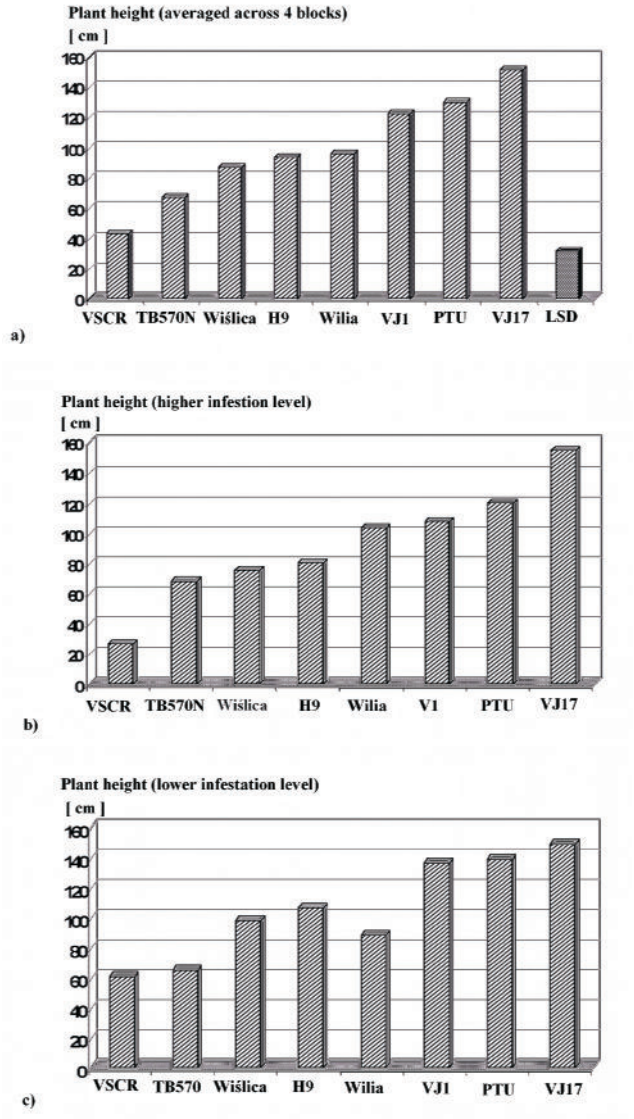

Fig. 1. Plant height of eight flue–cured tobacco varieties grown on a field naturally infested with black root rot: (a) averaged across all four blocks of the experiment, (b) averaged across two blocks with lower infestation, (c) averaged across two blocks with higher infestation

quite conspicuous on the part of the field more heavily infested. With regard to response with plant height to black root rot four groups could be distinguished: very resistant – VJ 17, moderately resistant – VJ 1 and PTU, moderately susceptible – Wilia, H9, Wiślica, susceptible – TB–570N. On this relative scale the susceptible check Virginia SCR could be classified as very susceptible.

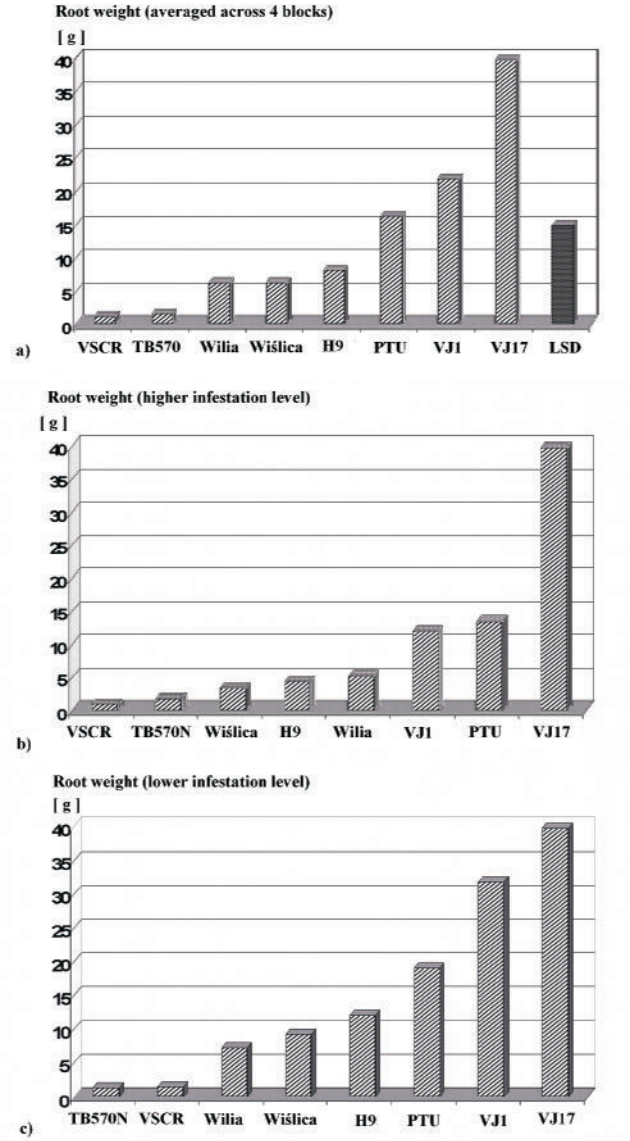

Fig. 2. Root weight of eight flue–cured tobacco varieties grown on a field naturally infested with black root rot: (a) averaged across all four blocks of the experiment, (b) averaged across two blocks with lower infestation, (c) averaged across two blocks with higher infestation

Differences in root weight were even more pronounced than those in plant height and they followed a similar pattern of response to black root rot (Fig 2). Compared to the other entries, by far the heaviest root system was grown by VJ 17, especially on the two blocks with higher infestation. Root weight of VJ 17 was practically unaffected by black root rot level (Fig 2a and 2b) but that of the second most resistant entry (VJ 1)

was, and to a very high degree. At lower black root rot infestation the root weight of VJ1 was more than double compared to that at higher black root rot level.

Based on root weight response, the division into resistance groups of the varieties studied was roughly similar to that based on plant height. VJ 17 stood alone on the two more infested blocks with root weight three times as high as that of the next in turn PTU (Fig. 2a). VJ and PTU were moderately resistant and had similar root weights at high infestation level but VJ 1 seemed to tolerate a lower black root rot level better. Wiślica, H9 and Wilia made up the susceptible group whereas there was no difference between Virginia SCR and TB–570N, both showing simi–lar highly reduced root weight regardless of black root rot level.

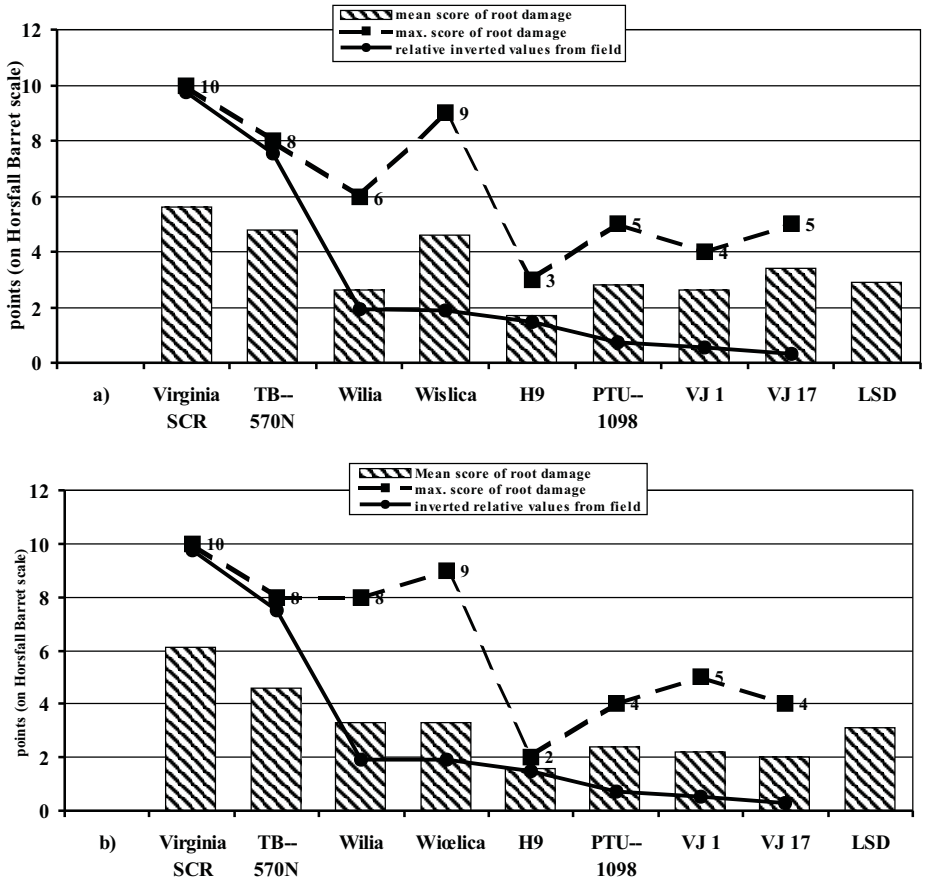

Fig 3. Mean and maximum scores of root damage by *Thielaviopsis basicola* in pot culture of 8 to–bacco varieties: a) direct inoculation of roots, b) inoculation of the growth medium

Response to artificial infection with black root rot spores in pot culture

Based on the visual score of black root rot symptoms following inoculation of the roots of the transplants prior to transferring them to pots the highest damage was found in VSCR, TB–570N and Wiślica, the lowest in H9, the response of other varieties being intermediate and similar (Fig. 3a). The correspondence with the results from the field, as visualized by a line representing inverted relative values of average root weight (comp. Fig. 2a) was very poor. It was slightly better when maximum rather than mean scores were considered.

The fit with the results from the naturally infested field was much better when the transplants were transferred into pots containing *Th. basicola*–inoculated sand (Fig. 3b). The response generally followed the pattern observed in the field, except H9 which again showed the least damage. Unlike in the treatment involving inoculation of the roots, the average score of root damage provided a better estimation of performance under field conditions than the maximum scores for individual plants.

Response with root weight to black root rot infection either through direct inoculation of the roots or through inoculation of the growth medium compared with analogous data from the field test is shown in Fig. 4. As with visual scores, inoculation of the growh medium gave a better fit with the results from the field compared to direct inoculation of the roots. The most outstanding deviation were the results from TB–570N, which was the second worst performer in the field but rated as fourth best in this test, practically on a pair with VJ 1 and PTU–1098, considerably black root rot tolerant entries in the field test.

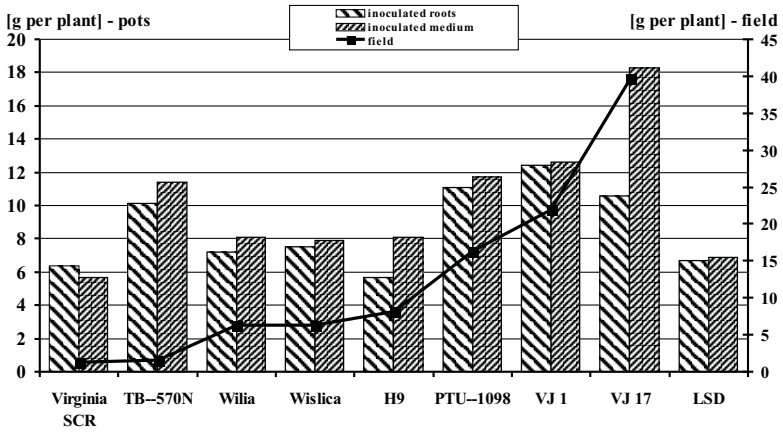

Fig. 4. Root weight in pot culture of 8 tobacco varieties after 27 days of exposure to *Thielaviopsis basicola* by either direct inoculation of roots or inoculation of the growth medium (compared with results from naturally infested field culture)

Generally, the pot test gave a pretty good estimation of high resistance in the field but failed to differentiate between different levels of suscep–tibility.

Chlamydospore test – inoculation of seedlings in Petri dishes

After 7 days of incubation with Th. basicola the highest counts of chlamydospores were obtained from Virginia SCR and the lowest from VJ 17 which corresponded well with data on black root rot susceptibility obtained from the field (Fig. 5a). Relatively low chlamydospore counts

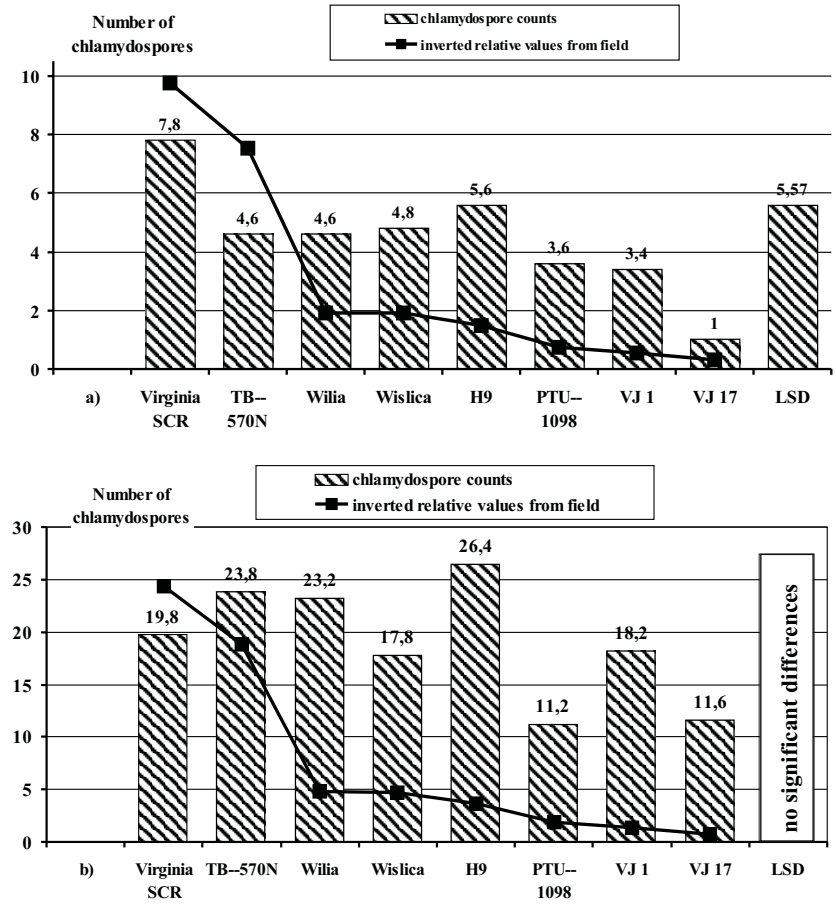

Fig. 5. Chlamydospore counts on the roots of seedlings of 8 tobacco varieties after incubation for 7 (a) and 10 days (b) with spores of *Th.basicola*

were obtained from PTU–1098 and VJ1, two entries in the study which also showed relatively good tolerance of PVY in the field. The results for the remaining entries were similar and showed little correspondence with black root rot resistance data from the field. The counts obtained after 10 days of incubation (Fig. 5b) showed an erratic pattern and little fit with the data from the field.

DISCUSSION

Evaluation of resistance to black root rot at post–transplant stage is commonly done either through direct inoculation of the roots (Miki and Katsuya 1996) or through the inoculation of the medium (Samek and Jankowski 1987, 1988; Wilkinson *et al*. 1992).

Results obtained in this study indicate that the greenhouse testing of resistance to black root rot provides consistently reliable assessment of the genotype's capacity to resist the disease under natural field conditions only when one of the two extreme responses – high susceptibility or high resistance – is involved. With intermediate types of response the artificial inoculation–based estimations became more difficult. A better assessment of the field performance against black root rot was obtained when the growth medium rather than the roots were inoculated. Samek and Jankowski (1987, 1988) also used inoculation of the medium at the same rate as used in this study (20.000 propagules/1g soil) and reported satisfactory estimation of what they called "field tolerance" of the disease. Likewise Wilkinson *et al*. 1995) found the greenhouse test to give good evaluation of differences in genetic resistance to black root rot in burley tobacco genotypes but the investigators used much lower rates of medium inoculation (100 chlamydospores per 1 g soil mixture). In this study the order of the intensity of black root rot symptoms in the greenhouse roughly followed that observed in the field, especially for entries that showed moderate resistance in the field. It became more erratic for entries that based on field results could be classified as moderately susceptible. The results of chlamydospore test carried out as part of this study showed practically no consistency with the results from the field. Tosi and Zazzerini (1991) also found differences between the chlamydospore test and the greenhouse test regarding the assessment of resistance to black root rot of particular genotypes although they did not question the usability of the test. The problem was further complicated by the fact that the order of response to black root rot as observed in the field varied in some cases with disease intensity i. e. some entries were more sensitive to high level of black root rot infestation than others. Another factor that may have influenced the results of this comparison is that the Th. basicola isolate used for the inoculation came from the experiment plots of the institute situated about 150 km away from the field experiment site so there could be different strains involved. Different genotypes may vary in their response to different strains of the black root rot pathogen (Miki and Katsuya 1996, Ohashi and Imoto 1983).

CONCLUSIONS

1. Under natural field infection with black root rot the response of 8 cultivars of flue–cured tobacco ranged from very susceptible

(VSCR, TB–570N) to resistant (V17), the remaining entries showing different degrees of intermediate response
2. Some varieties were more sensitive than others to an increased level of black root rot in the soil
3. Under artificial inoculation in pot culture the pattern of response was fully consistent with field results only for the very susceptible vs. resistant entries. Inoculation of the sand gave better fit with the results from the field than direct inoculation of roots.
4. The results of "chlamydospore test" showed good consistency with field results for the most resistant and most susceptible entries only when chlamydospore counts were made after 7 days of incubation.

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