# IDENTIFICATION OF SOME CULTIVARS OF *BRASSICA NAPUS* WITH RESISTANCE AT *VERTICILLIUM LONGISPORUM*

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***Abstract:*** *Verticillium longisporum is a soil born vascular fungal pathogen on Brassicas oil crops (Heale et al. 1999). Due of the high demand for rapeseed oil, the area under oilseed rape cultivation continues to rise dramatically (Eynck et al 2008). The current European cultivar poses a low level of resistance for this disease, so a severe infection can produce major yield losses. The fungus is able to survive in the soil for several years through the production of microsclerotia and because until now there are no approved fungicide available for V. longisporum resistant cultivars of oilseed rape are required. Resistance to Verticillium spp. is known from several other crops like tomato (Diwan et al. 1999), potato (Jansky et al. 2004), cotton (Bolek et al. 2005), and strawberry (Lynch et al. 1997). At the present, there are no resistant oilseed rape cultivars available for this disease, so the identification of some new resistance sources is very important for further studies in oilseed rape breeding process. The aim of this study was to identify some oilseed rape cultivars with resistance to Verticillium longisporum. For this purpose we tested a number of 39 cultivars of oilseed rape cultivars for their resistance to Verticillium longisporum by artificial infection. The experiments were conducted in the climatic chamber under controlled condition. Ten-days seedlings were artificial infected with the pathogen by root deep inoculation. The winter oilseed rape varieties Express (less susceptible) and Falcon (highly susceptible) were used as reference controls in our experiment. Disease scores were taken weekly for each plant for a four week period using an assessment key with nine classes. Based on this data for each accession was calculated area under disease progress curve (AUDPC). Analyzing the obtained data, we identified a number of 7 cultivars with a high level of resistance which could be some new resistance sources that could be used in the future for in the breeding programs for obtaining some resistant oilseed rape cultivars to Verticillium longisporum.*

***Key words:*** *resistance, Verticillium longisporum, Brassica napus, AUDPC*

## INTRODUCTION

One of the most important diseases of oilseed rape (*Brassica napus*) from Europe is consider being verticillium wilt, caused by the fungal pathogen *Verticillium longisporum*. The disease is present in Germany and Sweden, but has been also reported in some other countries such Poland, France, Russia and Ukraine. The funguses infect the oilseed rape plants through the roots and colonize the xylem vessels.

The objective of this work was to evaluate a number of 39 cultivars of oilseed rapes for resistance to *Verticillium longisporum* under controlled condition in order to identify the most resistance cultivars which will be used in further studies in breeding programs.

## MATERIAL AND METHODS

The artificial infection was performed with the *V longisporum* isolate VL 43 which was provided by division of Plant Pathology and Plant Protection, Gottingen, Germany. For long-term storage we prepared a conidial suspension in PDB (potato dextrose broth) supplemented with 25% glycerol and stored at -80$^0$C. The inoculum suspension was made by adding 500 μl spore stock solution in 250 ml PDB medium. The cultures were subsequently incubated 7 days at $23^0$C on a rotary shaker. After 7 days the culture was filtered and using a haematocytometer we determined the spore concentration. For the inoculation we diluted the solution at $1x10^6$ spore $mL^{-1}$.

A number of 39 winter oilseed rape cultivars selected from collection of accessions from Center of Genetically Resources Netherland were tested for the *Verticillium longisporum* resistance by artificial inoculation. The winter oilseed rape varieties Express (less susceptible) and Falcon (highly susceptible) were used as reference control in our experiments. The seeds were two times surface sterilized by immersion in 70% ethanol for 2 minutes. After the sterilization, the seeds were washed with tap water and then sowed in silica sand. After 10 days, the roots of the plans were carefully washed from the sand. Inoculation was performed by cutting 2 cm from the roots and holds them for 30 min in the spore suspension. Plants from the controls were also cut and hold 30 min in tap water. For each cultivar we used 10 plants inoculated and 10 controls. Plantlets were transferred after inoculation in pots into a mixture of sand, peat and compost (1: 1:2) and grown in a climatic chamber at 230C with a light/dark cycle of 1/10. Every week we take the disease scores using an assessment key with nine classes as described by EYNCK et al 2007 (table 1). Because the disease produced by *Verticillium longisporum* reduces the plant growth, the plant height was measured at 28 days after inoculation.

For each accession, area under disease progress curve (AUDPC) was calculated from the disease severity values.

*Table 1*

Assessment key for scoring disease severity

| Score | Symptom development |
|---|---|
| 1 | No symptoms |
| 2 | Slight symptoms on the oldest leaves (yellowing, black veins) |
| 3 | Slight symptoms on the next younger leaves |
| 4 | About 50% of the leaves show symptoms |
| 5 | More than 50% of the leaves show symptoms |
| 6 | Up to 50%of the leaves are dead |
| 7 | More than 50% of the leaves are dead |
| 8 | Only apical meristem is still alive |
| 9 | The plant is dead |

**RESULTS AND DISCUSSIONS**

After the artificial infection with *Verticillium longisporum*, typical symptoms like asymmetric yellowing of leaves and early stunting was observed. Plants of the control variant were also scored in order take into account the unspecific symptoms occurring during the natural ageing process which varied between the cultivars. A large variation of resistance to *V.longisporum* was observed among the tested cultivars. Resistance responses of the tested cultivars along with the oilseed rape controls “Express” and “Falcon” measured by AUDPC values are shown in figure1.

We identify 7 cultivars with a high level of resistance, with the AUDPC values smaller than the cultivar Express. The most resistant cultivar to the disease proved to be the cultivar “RBN 03”, with the smallest value of the AUDPC of 0.10. The next six cultivars showed AUDPC values between 0.34 - 0.79 and have a high level of resistance to *Verticillium* *longisporum* and are proved to be some promising sources of resistance in our further studies.

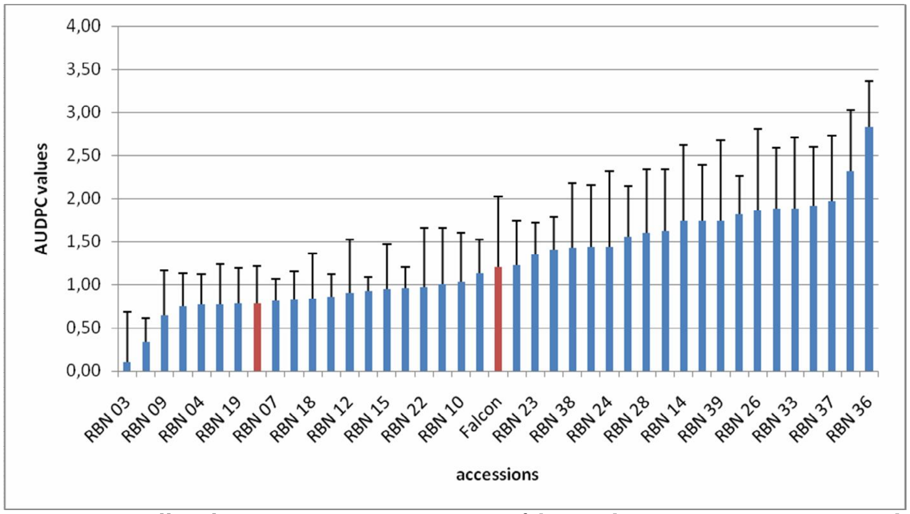


Figure 1: *Verticillium longisporum* resistance response of the tested *Brassica napus* accessions and reference cultivars Express and Falcon (red) measured by area under disease curve (AUDPC). Columns and whiskers present mean values and standard errors from 10 infected plants of each accession

A number of 12 cultivars showed less resistance level than the cultivar Express and better resistance level than the cultivar Falcon. From the total of the 40 tested accessions 50% proved to be more sensible than the cultivar Falcon so these cultivars will be excluded from our further studies. The most susceptible cultivar to *V. longisporum* proved to be "RBN 36" with the great AUDPC value.

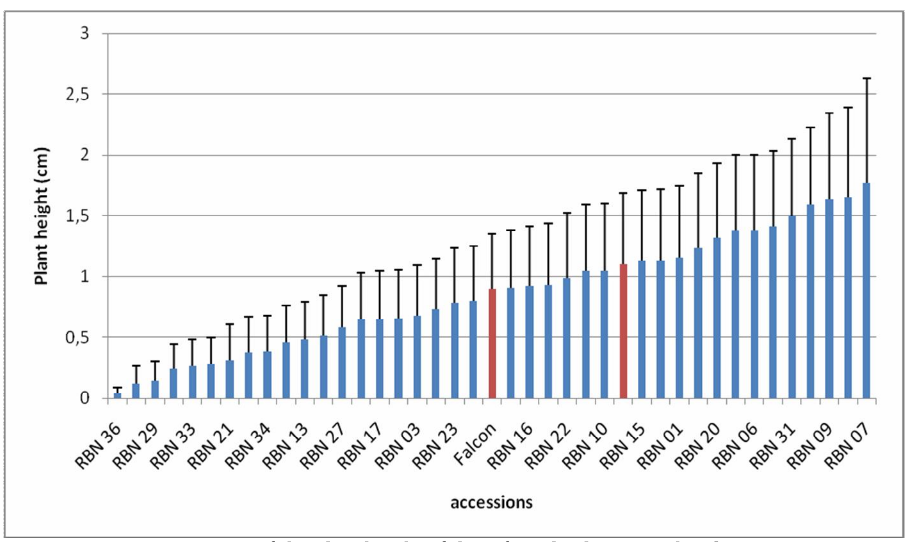


Figure 2. Evoution of the plant height of the infected cultivars with *V.longisporum*

During our experiments it can also be observed (figure2) that *V. longisporum* influenced the plant growth. At the infected cultivars the, plant growth was reduced compared with the control plants.

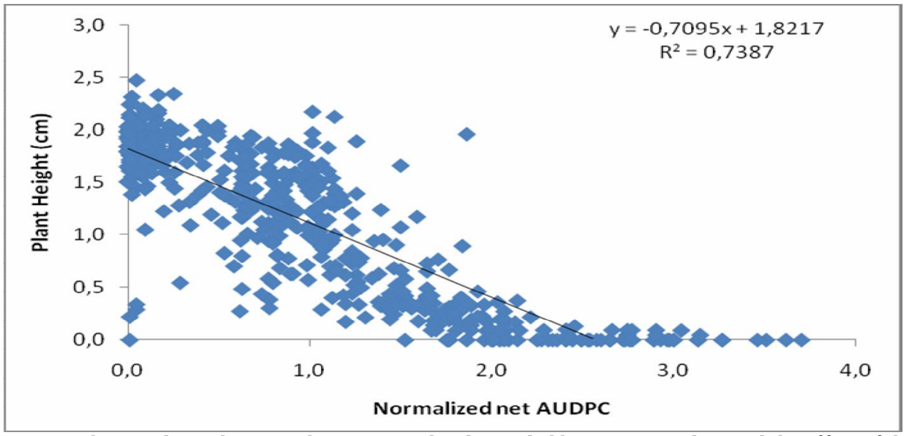


Figure 2. The correlation between the resistance level revealed by AUDPC values and the effect of the infection on the plant height

It can also be observed (figure2) a positive correlation between the resistance levels assessed by calculation of AUDPC values from symptoms scores and the effect of the infection on the plant height. As it can be observed if the infection in high, the plant height is smaller.

## CONCLUSIONS

The increase of the areas cultivated with oilseed rape in Europe will cause also the spread of this pathogen this why resistant cultivars to this disease are required. For this purpose a first step is the screening of a large number of accessions for *Verticillium* resistance that could be used in the future for obtaining new resistant cultivars. Breeders are have made a great effort to identify sources of resistance o *V.longisporum* in the primary oilseed rape gene pool, but without so much success (HAPPSTADIUS et al, 2003).

Recently some promising genotypes of *B. oleracea and Brassca rapa* with enhanced resistance to *Verticillium* ssp. were identified (DIXELIUS et al., 2005; RYGULLA et al.;2007).

In the present study, we tested a number of 39 cultivars of *Brassica napus* to *Verticillium longisporum* in order to identify some new resistance sources that will be used in our further studies.

The cultivars tested in this study showed a differential variation in resistance to *V. longisporum.*

In this study, we identified 7 cultivars with a high level of resistance which we will use them in our further studies.

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