# Inheritance and Efficiency of Crown Rust Resistance in the Line *Pc 50-4* (*Avena sterilis* L.)

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**Abstract**: *Pc 50-4*, gene of resistance to oat crown rust, is a member of the set of genes transferred to cultivated *Avena sativa* L. from wild *Avena sterilis* L. In our study, a high level of the efficiency of *Pc 50-4* in the control of a number of pathotypes originating from different regions of Europe and Middle East, collected in 2000–2004, is demonstrated. Only 5 pathotypes from different geographical areas were virulent to *Pc 50-4* out of the total of 71 tested isolates. The efficiency of the gene *Pc 50-4* is 0.9296. This value is well comparable with the very high efficiency of the set of genes which are commonly used as differentials in the Research Institute of Crop Production in Prague as well as with genes successfully used in breeding resistance programmes in North America. Hybridological F2 and F3 analyses performed in field and glasshouse conditions show that in the *Pc 50-4* line the resistance to two recently identified pathotypes of oat crown rust is conditioned by one major gene as well.

**Keywords**: oat; major resistance genes; *Puccinia coronata* f.sp. *avenae*; pathotype

Oat crown rust, caused by the fungus *Puccinia coronata* Cda. f.sp. *avenae* Erikson, is the most widespread and harmful disease of cultivated oat in many regions of the world (Simons 1985; Harder & Haber 1992). The disease reduces both the yield and the quality of grain and forage (Šebesta 1971; Šebesta *et al.* 1972; Šebesta & Sýkora 1974) and increases lodging (Ohm & Shaner 1992).

Genetic resistance (Šebesta 1991) is the most economical and ecological measure of crown rust control (Harder & Haber 1992). Information about virulence genes in the pathogen race population, efficiency of resistance genes and their relationships have been used in resistance breeding programmes. The standard set of ten differentials (Simons & Murphy 1955) was widely used for oat crown rust research till the 1960's. Some of the differentials were also important donors of resistance in breeding programmes (Frey *et al.* 1977). However, by the end of the 1950's, none of the known sources of crown rust resistance present in cultivated oat could provide adequate protection against the disease (Martens & Dyck 1989). The pathogen was indicated to be highly variable in virulence and to be able to rapidly evolve new virulent pathotypes that overcome commonly used resistance genotypes (Chong & Kolmer 1993; Zhu & Kaeppler 2003).

Wild populations of *Avena sterilis* L. originating from North Africa, Mediterranean Region, and Middle East proved to be rich sources of crown rust resistance. A significant increase in the number of identified novel genes has been recorded within continuous research, and more than 90 crown rust resistance genes are now available (http://www.cdl.umn.edu/res_gene/res_gene.html). Many of these genes were important for resistance breeding in North America (Martens

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& Dyck 1989; McMullen & Patterson 1992; Chong 2000).

*Pc 50-4* is one of the identified resistance genes (Rajhathy *et al*. 1966). The isoline *Pc 50* (Pendek × CW 486-1) is a member of the set of nearly isogenic lines of cultivar Pendek into which crown rust resistance genes were transferred from *Avena sterilis* L. by backcrossing (Fleischmann & Baker 1971). Šebesta and Harder (1983) found out segregation in resistant and susceptible plants in the isoline *Pc 50* in relation to some crown rust isolates. Reselection of *Pc 50* into *Pc 50-2* and *Pc 50-4*, each possessing a different major gene, was carried out by Šebesta (1983). The reselected lines *Pc 50-2* and *Pc 50-4* showed to be homozygous for a crown rust reaction (Šebesta & Zwatz 1980).

The aim of this study was to clarify the current efficiency of the gene *Pc 50-4* using different isolates of oat crown rust obtained in 2000–2004, and to compare this value with the efficiency of oat differentials used in the Research Institute of Crop Production (RICP) in Prague. We also focused on the inheritance of *Pc 50-4* in $F_2$ and $F_3$ generation to two new rust isolates. The results are supposed to be the basic information for further molecular analyses of *Pc 50-4*.

## MATERIALS AND METHODS

### *Puccinia coronata* f.sp. *avenae*

Oat crown rust isolates collected in 2000–2004 were used to assess the efficiency of the line *Pc 50-4*. Crown rust pathotypes were obtained from trials and commercial fields in various countries of Europe and Middle East (Austria, Belarus, Czech Republic, Estonia, Hungary, Israel, Serbia and Monte Negro, Sweden). Isolates of these pathotypes are kept in the RICP collection.

### Assessment of *Pc 50-4* gene efficiency

Analyses were performed in the juvenile stage of the plant in glasshouse conditions. Oat crown rust isolates for testing were multiplied on a susceptible oat cultivar. By means of a glass cyclone separator, the first fully expanded seedling leaves of *Pc 50-4* line were inoculated with different urediospore/talc mixtures. After inoculation, the plants were put into glass cylinders to avoid any contamination, placed on the greenhouse bench and incubated at a temperature of 18°C and high humidity enabling the urediospore germination for 2 days. After incubation, the same greenhouse temperature was maintained and natural daylight was complemented by 16 h photosynthetically active radiation emitted by cool-white fluorescent tubes (Šebesta 1972). In 10–14 days after inoculation, the rust reactions were scored using the scale described by Šebesta (1991). Infection types from 0 to 2 indicated resistance while types 3 and 4 denoted susceptibility. The efficiency of resistance was calculated as the ratio of avirulent isolates out of the number of all tested isolates.

### Analysis of inheritance of *Pc 50-4* line to crown rust

The cross *Pc 50-4* × Atego was used for inheritance analysis. Cv. Atego was chosen due to its high susceptibility to all oat crown rust pathotypes tested so far. $F_2$ and $F_3$ generations were used for inheritance analyses in greenhouse and field conditions. The infection technique as well as temperature and light conditions in the greenhouse corresponded with the procedure mentioned above. Two pathotypes avirulent to *Pc 50-4* line and virulent to Atego were applied.

Field infection was realized by the application of water suspension with urediospores into the apical point of the plant (through incision) at the beginning of the stem extension stage. Susceptible oat cultivars were used as natural spreaders of crown rust infection.

The Chi square statistic was used to test the goodness of fit of expected segregation ratios.

## RESULTS AND DISCUSSION

### Assessment of gene *Pc 50-4* efficiency

In total 71 isolates of different oat crown rust pathotypes were used to define the current efficiency of the gene *Pc 50-4*. The gene reaction with infection types and the origin of pathotypes are presented in Table 1.

The reaction of *Pc 50-4* was evaluated as resistant to 66 isolates, only the "0," infection type was observed (green leaves without any flecks or pustules). A susceptible reaction (large pustules, rich sporulation) was recorded with 5 isolates only. These 5 crown rust isolates originated from different geographical areas, from the Czech Re-

Table 1. The efficiency of the gene *Pc 50-4* in the control of oat crown rust pathotypes from various countries of Europe and Middle East

| Oat crown rust isolate | Origin | Reaction | Infection type | Oat crown rust isolate | Origin | Reaction | Infection type |
|---|---|---|---|---|---|---|---|
| 3-00 | AUT | R | 0, | 21-00 | HU | R | 0, |
| 4-00 | AUT | R | 0, | 1-04 | HU | R | 0, |
| 5-00 | AUT | R | 0, | 45-00 | ISR | R | 0, |
| 5-00/3-2 | AUT | R | 0, | 46-00 | ISR | R | 0, |
| 5-00/3-4 | AUT | R | 0, | 47-00 | ISR | R | 0, |
| 10-00 | AUT | R | 0, | 25-04/1 | ISR | R | 0, |
| 11-00 | AUT | R | 0, | 25-04/2 | ISR | R | 0, |
| 15-00 | AUT | R | 0, | 27-04/1 | ISR | R | 0, |
| 16-00 | AUT | S | **4** | 28-04 | ISR | R | 0, |
| 29-00 | BLR | R | 0, | 29-04 | ISR | S | **4** |
| 39-00 | BLR | R | 0, | 59-99 | SWE | R | 0, |
| 41-00 | BLR | R | 0, | 74-99 | SWE | R | 0, |
| 1-02 | CZ | R | 0, | 101-99 | SWE | R | 0, |
| 2-02 | CZ | R | 0, | 102-99 | SWE | R | 0, |
| 3-02 | CZ | R | 0, | 102-99/1 | SWE | R | 0, |
| 4-02 | CZ | R | 0, | 23-00 | SWE | R | 0, |
| 5-02 | CZ | R | 0, | 35-00 | SWE | R | 0, |
| 7-02 | CZ | S | **4** | 20-00 | YU | R | 0, |
| 8-02 | CZ | R | 0, | 28-00 | YU | R | 0, |
| 4-04 | CZ | R | 0, | 4-01 | YU | R | 0, |
| 5-04 | CZ | R | 0, | 12-04 | YU | R | 0, |
| 6-04 | CZ | R | 0, | 13-04 | YU | R | 0, |
| 7-04 | CZ | R | 0, | 14-04 | YU | R | 0, |
| 8-04 | CZ | R | 0, | 16-04 | YU | R | 0, |
| 9-04 | CZ | R | 0, | 15-04 | YU | S | **4** |
| 10-04 | CZ | R | 0, | 16-04 | YU | R | 0, |
| 11-04 | CZ | R | 0, | 17-04 | YU | R | 0, |
| 30-04 | CZ | R | 0, | 18-04 | YU | R | 0, |
| 74-99/1 | EST | R | 0, | 19-04 | YU | R | 0, |
| 44-00 | EST | R | 0, | 19-04/2 | YU | R | 0, |
| 32-04 | EST | S | **4** | 20-04 | YU | R | 0, |
| 33-04 | EST | R | 0, | 21-04 | YU | R | 0, |
| 34-04 | EST | R | 0, | 22-04 | YU | R | 0, |
| 35-04 | EST | R | 0, | 23-04 | YU | R | 0, |
| 36-04 | EST | R | 0, | 24-04 | YU | R | 0, |
| 37-04 | EST | R | 0, | | | | |

AUT = Austria; BLR = Belarus; CZ = Czech Republic; EST = Estonia; ISR = Israel; HU = Hungary; SWE = Sweden; YU = Serbia and Monte Negro

R = resistant reaction (infection type 0, 0; 1, 2); S = susceptible reaction (infection type 3, 4)

public, Austria, Serbia and Monte Negro, Estonia and Israel. Šebesta (1983) did not detect any crown rust pathotype originating from Austria, Yugoslavia or Czechoslovakia virulent to *Pc 50-4*. These observations are in accordance with the fact that *P. coronata* f.sp. *avenae* is highly variable in virulence and can rapidly evolve new pathotypes that overcome commonly used resistance genes (Chong & Kolmer 1993; Zhu & Kaeppler 2003). Despite of an increase in the occurrence of virulent pathotypes, the efficiency of *Pc 50-4* is still very high, the current evaluation is 0.9296.

## Comparison of resistance efficiency of *Pc 50-4* with other *Pc* resistance genes

The efficiency of *Pc 50-4* was compared with the efficiency of differentials carrying *Pc* genes which are currently used in RICP to determine the range and combination of virulence of oat crown rust pathotypes. Twenty-eight differentials were used for comparison; their designation, genealogy and calculated efficiency are included in Table 2.

According to the value of resistance efficiency the differentials were divided into four groups. The

Table 2. The list of differentials used in the RICP, ranked according to the efficiency level

| Gene/cv. designation | Original source | Line | Efficiency of the gene | |
|---|---|---|---|---|
| *Pc 40* | *A. sterilis* F-83 | Pendek × Pc40 | 0.2571 | |
| *Pc 45* | *A. sterilis* F-169 | Pendek × Pc45 | 0.3000 | low |
| cv. Azur | [(Hin. × Veles) × Fl.] × S 325/81 | | 0.3732 | |
| *Pc 46* | *A. sterilis* F-290 | Pendek × Pc46 | 0.4000 | |
| VIR 343-2 | A. sterilis | Reselection RICP Prague | 0.4718 | |
| *Pc 54* | *A. sterilis* CAV 1832 | Pendek × Pc54 | 0.5286 | |
| VIR 343-1 | *A. sterilis* | Reselection RICP Prague | 0.6338 | |
| *Pc 51* | *A. sterilis* Wahl No. 8 | Iowa isolines X270 & X434 | 0.6571 | medium |
| *Pc 64* | *A. sterilis* CAV 4248 | Makuru//Sun II Pc64 | 0.7125 | |
| *Pc 67* | *A. sterilis* CAV 4656 | Makuru//Sun II Pc67 | 0.7254 | |
| *Pc 38* | *A. sterilis* CW491-4 | Pendek × Pc38 | 0.7436 | |
| *Pc 56* | *A. sterilis* CAV 1964 | Pendek × Pc56 | 0.7887 | |
| cv. Vok | [(Fl. × Ardo) × (Fl. × KR-81-1122)] × {(Fl. × Ardo) × [Fl. × (KR-81-1010 × Dr.)]} | | 0.8028 | |
| *Pc 96* | *A. sativa* | | 0.8571 | high |
| *Pc 61* | *A. sterilis* PI 287211 | Coker 234 | 0.8732 | |
| *Pc 60* | *A. sterilis* PI 287211 | Coker 227 | 0.8803 | |
| *Pc 50-4* | *A. sterilis* CW-488 | Pendek × Pc50 | 0.9296 | |
| *Pc 55* | *A. sterilis* CAV 4963 | Pendek × Pc55 | 0.9296 | |
| *Pc 58* | *A. sterilis* PI 295919 | TAM-O-301 | 0.9296 | |
| *Pc 50-2* | *A. sterilis* CW-487 | Pendek × Pc50 | 0.9437 | |
| *Pc 62* | *A. sterilis* CAV 4274 | Fraser Pc62 | 0.9718 | |
| *Pc 39* | *A. sterilis* F-366 | Pendek × Pc39 | 0.9789 | very high |
| *Pc 48* | *A. sterilis* F-158 | Pendek × Pc48 | 0.9859 | |
| *Pc 54-1* | *A. sterilis* CAV 1833 | Pendek × Pc54 | 0.9859 | |
| *Pc 59* | *A. sterilis* PI 296244 | TAM-O-312 | 0.9859 | |
| *Pc 68* | *A. sterilis* CAV 4904 | Makuru//Sun II Pc68 | 0.9859 | |
| *Pc 52* | *A. sterilis* Wahl No. 2 | Iowa isoline X421 | 1.0000 | |
| *Pc 94* | *A. strigosa* RL1697 | | 1.0000 | |

Table 3. F2 seedling and adult plant segregation for crown rust reaction in the cross *Pc 50-4* × Atego

| Test | Pathotype | No. of plants | | | Expected ratio | *P* |
|---|---|---|---|---|---|---|
| | | R | **S** | Σ | | |
| Glasshouse 05 - 1 | 30-04 | 201 | 67 | 268 | 3:1 | 1.00 |
| Glasshouse 05 - 2 | 15-04 | 260 | 97 | 357 | 3:1 | 0.5–0.3 |
| Field 05 | 30-04 | 46 | 12 | 58 | 3:1 | 0.5–0.3 |

R = resistant reaction; S = susceptible reaction; Σ = total number of plants; P = probability according to Chi square

first group with low efficiency containing the genes *Pc 40*, *Pc 45*, and cv. Azur had the value of efficiency 0–0.399. The second group with medium efficiency comprising the genes *Pc 38*, *Pc 46*, *Pc 51*, *Pc 54*, *Pc 56*, *Pc 64*, *Pc 67*, accessions VIR 343-1 and VIR 343-2 had the value of efficiency 0.400–0.799. The third group with high efficiency having the value 0.800–0.899 contained *Pc 60*, *Pc 61*, *Pc 96* and the cv. Vok. The last group with very high efficiency had the value 0.900–1.00. This group consisted of *Pc 39*, *Pc 48*, *Pc 50-2*, *Pc 50-4*, *Pc 52*, *Pc 54-1*, *Pc 55*, *Pc 58*, *Pc 59*, *Pc 62*, *Pc 68* and *Pc 94*.

As demonstrated, *Pc 50-4* with the efficiency value 0.9296 is comparable with the genes with the highest indicated efficiency to oat crown rust pathotypes coming from different regions of Europe and Middle East. Therefore, it is substantiated to use also this gene in the Czech breeding programmes of oat crown rust.

Problems of oat resistance to crown rust were successfully solved in breeding programmes in Canada and USA. Genes such as *Pc 48*, *Pc 68* and also *Pc 94*, *Pc 96* were widely used as sources of crown rust resistance (Chong & Zegeye 2004; Chong 2000; McMullen & Patterson 1992; Martens & Dyck 1989). These four genes were included in our analyses for a number of years, first *Pc 48* and *Pc 68* and now *Pc 94* and *96* since 2003. As obvious from our results, these genes are also very effective in the control of European pathotypes of oat crown rust. In our experiment *Pc 96* was evaluated as very effective with the value 0.857; Chong and Brown (1996) reported the resistance efficiency of this gene in the control of even more than 94% isolates of oat crown rust from regions in North America. The results of our analyses put the genes *Pc 48*, *Pc 68* and *Pc 94* into the same group as *Pc 50-4* studied by us, i.e. into the group with very high efficiency. The efficiency values reached 0.9859–1.00. The monitoring of the genes *Pc 94* and *Pc 96* has been carried out for about 3 years; our results indicate the possibility of using these genes in Czech breeding programmes of oat as well.

### Analysis of inheritance of *Pc 50-4* line to crown rust

Further objective of this study was to clarify the inheritance of crown rust resistance in the *Pc 50-4* line. 268 or 357 $F_2$ plants (*Pc 50-4* × Atego) were tested in two glasshouse experiments. Pathotypes avirulent to *Pc 50-4* line and virulent to Atego designated 30-04 and 15-04 were applied. The third analysis covering 58 plants was carried out at the adult stage in field conditions. Plants were separately inoculated with pathotype 30-04. Segregating plants were evaluated as resistant (infection type 0,) and susceptible with infection type 4.

Table 3 shows the numbers of resistant and susceptible plants; plants in $F_2$ generation segregated at the ratio 3:1, which is in accordance with the monofactorial inheritance of resistance of the *Pc 50-4* line. This indicates that the *Pc 50-4* line does not contain any other independent resistance gene(s) effective in the control of the used pathotypes. Resistance to the applied pathotypes is obviously controlled by the gene *Pc 50-4*. Monofactorial inheritance of resistance was also confirmed by a hybridological analysis of the $F_3$ generation. In total 52 families were analysed. The ratio of $F_3$ families 1:2:1 (R:Segr:S) was found in the seedling stage ($P$ = 0.8–0.7). These results confirm previous data on the inheritance of *Pc 50-4* resistance to crown rust (Šebesta 1983).

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**References:**


Chong J. (2000): Incidence and virulence of *Puccinia coronata* f.sp. *avenae* in Canada from 1996 to 1998. Canadian Journal of Plant Pathology, **22**: 99–109.

Chong J., Brown P.D. (1996): Genetics of resistance to *Puccinia coronata* f. sp. *avenae* in two *Avena sativa* accessions. Canadian Journal of Plant Pathology, **18**: 286–292.

Chong J., Kolmer J.A. (1993): Virulence dynamics and phenotypic diversity of *Puccinia coronata* f.sp. *avenae* in Canada from 1974 to 1990. Canadian Journal of Botany, **71**: 248–255.

Chong J., Zegeye T. (2004): Physiologic specialization of *Puccinia coronata* f.sp. *avenae*, the cause of oat crown rust, in Canada from 1999 to 2001. Canadian Journal of Plant Pathology, **26**: 97–108.

Fleischmann G., Baker F.J. (1971): Oat crown rust differentiation: replacement of the standard differential varieties with a new set of single resistance gene lines derived from *A. sterilis.* Canadian Journal of Botany, **49**: 1433–1437.

Frey K.J., Browning J.A., Simons M.D. (1977): Management systems for host genes to control disease loss. Annals of the New York Academy of Sciences, **287**: 255–274.

Harder D.E., Haber S. (1992): Oat diseases and pathological techniques. In: Marshall H.G., Sorrells M.E. (eds): Oat Science and Technology. Agron. Monogr. 33. American Society of Agronomy, Inc., Madison, Crop Science Society of America, Inc., Madison, 307–425.

Martens J.W., Dyck P.L. (1989): Genetics of resistance to rust in cereals from a Canadian perspective. Canadian Journal of Plant Pathology, **11**: 78–85.

McMullen M.S., Patterson F.L. (1992): Oat cultivar development in the USA and Canada. In: Marshall H.G., Sorrells M.E. (eds): Oat Science and Technology. Agronomy Monograph No. 33. American Society of Agronomy, Inc., Madison, Crop Science Society of America, Inc., Madison, 573–612.

Ohm H.W., Shaner G. (1992): Breeding oat for resistance to diseases. In: Marshall H.G., Sorrells M.E. (eds): Oat Science and Technology. Agronomy Monograph No. 33. American Society of Agronomy, Inc., Madison, Crop Science Society of America, Inc., Madison, 657–698.

Rajhathy T., Zillinsky F.J., Hayes J.D. (1966): A collection of wild oat species in the Mediterranean Region. Report of the Canada-Welsh Expedition, Canadian Department of Agriculture, Ottawa Research Station.

Simons M.D. (1985): Crown rust. In: Roelfs A.P., Bushnell W.R. (eds): The Cereal Rusts: Diseases, Distribution, Epidemiology, and Control. Vol. 2. Academic Press, Orlando, Florida, 131–172.

Simons M.D., Murphy H.C. (1955): Certain combinations of oat varieties as crown rust differentials. Techn. Bull., **1112**: 1–22.

Šebesta J. (1971): Effect of stem rust and crown rust on the yield of oats. (in Czech) Ochrana rostlin, **7:** 253–260.

Šebesta J. (1972): Infektionsmethoden zur Selektion auf Rostresistenz des Getreides im Gewächshaus und im Freiland. Bericht Arbeitstagung 1972 Arbeitsgemeinschaft der Aaatzuchtleiter, Gumpenstein (Austria), November 1972, 185–197.

Šebesta J. (1983): Race-specific expression of oat crown rust resistance conditioned by major and minor genes. Euphytica, **32**: 857–861.

Šebesta J. (1991): Hodnocení chorob polních plodin z hlediska šlechtění na odolnost. In: Studie VTR, Rostlinná výroba, No. 10, ÚVTIZ, Prague.

Šebesta J., Harder D.E. (1983): Occurrence and distribution of virulence in *Puccinia coronata* var. *avenae* in Europe, 1977–1980. Plant Disease, **67**: 56–59.

Šebesta J., Sýkora J. (1974): The harmfulness of rusts to oats as a racial and varietal peculiarity. (in Czech) Ochrana rostlin, **10**: 265–269.

Šebesta J., Zwatz B. (1980): Virulenz der mitteleuropäischen Rassenpopulationen des Schwarzrostes des Hafers (*P. graminis* Pers. f.sp. *avenae* Erikss. et Henn) unter besonderer Berücksichtigung der Wirksamkeit der Resistenzgene. Pflanzenschutzberichte, **XLVI**, 1/5: 1–41.

Šebesta J., Mouchová H., Sýkora J. (1972): Effect of rusts on protein and bound amino acids content in oat kernels. (in Czech) Ochrana rostlin, **8**: 5–10.

Zhu S., Kaeppler F. (2003): Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. Crop science, **43**: 358–366.

Catalogue of Rust Resistance Genes in Small Grains. [cit. 6.10. 2005], available from Internet: http://www.cdl.umn.edu/res_gene/res_gene.html


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