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The Response of Selected Winter Wheat Cultivars to Artificial Infection with *Septoria tritici* under Field Conditions

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Abstract: The response of 26 winter wheat cultivars to infection with *Septoria tritici* was studied in 1997–2000 field experiments at the location Prague-Ruzyně. The method of pathogen cultivation and of inoculation technique is described in detail. *Septoria* disease progress (SDP) and infected leaf area (ILA) were estimated on a 0–9 scale. By multiplying both parameters the coefficient of infection (CI) was calculated. To minimize the effect of experimental year micro-irrigation system was necessary. The highly virulent Czech isolate RULI was used for inoculation during the whole period. In 2000 this isolate was used in mixture with other two isolates, but this did not affect the results of cultivar evaluation. The ANOVA revealed significant cultivar and year effects. Cultivar by year interaction mean squares were of relatively lower magnitude, but statistically significant for all examined traits. Based on the analysis of multiple comparisons, the highest number of significant differences between cultivars was observed for the rate of disease spread (SDP) in plant stands. The highest resistance level showed the cultivars Arina, Hereward, Sida and Kavkaz. The Swiss cultivar Arina may be used as a source of combined resistance to the occurring pathogens causing leaf and head blights. A high resistance level was associated with later heading and greater plant height. The early and short genotypes showed on average a more rapid penetration of infection into upper parts of a plant and great variability in the examined resistance parameters, which indicates the possibility to obtain in these categories also materials with acceptable resistance level.

Keywords: winter wheat; *Septoria tritici*; resistance of cultivars; field infection tests

Septoria tritici blotch of wheat caused by the pathogen *Septoria tritici* Rob ap ud Desm. (teleomorph *Mycosphaerella graminicola* [Fuckel] J. Schroeter in Cohn) is an important disease in many wheat producing areas of the world. The disease can result in severe crop damage during summer, leading to substantial yield losses (KING *et al.* 1983; THOMAS *et al.* 1989), which in epidemic years may reach 30–50% (KOLOMIETS 1999). HORČÍČKA *et al.* (2001) estimated in the conditions of Central Europe the average reduction of kernel weight per spike due to infection of *Septoria* and *Pyrenophora* diseases to be 16% and these authors stress the importance of resistant cultivars to minimize yield losses. In the conditions of Western Australia LOUGHMAN *et al.* (1999) found that susceptible wheat cultivars suffered 20–50% yield loss, while yield loss of resistant cultivars was 0–15%. Yield losses are mainly attributable to infections of the upper leaves after repeated asexual reproduction cycles of the fungus, during which pycnidia produce

splash-dispersed pycnidiospores. The role of the teleomorph stage, *Mycosphaerella graminicola*, in epidemics of *Septoria tritici* blotch on winter wheat was studied in the UK by HUNTER *et al.* (1999). *Septoria tritici* blotch is regarded as a wet weather disease, as moisture is required in all phases of the disease cycle (SHANER & FINNEY 1976; THOMAS *et al.* 1989).

Complete resistance to *Septoria tritici* was reported by MIELKE (1995) in all examined triticale cultivars, but many sources of resistance are also available in wheat (NELSON & MARSHALL 1990; van GINKEL & RAJARAM 1999; GILCHRIST *et al.* 1999; BROWN *et al.* 1999). However, conflicting reports were found in the literature regarding the nature of genetic resistance to *Septoria tritici* blotch (CAMACHO-CASAS *et al.* 1995). These range from simple Mendelian genetics to complex quantitative inheritance patterns. Single resistance genes, showing partial to strong dominance, were reported in the spring wheat cultivar Tadinia by SOMASCO *et al.* (1996) and in the re-

sistant line 72626E2-12-9-1 by XUEYI *et al.* (1998). WILSON (1985) proposed three different genes conferring resistance to *Septoria tritici* blotch. The inheritance studies, using the cultivars Kavkaz and Aurora as resistance sources, showed prevalence of additive-dominant gene effects (GONZALES *et al.* 2000). In general, VAN GINKEL and RAJARAM (1999) postulated that additive gene effects contribute more to resistance than dominance effects. Failure to transfer satisfactory levels of resistance to *Septoria tritici* blotch in some crosses was noted by EYAL *et al.* (1987), who suggested the presence of modifying genes that affect the expression of resistance genes.

Resistance has been often found associated with greater plant height and late maturity, which may suggest an escape mechanism rather than true genetic resistance (CAMACHO-CASAS *et al.* 1995). In the experiments of BALTAZAR *et al.* (1990) the most susceptible plants were observed in populations carrying the *Rht1* gene, while the presence of *Rht2* was generally associated with a relatively higher level of resistance (similarly VAN GINKEL & RAJARAM 1999). It proved possible to select shorter, earlier maturing wheat cultivars with acceptable levels of resistance, but these authors stressed the importance of choosing an appropriate dwarfing source. However, the association of resistance with certain morphological traits, such as plant height, canopy structure and earliness, may interfere with a reliable assessment of true resistance (ARAMA *et al.* 1999).

Success in breeding for resistance to *Septoria tritici* blotch depends undoubtedly, besides the availability of suitable resistance sources, on a reliable method of resistance testing. Particularly the composition and 'quality' of inoculum is the key factor determining the rate of disease development in the field. EYAL (1992) studied the response of field-inoculated wheat cultivars to mixtures of *Septoria tritici* isolates. Cultivars exposed to mixtures of isolates expressed differential response in pycnidial coverage compared to single isolate response. The suppression of symptoms in an isolate mixture relative to the most virulent component may be indicative of differential aggressiveness of isolates regardless of their virulence, which may affect screening and selection procedures in breeding for resistance. Significant isolates by cultivar interactions were found for both the induced necrosis and number of pycnidia on primary leaves in the experiments of KEMA *et al.* (1995). The authors selected by means of a cluster analysis three isolates significantly different in both disease parameters and these three isolates were used to inoculate a field experiment. Strong cultivar by isolate interactions, found in experiments of BROWN *et al.* (1999), were stable over the environments and, therefore, breeders must take account of the possibility that certain resistances may not be durable. The virulence of six isolates (five obtained from DLO-RIPP,

Wageningen; one Czech isolate RULI) was studied in trials with 30 winter wheat cultivars (ŠÍP *et al.* 1997). The cultivars were more susceptible to isolates RULI, 323 and 94269 than to isolate 001. The entries Arina, Apollo, NSL 92, CH 76337 and SG-RU 5007 were found resistant to most isolates, while CH 95413, RU-586-96 and Galaxie were susceptible to all isolates.

The objectives of the present study were to introduce a suitable method of inoculation under field conditions and evaluate the response of the selected winter wheat cultivars to the infection with *Septoria tritici* in trials conducted over four years.

MATERIAL AND METHODS

Plant material and experimental design. The experiment included 22 winter wheat cultivars registered in the Czech Republic, the Swiss cultivar Arina and the check cultivars Hereward, Kavkaz (resistant) and Galaxie (susceptible). The cultivar Arina was included in the experiments as a potential source of combined resistance to *Septoria tritici*, *Stagonospora nodorum*, *Pyrenophora tritici repentis* and *Fusarium head blight*. All cultivars were grown from 1997 to 2000 in a field trial located at Prague-Ruzyně. The trial consisted of randomised complete blocks with two replications. The plots were 2 m long with six rows spaced 20 cm. The blocks of plots were separated by a 1.2-m wide strip and the adjacent plots within blocks were separated by one empty row. To minimize the effect of neighbouring plots on the spread of disease, a central 1-m wide strip in each plot was inoculated with the fungus and evaluation was based on data obtained from the inner part of the plot.

Isolates, pathogen cultivation and inoculation technique. In all four years the highly virulent Czech isolate RULI (ŠÍP *et al.* 1997) was used for inoculation. Because of a less successful inoculation in 1999, we supplemented this isolate by other two isolates obtained from different locations in the Czech Republic. The resulting inoculum was highly infectious in 2000.

The fungus was propagated by methods obtained from IPO-DLO Wageningen. The fungus isolates were grown in Petri dishes on a solid V8 medium, containing 0.5 l of vegetable juice (Ewig, Granini or Happy Day), 0.5 l of demineralized water and 25 g of agar. For field inoculations the *Septoria tritici* culture was transferred to a liquid yeast-glucose medium (10 g of glucose, 30 g of Difco yeast extract and 1 l of demineralized water). The medium was prepared in Erlenmeyer flasks, sealed with a cotton plug and autoclaved at 110°C for 20 min. The flasks were shaken on a shaker for 5–10 days at 20 ± 2°C, depending on the culture. The shaking was stopped the evening before inoculation to allow the spores to settle. The clear

supernatant was removed in the morning and the purity of the sedimented inoculum examined under the microscope. The concentration of the inoculum was $0.5\text{--}1 \times 10^6$ spores per ml. Each year the plants were inoculated twice (Table 1) by spraying from a distance of approximately 1 m above the plants. The first inoculation was done at the flag leaf stage (growth stages ≥ 47) and the second inoculation seven days later (Table 1). A micro-irrigation system was used to provide sufficient wetness in the plant stands. Irrigation started 24 h before the first inoculation. The irrigation consisted of cycles of one minute spraying, followed by a break of 14 or 29 minutes, depending on the weather conditions.

Evaluation of symptoms and data analysis. The data of 1999 were excluded from the analysis, because of very low infestation of plants in this year. The symptoms of *Septoria tritici* infection were assessed in each year at three dates, given in Table 1. However, only data obtained at the date, when the infection culminated, were analysed. The severity of symptoms in the stands was evaluated using a two-digit scale, recommended by EYAL *et al.* (1987). This scale was found very helpful in explaining yield losses caused by the disease (VAN BEUNINGEN & KOHLI 1990). Each digit was recorded in linear steps from 0 to 9, with 9 indicating the highest symptom severity. The first digit, specified as "Septoria disease progress" (SDP), indicated the relative height of disease on

the plant and was an estimate of the rate of disease spread in a certain period of time. The second digit, specified as "infected leaf area" (ILA), was an estimate of the coverage of the upper four leaves by chlorotic or necrotic symptoms, relative to the leaf area (BALTAZAR *et al.* 1990). To obtain a single reasonable resistance parameter, a coefficient of infection (CI) was derived by multiplying both digits (VAN BEUNINGEN & KOHLI 1990). We used the analysis of variance (ANOVA) to separate the effects of cultivar, year and cultivar by year interactions for the three examined parameters. Duncan's multiple range test was used to calculate the statistical significance of cultivar and year ranking.

RESULTS AND DISCUSSION

Course of infection in different years. The ANOVA results (Table 2) showed significant cultivar and year effects in all the examined traits. Much lower than year and cultivar mean squares, but also statistically significant, were the mean squares for cultivar by year interactions. Both ANOVA and the Duncan test (Table 3) show a considerably lower disease incidence in 1997 than in 1998 and 2000. The highest SDP was observed in 1998 and the highest ILA in 2000. Nevertheless, the inter-annual correlation coefficients, given in Table 4, were highly significant for all the examined characteristics. As to the coefficient of infection and infected leaf area, the relation between the years 1998 and 2000 was closer than between 1997 and 1998, and between 1997 and 2000 (Table 4). This might be due to relatively lower infestation, leading to a lower genotype variation in conditions of 1997 (Fig. 1). It also follows from the analysis, that adding two fresh isolates to the 'RULI' inoculum in 2000, in response to the unsatisfactory results in 1999, did not significantly influence the ranking of cultivars and was associated with the highest infestation of leaf area, which is desirable for better differentiation between the cultivars. Much lower differences between resistant and susceptible cultivars

Table 1. Mean pentad temperature ($^{\circ}\text{C}$), and dates of inoculation and symptom evaluation in 1997, 1998 and 2000

| | | 1997 | 1998 | 2000 |
|---|-------|-------|-------|-------|
| May | 17–21 | 18.4 | 13.7 | 13.4 |
| | 22–26 | 10.9 | 10.8 | 15.6 |
| | 27–31 | 9.8 | 17.8 | 12.6 |
| June | 1–5 | 13.7 | 18.7 | 21.0 |
| | 6–10 | 17.7 | 19.8 | 17.9 |
| | 11–15 | 17.9 | 12.7 | 21.7 |
| | 16–20 | 13.5 | 14.8 | 18.2 |
| | 21–25 | 13.6 | 18.5 | 19.5 |
| | 26–30 | 19.5 | 18.7 | 13.0 |
| July | 1–5 | 16.8 | 15.1 | 18.8 |
| | 6–10 | 16.3 | 13.4 | 15.2 |
| Mean temperature ($^{\circ}\text{C}$) | | 15.3 | 15.8 | 17.0 |
| Date of inoculation | 1. | 4.6. | 25.5. | 24.5. |
| | 2. | 11.6. | 1.6. | 31.5. |
| Date of evaluation | 1. | 30.6. | 18.6. | 15.6. |
| | 2. | 5.7. | 26.6. | 20.6. |
| | 3. | 15.7. | 8.7. | 26.6. |

Table 2. ANOVA mean squares for CI (coefficient of infection), SDP (Septoria disease progress) and ILA (infected leaf area)

| Source of variation | df | CI | SDP | ILA |
|---------------------|----|-----------|---------|---------|
| Cultivar (C) | 25 | 693.08** | 18.25* | 8.98** |
| Year (Y) | 2 | 1239.65** | 27.89** | 37.19** |
| C \times Y | 50 | 132.75** | 2.69** | 1.81** |
| Residual | 78 | 24.42 | 0.49 | 0.43 |

** $P < 0.01$

Table 3. Cultivar and year ranking for the examined characters, and inclusion of cultivars and years into homogeneous groups (Duncan $P = 95\%$)

| Cultivar/ Year | Country of origin | Average CI* | Homoge- neous group | Average SDP | Homoge- neous group | Average ILA | Homoge- neous group |
|-------------------|----------------------|----------------|------------------------|----------------|------------------------|----------------|------------------------|
| Arina | Switzerland | 0.58 | a | 0.83 | a | 0.67 | a |
| Hereward | England | 1.92 | a | 1.17 | ab | 1.58 | abc |
| Sida | Czech Republic | 3.04 | ab | 1.75 | abcd | 1.42 | abc |
| Kavkaz | SU | 3.50 | ab | 1.50 | abc | 2.00 | abcde |
| Ina | Czech Republic | 4.50 | abc | 2.33 | abcde | 1.42 | ab |
| Ritmo | The Netherlands | 6.04 | abcd | 2.42 | abcde | 1.75 | abcd |
| Samara | Czech Republic | 6.63 | abcd | 2.91 | bcdefg | 2.17 | abcdef |
| Versailles | Netherlands | 7.00 | abcd | 2.75 | bcdef | 2.17 | abcdef |
| Ilona | Slovakia | 7.75 | abcd | 3.17 | cdefgh | 2.33 | bcdef |
| Athlet | Germany | 8.92 | abcd | 2.75 | bcdef | 2.67 | bcdefg |
| Vlasta | Czech Republic | 10.58 | abcde | 3.67 | efghij | 2.75 | bcdefg |
| Mona | Czech Republic | 11.33 | abcde | 3.83 | efghijk | 2.75 | bcdefg |
| Siria | Czech Republic | 12.58 | abcdef | 3.50 | defghi | 3.00 | bcdefg |
| Ebi | Germany | 12.67 | abcdef | 3.50 | defghi | 3.58 | efg |
| Brea | Czech Republic | 12.83 | abcdef | 4.33 | fghijkl | 2.83 | bcdefg |
| Estica | The Netherlands | 12.83 | abcdef | 3.83 | efghijk | 3.17 | cdefg |
| Alka | Czech Republic | 15.83 | bcdef | 5.33 | jklmn | 2.83 | bcdefg |
| Boka | Czech Republic | 16.38 | cdef | 4.75 | hijkl | 3.42 | defg |
| Saskia | Czech Republic | 16.71 | cdef | 4.67 | ghijkl | 2.58 | efg |
| Astella | Slovakia | 17.98 | def | 5.50 | klmno | 3.25 | cdefg |
| Alana | Czech Republic | 18.46 | def | 4.83 | hijklm | 3.50 | efg |
| Bruta | Czech Republic | 21.96 | ef | 5.25 | ijklm | 4.17 | fhi |
| Samanta | Czech Republic | 24.08 | fg | 5.83 | lmno | 3.75 | fgh |
| Galaxie | France | 33.33 | gh | 6.50 | mno | 5.17 | hi |
| Šárka | Czech Republic | 37.83 | h | 7.00 | no | 5.41 | i |
| Hana | Czech Republic | 42.75 | h | 7.17 | o | 5.58 | i |
| 1997 | | 8.51 | a | 3.24 | a | 2.07 | a |
| 2000 | | 16.95 | b | 3.74 | a | 3.75 | c |
| 1998 | | 16.99 | b | 4.68 | b | 3.06 | b |

*CI (coefficient of infection) = SDP (Septoria disease progress) × ILA (infected leaf area)

SDP – disease level in terms of plant height: 0–9 scale (9 – the highest rate of disease spreading)

ILA – percentage of necrotic and chlorotic leaf area: 0–9 scale (0 – 0%; 1 – 10%; 2 – 20% etc.)

than in 1998 and 2000 were observed in 1997 (Fig. 1). It is likely that other factors than isolate composition of inoculum influenced the severity of infection in single years. The use of mixtures of isolates may help to improve the inoculum quality, but according to our experience, this may also be obtained with a single highly virulent isolate (1998). As reported earlier (ŠÍP *et al.* 1997), the highly resistant and susceptible cultivars responded similarly to the virulent isolates RULI, 323 and 94269, and the mis-

classification of a genotype was particularly due to the use of isolate 001 with lower virulence. The use of isolate mixtures may lead in comparison with the most virulent component to the suppression of symptoms (EYAL 1992). The presence of significant cultivar by isolate interactions had often been detected (MIELKE 1995; KEMA *et al.* 1995; BROWN *et al.* 1999) and must be taken into consideration. However, there are undoubtedly many other factors that influence the response of a cultivar in a cer-

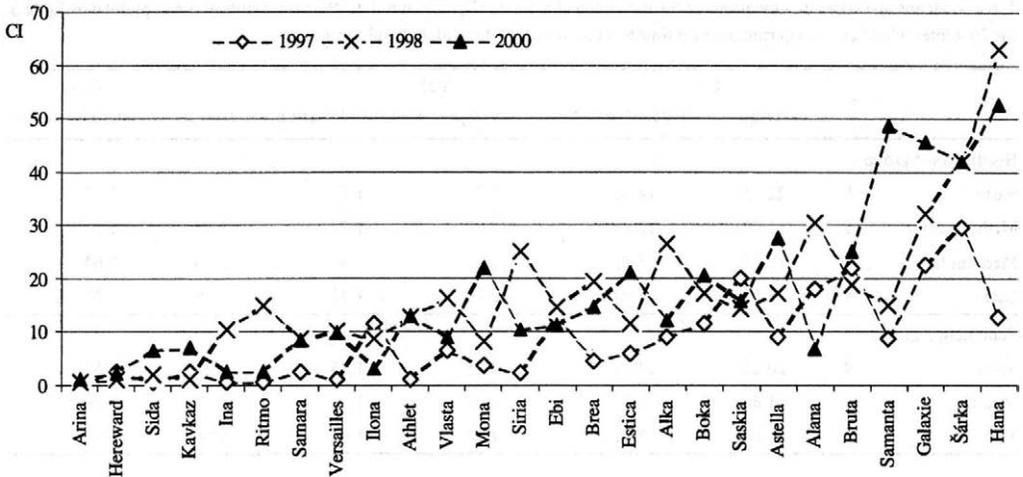


Fig. 1. Coefficients of infection (CI) of the examined 26 cultivars in different years

tain year. Differences in cultivar response to infection between years were in these experiments particularly evident for genotypes with medium resistance or susceptibility (Fig. 1). It cannot be concluded from these experiments that the use of a mixture of isolates was superior to the use of a single virulent isolate, but it was possible with the use of highly virulent inoculum and in conditions suitable for disease development to differentiate better between genotypes. Anyway, it is necessary to mention that multi-year comparisons are needed to obtain a more accurate resolution, especially in the case of medium resistant genotypes.

Under conditions of sufficient leaf wetness, reached by appropriate irrigation, temperature might be the main factor influencing the course of infection (Table 1). WAINSHILBAUM and LIPPS (1991) reported that infection with *S. tritici* was in comparison with *S. nodorum* infection much more influenced by temperature than by the growth stage at the time of inoculation.

Differences in cultivar response to the infection. Coefficients of infection (CI) of the examined cultivars in different years are given in Fig. 1. Differences in cultivar response to artificial infection with *Septoria tritici* are evident from the mean values and multiple comparisons that are available in Table 3 for the three examined characters. It is clear that the highest number of statistically significant differences between cultivars could be detected in SDP, measuring the rate of disease spreading within a plant. This character is very important in terms of tolerance to this disease, because yield losses due to infection are highly conditioned by the height of disease on the plant. SHAW and ROYLE (1989) have found that only the top two leaves contributed to yield loss. The

Table 4. Interannual correlations for the examined characters ($n = 26$)

| | Coefficient of infection (CI) | Septoria disease progress (SDP) | Infected leaf area (ILA) |
|-----------|-------------------------------|---------------------------------|--------------------------|
| 1997/1998 | 0.60** | 0.63** | 0.53** |
| 1997/2000 | 0.59** | 0.72** | 0.54** |
| 1998/2000 | 0.69** | 0.64** | 0.67** |

** $P < 0.01$

coefficient of infection, combining both SDP and ILA, clearly separated the susceptible cultivars, but the first resistant group 'a' was large, evidently due to lower infestation in 1997, when a relatively lower infected area was detected also in certain cultivars that can be classified as medium resistant or medium susceptible according to SDP. In spite of the fact that SDP was positively correlated with ILA ($r = 0.93$; $P < 0.01$), obtaining a high severity of infection in every year is undoubtedly a very important prerequisite for making reliable statements about resistance. According to VAN BEUNINGEN and KOHLI (1990) the data could be analysed only from environments where the infection score of the most susceptible entry reached at least 60% of the maximum of the evaluation scale and the frequency of "zero-disease" was low. However, the correlation coefficients given in Table 4 imply, that the evaluation in 1997 did not lead to distorted estimates of resistance, although the disease level was low and the variability of data large.

The highest resistance level was detected in the cultivars Arina, Hereward, Sida and Kavkaz, and in two of

Table 5. Means and standard deviations of the examined characters CI, SDP and ILA (the used symbols are explained in Table 3) for 26 winter wheat cultivars arranged into four heading date and three plant height groups

| | n | CI | | SDP | | ILA | |
|--------------------|----|---------|--------------------|---------|--------------------|---------|--------------------|
| | | average | standard deviation | average | standard deviation | average | standard deviation |
| Heading date group | | | | | | | |
| Early | 5 | 22.63 | 14.92 | 5.23 | 1.71 | 3.82 | 1.47 |
| Medium early | 5 | 21.02 | 12.09 | 5.02 | 1.73 | 3.47 | 1.53 |
| Medium late | 8 | 10.17 | 5.23 | 3.47 | 1.36 | 2.55 | 0.64 |
| Late | 8 | 8.55 | 5.97 | 2.76 | 1.33 | 2.39 | 1.03 |
| Plant height group | | | | | | | |
| Short | 9 | 20.25 | 14.11 | 4.78 | 1.85 | 3.49 | 1.50 |
| Medium | 12 | 11.60 | 7.11 | 3.63 | 1.50 | 2.67 | 0.89 |
| Tall | 5 | 9.31 | 7.23 | 2.90 | 1.67 | 2.50 | 1.21 |

three years also in Ina and Ritmo. The most susceptible were the check cultivar Galaxie and also the cultivars Hana and Šárka. The detection of the highest resistance level in Arina is particularly important for breeding, because this Swiss cultivar was found highly resistant also to leaf blotch caused by *Septoria nodorum*, to tan spot caused by *Pyrenophora tritici-repentis* and to Fusarium head blight (ŠÍP & STUHLÍKOVÁ 1997). A high resistance of Arina to *Septoria tritici*, comparable with the resistance of cultivars Veranopolis, Kavkaz or Frontana, was also reported by BROWN *et al.* (1999). However, compared with Central European cultivars, Arina is tall and late.

Effect of cultivar heading date and plant height on the resistance level. Because resistance to this disease has frequently been found associated with greater plant height and later maturity, the examined cultivars were arranged for further statistical analyses into four groups according to heading date and three groups according to plant height. The variation ranges of the included cultivars were for heading date approximately 14 days and for plant height approximately 25 cm. Late and tall cultivars demonstrated a lower disease progression compared to early and short cultivars (Table 5). Greater variability in the examined resistance parameters was obtained in groups of early and short genotypes than in late and tall groups. This indicates the detection of at least medium resistance level also among the earlier and shorter materials. The group of late cultivars included the relatively taller highly resistant cultivar Arina and medium resistant Ebi, but also the cultivars Hereward, Estica, Ritmo, Athlet and Versailles from the medium or short plant height group. The remaining cultivars with relatively higher resistance level, Sida, Kavkaz and Ina, could be classified as medium late or medium early. However, no

early and short cultivar was found highly resistant to this disease.

Data on days to heading of cultivars, obtained in the examined years, were correlated with the respective CI, SDP and ILA values. It is clear from Table 6 that the obtained correlation coefficients were negative and mostly significant, which indicates that resistance is more likely to be reached with later heading genotypes. It is also evident from these correlation studies that the early and usually shorter genotypes might be characterized by a more rapid penetration of a disease into upper parts of the plant, which was indicated by a closer relation obtained for SDP.

It was not possible with the examined cultivar set to study the relation between the presence of the dwarfing genes *Rht1* and *Rht2* and resistance to *Septoria tritici* blotch, as did BALTAZAR *et al.* (1990). Insensitivity to applied gibberellic acid (GA), showing the presence of these genes, was found only in eight of the 26 genotypes and the number of genotypes carrying a particular *Rht* gene was too small. The GA sensitive and GA insensitive groups did not differ substantially in resistance lev-

Table 6. Coefficients of correlation between days to heading and the examined characters CI, SDP and ILA in different years

| Year | Coefficient of infection (CI) | Septoria disease progress (SDP) | Infected leaf area (ILA) |
|------|-------------------------------|---------------------------------|--------------------------|
| 1997 | -0.34* | -0.59** | -0.15 |
| 1998 | -0.34* | -0.37* | -0.36* |
| 2000 | -0.51** | -0.59** | -0.41* |

* $P < 0.05$, ** $P < 0.01$

els (CI: sensitive group – 13.9; insensitive group – 12.3, with respective standard deviations 10.0 and 11.3).

CAMACHO *et al.* (1995), who compared the effects of plant height and heading date on disease severity, found that the direct effect of heading date was larger. When the associations were examined in relation to grain yield, a negative association was observed between heading date and yield, whereas positive association was noted between plant height and yield. A higher spread of the disease in the early genotypes may also be due to more favourable weather conditions (cooler and wet enough) in decisive stages of plant growth and development. When assessed at the same developmental stage, ARAMA *et al.* (1999) found the disease build up independent of heading date, but strongly dependent on the resistance level; there was no indication that early heading cultivars were more susceptible than late heading cultivars. Though in our experiments the period of evaluation of symptoms (in three terms – Table 1) was large enough to enable the full development of the disease in both the early and late genotypes, the effects of different developmental patterns of genotypes and, therefore, exposure to different conditions for disease development, could not be excluded in field experiments. Resistance studies in the progenies of crosses between late maturing, highly resistant genotypes and early genotypes may be helpful from these aspects. However, CAMACHO *et al.* (1995) have found no resistant progeny as early and short as the susceptible parent, but they conclude that a compromise might be reached for these traits to achieve an acceptable level of resistance.

Breeding for resistance to *Septoria tritici*. Numerous sources of resistance to *S. tritici* were detected in many parts of the world. Three main groups of sources of resistance were successfully used to introduce resistance in the CIMMYT breeding programme: a) Russian winter wheat lines; b) lines from the Southern Cone of South America and b) to lesser extent also the lines from the USA (GILCHRIST *et al.* 1999). Other, but rather long term possibilities, consist in the use of resistance genes from *Triticale* and *Triticum diccocon*, in the introgression of resistance from synthetic hexaploids and derivatives into susceptible stocks and in the increase of variability of the resistance base particularly with the use of geographically distant germplasm (e.g. Chinese lines). It is encouraging for the European breeding programmes that, as reported by BROWN *et al.* (1999), a wide range of European wheat germplasm from several countries, having good level of resistance, is available to breeders. Though there are at least five confounding factors that complicate selection for resistance (VAN GINKEL & RAJARAM 1999), the striking success of e.g. the Swiss breeding programme (giving rise also to the cultivar Arina, studied here) is a result of long term selection for foliar disease

resistance, using both artificial and natural inoculation (BROWN *et al.* 1999). The results of this study and results of other experiments mentioned here indicate that great attention should be paid particularly to reaching a high level of resistance in different genotype categories of maturity and plant height and to obtaining more durable resistance, effective against different isolates of the pathogen (pyramiding of resistance genes). The diversity of isolates at the field level is of paramount importance. The breeder has to take it into consideration when planning artificially inoculations, or rely on multisite testing (VAN GINKEL & RAJARAM 1999). In general, two breeding strategies may be effective, similarly as with other pathogens: either to combine specific resistance genes or to select for a high level of quantitative resistance. Until now, mainly sources with undefined genetic basis of resistance were predominantly used. Recent results of studies on the structure of pathogen populations (EYAL 1999), together with newly detected differences in the genetic base of resistance to this pathogen in the world collection, will enable to concentrate the future work on combining accumulated resistances that are based on different genes and/or different resistance mechanisms (VAN GINKEL & RAJARAM 1999) so as to obtain satisfactory and more durable resistance to septoria foliar blights, which can be speeded up with the use of available molecular tools.

It can be concluded that the progress in resistance breeding is unlikely to be limited by the availability of resistance sources, but major difficulty still lies in a rapid introduction of resistance into desirable plant types. In field infection tests, the results of which are presented here, only a smaller amount of material can be examined. Rapid screening tests are evidently needed that would enable selection in earlier hybrid generations. It is encouraging, that the correlation between seedling and adult host response has been substantiated by several investigators (EYAL 1999; KEMA & VAN SILFHOUT 1997). Until now, however, seedling tests mainly serve as a supplementary measure and are an excellent tool for detailed, controlled studies on a multitude of biological issues associated with host-pathogen interactions. It is particularly important for the application of seedling tests as a screening measure to guarantee the conformity of the used virulence spectrum of isolates to the spectrum occurring under field conditions (EYAL 1999).

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Souhrn

ŠÍP V., STUHLÍKOVÁ E., CHRPOVÁ J. (2001): **Reakce vybraných odrůd pšenice ozimé na umělou infekci houbou *Septoria tritici* v polních podmínkách.** Czech. J. Genet. Plant Breed., 37: 73–81.

Reakce na infekci 26 odrůd pšenice ozimé houbou *Septoria tritici* byla hodnocena v polních pokusech na stanovišti Praha-Ruzyně v období 1997–2000. K dispozici pro další využití je podrobný popis metody kultivace patogena a způsobu inokulace rostlin. Rychlost šíření infekce a napadení listové plochy byly posuzovány na základě devítibodové stupnice a vypočítán koeficient infekce jako násobek obou charakteristik. Pro minimalizaci vlivu pokusného ročníku bylo nezbytné zajistit umělé zavlažování porostů rosením a vysokou infekčnost, která může záviset také na teplotě a růstové fázi rostlin. Vysoce virulentní český izolát RULI byl použit pro inokulaci v celém období. Použití tohoto izolátu ve směsi s dalšími dvěma izoláty v roce 2000 neovlivnilo výsledky testování odrůd. Analýzami rozptylu byly prokázány statisticky významné rozdíly v reakci odrůd i vlivy ročníků. Relativně nižší, avšak významné u všech znaků byly průměrné čtverce pro interakce odrůdy s rokem. Na základě analýzy mnohonásobných porovnání byl nejvyšší počet homogenních skupin odrůd prokázán u znaku rychlost šíření infekce. Nejvyšší úroveň rezistence byla zjištěna u odrůd Arina, Hereward, Sida a Kavkaz. Švýcarská odrůda Arina může být využita ve šlechtitelských programech jako zdroj kombinované rezistence k vyskytujícím se listovým skvrnitostem působeným též houbami *Stagonospora nodorum* a *Pyrenophora tritici-repentis*, jakož i k fuzariózám klasu. Vyšší stupeň rezistence odrůd byl spojen s pozdějším metáním a větší délkou rostliny. Ranější a kratší genotypy vykazaly v průměru rychlejší šíření infekce do horních pater rostliny a vyšší variabilitu ve sledovaných ukazatelích rezistence, což indikuje možnost získání přijatelné úrovně rezistence také v těchto kategoriích.

Klíčová slova: pšenice ozimá; *Septoria tritici*; rezistence odrůd; polní infekční testy

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Heat Induced Susceptibility of *mlo*-Barley to Powdery Mildew (*Blumeria graminis* D.C. f.sp. *hordei* Marchal)

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Abstract: Primary leaf segments of two *Mlo* barley lines, the extremely susceptible SM4142 and the medium susceptible Diamant, and of two *mlo*-barley lines, the less resistant Apex and the more resistant HLN70-8, were inoculated with the mildew isolates PV-4 and HL-3, differing in their level of partial *mlo*-virulence. The leaf segments, laid out in Petri-dishes on an agar medium, were exposed for 30, 60 and 120 min to 36°C prior to uniform inoculation with conidia of the mildew isolates in a settling tower. After 5–6 days of incubation at 17–18°C the colonies were counted and compared with counts on simultaneously inoculated segments without heat treatment. The heat treatments resulted in increased susceptibility of all tested varieties to both mildew cultures. The effect was largest with the most resistant variety and the less *mlo*-virulent mildew culture. The largest observed increase was by one order of magnitude. In contrast, cooling the leaf segments to 1°C prior to inoculation for 30, 60 or 120 min did not have a significant effect with the culture PV-4 and only a slight effect with HL-3. The heat induced susceptibility was temporary and declined within 13 hours to approx. 130%, relative to not heat-treated leaf segments. Since the heat induction operates at temperatures occurring in nature, it may contribute to occasionally observed outbreaks of mildew in fields of *mlo*-varieties. Heat induced susceptibility also can be utilised for collecting partially *mlo*-virulent pathotypes from agricultural environments.

Keywords: barley; powdery mildew; *Blumeria graminis*; *mlo*; resistance; heat induced susceptibility

In contrast to resistance genes in barley, causing hypersensitive host reactions to powdery mildew, the resistance based on the *mlo*-gene is quantitative, reducing the number of successful infections per 100 spores of not adapted isolates by a factor of approximately 500 (ANDERSEN 1991). By experience, the percentage of successful infections of avirulent mildew isolates on *mlo*-hosts is difficult to reproduce and the results vary by more than one order of magnitude. Occasional outbreaks of mildew in fields of *mlo*-varieties never have been found due to *mlo*-virulent pathotypes, but to unknown environmental factors (LYNKGKJAE *et al.* 2000). Several non-genetic factors are known to affect the *mlo*-based resistance to mildew. The leaf cell types of *mlo*-plants exhibit very different levels of susceptibility to mildew (ANDERSEN 1991). Relief from water stress and to lesser extent Nitrogen supply are known to increase the mildew susceptibility of *mlo*-plants (BAKER *et al.* 1998, NEWTON & YOUNG 1996). Leaf treatment with mannose and some other chemicals (LYNKGKJAE *et al.* 1997) suppresses the *mlo*-based resistance. The present study deals with the effect of exposure of leaf segments of barley varieties with and without the *mlo*-gene to low and high tempera-

ture prior to artificial inoculation with two partially *mlo*-virulent mildew cultures and with the duration of heat induced susceptibility.

MATERIALS AND METHODS

Barley lines used in the experiments

Diamant, an old Czech variety without known resistance genes and susceptible under field and laboratory conditions

SM4142, a super-susceptible mutant of Diamant, on which approximately 2.5 times more mildew colonies than on its mother variety develop from the same amount of inoculum

HLN70-8, a *mlo*-mutant of Diamant, the genitor of all commercial barley varieties with the *mlo9*-allele (e.g. Alexis)
Apex, a variety with the *mlo11*-allele from an Ethiopian landrace, on which approx. three times more mildew colonies than on HLN 70-8 develop from the same amount of inoculum.

The seeds for the experiments were harvested in 2000 from small single plant progeny plots, selected for plant type and mildew reaction.

Mildew cultures

HL-3, a partially *mlo*-virulent culture, developed in the laboratory by almost 40 generations of mass screening from the *mlo*-avirulent culture GE-3 (SCHWARZBACH 1979). The culture was maintained for about 20 years at the Riso National Laboratory and obtained back in 1997. PV-4, a partially *mlo* virulent culture, collected from the air in southern Moravia in 1999 during a not published mildew monitoring experiment.

The culture HL-3 was originally 50–100 times more *mlo*-virulent than its ancestor GE-3 and had a 10% disease efficiency on *mlo*-hosts, compared with susceptible hosts. During the 23 years of its existence and travel between laboratories, HL-3 was frequently multiplied and maintained on *mlo*-hosts. Since re-inoculation is necessary at least once per month, HL-3 must have been multiplied for at least 280 conidial generations, partly on *mlo* hosts. The *mlo*-virulence of HL-3 likely increased further. In own recent experiments its disease efficiency on HLN70-8 was about 50–80% relative to the susceptible isogenic variety Diamant.

Conditions of growth and inoculation

Untreated seeds of the tested varieties were sown in plastic pots of 7 cm inner diameter in well mixed and sieved gardener soil, prepared uniformly in advance for all experiments. Forty seeds were arranged in each pot in a circle and covered with 15 mm of soil. The pots were irrigated by capillarity through the bottom and protected against infection by cellophane bags, using a wick system described earlier (OPPITZ & SCHWARZBACH 1974). The irrigation water contained 0.1% of Schering's WUXAL-SUPER nutrient solution. The seedlings were grown at 18–19°C at 30 cm distance from a cool white fluorescent source of 40 W output. Primary leaves of 9–10 days old seedlings were used for the experiments. Four 25 mm long leaf segments of each variety were laid out in a randomised arrangement in Petri-dishes on a

medium containing 0.3% agar, 20 ppm benzimidazole and 50 ppm WUXAL-SUPER nutrient solution. To ensure uniform inoculum quality and inoculation density, a sufficient number of segments of SM4142 was uniformly inoculated in advance in a settling tower with moderate inoculation density. The settling tower was a 65 cm high metal tube of 25 cm diameter, into which the mildew was blown by a thrust of 1500 ml of air through openings in the top. After 9 days of incubation at 17°C under dim fluorescent light two segments with sporulating colonies were used as the inoculum source for each settling tower infection. The inoculation density was examined under a microscope by counting the spores in the viewfield and, if necessary, adjusted. In this way, the inoculation density was usually around 850 spores/cm² and the differences between inoculations in an experimental series were not significant. The infected leaf segments were kept under continuous dim fluorescent light (13 W at a distance of 60 cm from the segments) at 17°C. After 5–6 days of incubation the colonies on the leaf segments were counted.

The heat treatment consisted in putting the closed Petri-dishes with leaf segments into a not illuminated thermostat adjusted to 36°C. After the specified period of time the Petri-dishes were allowed to cool at a glass plate at laboratory temperature for 5 minutes before inoculation.

Statistical evaluation

The variances in the experiments were very inhomogeneous, because the susceptibility of the barley lines differed by two orders of magnitude, the studied effects were not additive and additional stochastic errors were present if the numbers of colonies/cm² was low. No Analysis of variance was therefore performed. Instead, significance of effects was evaluated separately for the compared treatments, using the variance between replicates as the basis for comparisons. Differences between compared testaments or varieties were considered signif-

Table 1. Disease efficiency of the mildew cultures HL-3 and PV-4 on primary leaf segments of four barley varieties

| Host | | HL-3 | | | PV-4 | | |
|---------|---------------|--------------------------|-------------------------|-------|--------------------------|-------------------------|-------|
| Variety | allele | colonies/cm ² | relative (%) to Diamant | sign. | colonies/cm ² | relative (%) to Diamant | sign. |
| SM4142 | <i>Mlo</i> | 99.8 | 241.1 | a | 103.5 | 262.7 | a |
| Diamant | <i>Mlo</i> | 41.4 | 100.0 | b | 39.4 | 100.0 | b |
| Apex | <i>mlo</i> 11 | 35.7 | 86.2 | b | 8.0 | 20.3 | c |
| HLN70-8 | <i>mlo</i> 9 | 32.4 | 78.3 | b | 1.4 | 3.55 | d |

sign. – values with the same letter are not different at an error probability of $p \leq 0.01$

ificant, if their confidence intervals, calculated separately for the chosen probability level, did not overlap. This is a stronger test than the t-test, and has the benefit of safer conclusions and transparency. In many cases the observed effects were evident and beyond the need of statistical testing.

RESULTS

The interactions of the four barley lines with the two mildew cultures, expressed as colonies/cm² and relative to Diamant, are summarised in Table 1. The figures are pooled results from not temperature treated leaf segments of experiments described later.

The effect of temperature was first tested with the partially *mlo*-virulent culture PV-4. Within each step of treatment duration one Petri-dish was exposed to 1°C, one to laboratory temperature (18°C) and one to 36°C in a thermostat. After the specified period of time the three Petri-dishes with leaf segments were exposed simultaneously in a settling tower to uniformly dispersed mildew conidia

of PV-4. After incubation the colonies/cm² were evaluated and the results from temperature treated segments compared with those from non-treated segments. The Tables 2 and 3 summarize the effects of cooling or heating, respectively, of leaf segments prior to inoculation with the culture PV-4.

The results indicate clearly, that heat treatment at 36°C increased dramatically the susceptibility to the mildew culture PV-4 and that the higher the level of partial resistance, the higher was the relative increase in susceptibility. This is not surprising, since the higher the partial resistance, the higher is also the available space for the relative increase of susceptibility. The mildew culture HL-3 is considerable more *mlo*-virulent than PV-4. To investigate the relation of heat induction of susceptibility to the level of *mlo*-virulence, a similar series of experiments was performed with the culture HL-3. The Tables 4 and 5 summarise the effects of cooling or heating, respectively, of leaf segments prior to inoculation with the culture HL-3.

The results indicate a considerable increase in susceptibility by heat treatment prior to infection also with the more *mlo*-virulent culture HL-3. Although the relative

Table 2. Effect of leaf segment cooling on susceptibility to the mildew culture PV-4 (colonies/cm² on simultaneously infected leaf segments of four varieties)

| Host | Allele | Pooled non-treated segments | Duration of exposure to 1°C | | | Mean at 1°C | |
|---------|--------------|-----------------------------|-----------------------------|--------|---------|---------------------|--------------|
| | | | 30 min | 60 min | 120 min | col/cm ² | relative (%) |
| SM4142 | <i>Mlo</i> | 103.5 | 100.0 | 87.2 | 117.3 | 101.5 ns | 98 |
| Diamant | <i>Mlo</i> | 39.4 | 30.6 | 36.9 | 52.5 | 40.0 ns | 101 |
| Apex | <i>mlo11</i> | 8.0 | 6.1 | 7.8 | 8.1 | 7.3 ns | 92 |
| HLN70-8 | <i>mlo9</i> | 1.4 | 1.1 | 0.6 | 4.4 | 2.0 ns | 145 |

Comparison with non-treated segments: ns not significant at an error probability of $p \leq 0.05$

Differences between the three treatments are not significant

Differences in colonies/cm² between the four varieties are highly significant

Table 3. Effect of leaf segment heating on susceptibility to the mildew culture PV-4 (colonies/cm² on simultaneously infected leaf segments of four varieties)

| Host | Allele | Pooled non-treated segments | Duration of exposure to 36°C | | | Mean at 36°C | |
|---------|--------------|-----------------------------|------------------------------|--------|---------|---------------------|--------------|
| | | | 30 min | 60 min | 120 min | col/cm ² | relative (%) |
| SM4142 | <i>Mlo</i> | 103.5 | 134.6 | 164.8 | 157.2 | 152.2** | 147 |
| Diamant | <i>Mlo</i> | 39.4 | 46.8 | 41.3 | 93.9 | 60.7** | 154 |
| Apex | <i>mlo11</i> | 8.0 | 9.6 | 25.9 | 12.2 | 15.9** | 199 |
| HLN70-8 | <i>mlo9</i> | 1.4 | 5.9 | 7.1 | 8.5 | 7.2** | 512 |

Statistical significance:

Comparison with non-treated segments: **significant at error probability of $p \leq 0.01$

Differences between the three heat treatments are not significant

Differences between the four varieties are significant in colonies/cm²

Table 4. Colonies/cm² of culture HL-3 on simultaneously infected leaf segments of four varieties, exposed to 1°C prior to infection

| Host | Allele | Pooled non-treated segments | Duration of exposure to 1°C | | | Mean at 36°C | |
|---------|--------------|-----------------------------|-----------------------------|--------|---------|---------------------|--------------|
| | | | 30 min | 60 min | 120 min | col/cm ² | relative (%) |
| SM4142 | <i>Mlo</i> | 99.8 | 64.2 | 78.6 | 60.0 | 67.6* | 67.7 |
| Diamant | <i>Mlo</i> | 41.4 | 38.2 | 23.5 | 35.3 | 32.3 ns | 78.0 |
| Apex | <i>mlo11</i> | 35.7 | 29.5 | 33.1 | 31.1 | 31.3 ns | 87.4 |
| HLN70-8 | <i>mlo9</i> | 32.4 | 29.0 | 25.7 | 30.8 | 28.5 ns | 88.0 |

Statistical significance:

Comparison with non-treated segments: *significant at error probability of $p \leq 0.05$, ns not significant

Differences between the three cold treatments are not significant

SM4142 is significantly different from the remaining three varieties in colonies/cm²

Table 5. Colonies/cm² of culture HL-3 on simultaneously infected leaf segments of four varieties, exposed to 36°C prior to infection

| Host | Allele | Control (18°C) | Duration of exposure to 36°C | | | Mean at 36°C | |
|---------|--------------|----------------|------------------------------|--------|---------|---------------------|--------------|
| | | | 30 min | 60 min | 120 min | col/cm ² | relative (%) |
| SM4142 | <i>Mlo</i> | 99.8 | 190.9 | 183.3 | 150.0 | 174.7** | 175.0 |
| Diamant | <i>Mlo</i> | 41.4 | 57.6 | 116.9 | 92.3 | 88.9** | 214.8 |
| Apex | <i>mlo11</i> | 35.7 | 53.7 | 79.1 | 62.5 | 65.1** | 182.1 |
| HLN70-8 | <i>mlo9</i> | 32.4 | 57.3 | 66.8 | 76.9 | 67.0** | 206.8 |

Statistical significance:

Comparison with non-treated segments: **significant at error probability of $p \leq 0.01$

Differences between the three heat treatments are not significant.

SM4142 is significantly different from the remaining three varieties in colonies/cm²

Table 6. Duration of heat-induced susceptibility to mildew (Colonies/cm² of PV-4, relative to simultaneously infected not heat treated segments)

| Host | | Mean col/cm ² on control segments = 100 % | Coefficient of variation s/M | Susceptibility relative (%) to untreated segments at different time after heat treatment* | | | |
|-------------|--------------|--|------------------------------|---|------|-----|------|
| Barley line | allele | | | 0 h | 2 h | 5 h | 13 h |
| SM4142 | <i>Mlo</i> | 97.42 | 0.17 | 137 | 110 | 129 | 117 |
| Diamant | <i>Mlo</i> | 36.35 | 0.21 | 158 | 142 | 126 | 144 |
| Apex | <i>mlo11</i> | 4.51 | 0.18 | 381 | 460 | 140 | 146 |
| HLN70-8 | <i>mlo9</i> | 0.89 | 0.34 | 1291 | 1293 | 797 | 134 |

*Heat-treatment 1h at 36°C

Heat-treated and non-treated segments of the four varieties within the same time-step were inoculated simultaneously. Inoculation density was kept approx. at 850 spores/cm²

increase is smaller than with the culture PV-4, the actual level of susceptibility of the *mlo*-barley lines is higher than with PV-4.

The duration of induced susceptibility was tested in another experimental series. The mildew culture PV-4 was used, since with it the largest effect was observed. Only two leaf segments of each variety were laid out in Petri-

dishes, exposed for one hour to 36°C and stored for the specified periods of time at laboratory temperature. Immediately before inoculation two freshly cut leaf segments of each variety were supplemented to the Petri-dish, so that at inoculation four segments of each variety were in the dish. After inoculation and incubation as in the previous experiments the colonies/cm² on the heat treated

segments were compared with those on non-treated segments. The results are summarised in Table 6.

Still higher relative increase of susceptibility by heat treatment could be expected from the use of a *mlo*-avirulent culture. Unfortunately, no such culture was available. In recent agricultural environments in Europe pathotypes with a so low *mlo*-virulence as had GE-3, from which the partially *mlo*-virulent culture HL-3 has been developed, are not very frequent.

DISCUSSION

The temperature of 36°C was chosen arbitrarily and is not necessarily the most inducing temperature. It is, however, within the range of temperatures occurring locally on hot days in agricultural environments in Europe and harmless for the leaf tissue. Already a short exposure can raise the susceptibility considerably. On hot days also water stress can occur. Relief from water stress dramatically increases mildew susceptibility of *mlo*-varieties (NEWTON & YOUNG 1996; BAKER *et al.* 1998). So far we don't know, how heat induction and relief from water stress interact. It is possible, that both factors together boost susceptibility to levels permitting high levels of the disease in fields of *mlo*-barley. This would then explain the occasionally observed outbreaks of mildew in fields of *mlo*-barley. However, more research in this direction is needed.

The heat induction represents also a simple tool for monitoring and collecting partially *mlo*-virulent mildew pathotypes. The temperature of 36°C is harmless for the leaves and its effect persists for several hours. Petri-dishes with segments of *mlo*-barley can be exposed in advance to 36°C and then used directly for collecting in mobile spore traps on sampling tours.

Abstrakt

SCHWARZBACH E. (2001): **Teplem indukovaná náchylnost ječmene s genem *mlo* k padlí travního (*Blumeria graminis* D.C. f.sp. *hordei* Marchal).** Czech J. Genet. Plant Breed., 37: 82–86.

Segmenty primárních listů dvou linií ječmene s genem *mlo*, extrémně náchylné SM4142 a středně náchylné linie Diamant, a dvou linií ječmene s genem *mlo*, odolnější HLN70-8 a méně odolné Apex, byly infikovány izoláty PV-4 a HL-3 padlí travního, lišícími se úrovní parciální virulence vůči *mlo*. Listové segmenty, vyložené v Petriho miskách na agarovém médiu, byly vystaveny po 30, 60 a 120 min teplotě 36 °C a následně v sedimentační věži rovnoměrně infikovány konidiami zmíněných kultur padlí. Po 5–6 dnech inkubace při 17–18 °C byly vzniklé kupky padlí spočítány a srovnány s počtem kupek vyrostlých na současně infikovaných segmentech nevystavených předtím působení zvýšené teploty. U všech zkoušených linií ječmene a kultur padlí způsobila zvýšená teplota zvýšení náchylnosti k padlí. Největší nárůst, zhruba o jeden řád, byl zjištěn u nejodolnější linie ječmene (HLN70-8) a méně *mlo*-virulentní kultury PV-4. Ochlazení segmentů před infekcí po dobu 30, 60 a 120 min proti tomu nevedlo k průkaznému snížení náchylnosti vůči kultuře PV-4 a jen k mírnému snížení

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náchylnosti ke kultuře HL-3. Náchylnost indukovaná zahřátím byla dočasná a poklesla během 13 h zhruba na 130 % ve srovnání se segmenty nevystavenými zvýšené teplotě. Protože teplota 36 °C se vyskytuje ve venkovním prostředí, může se teplem indukovaná náchylnost k padlí podílet na občas pozorovaném intenzivnějším výskytu padlí v porostech odrůd s genem *mlo*. Teplem indukovaná náchylnost k padlí může být též využita pro sběr parciálně *mlo*-virulentních patotypů z venkovního prostředí.

Klíčová slova: ječmen; padlí travní; *Blumeria graminis*; *mlo*; odolnost; tepelná indukce náchylnosti

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The Search for Genetic Resources of Opium Poppy (*Papaver somniferum*) with High Thebaine Content and the Development of a Screening Method

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Abstract: Thebaine is an important source for the production of non-addictive analgesics in the pharmaceutical industry. The aim of the project was to develop a screening method for the determination of alkaloid composition in poppy and to find genetic resources of opium poppy (*Papaver somniferum*) with high thebaine content. A modified thin-layer chromatography appeared to be the most suitable method for the preliminary determination of alkaloid composition. This method was applied to breeding material and to varieties from the collection of poppy genetic resources. In the material from 1998 there were approximately 9% of plants with higher percentage of thebaine. In the material from the years 1999 and 2000 the proportion of such plants was about 15.5% and 11%, respectively. Most of the plants had besides thebaine also a high content of other alkaloids, predominantly morphine. Five plants were rich only in thebaine and one plant had a high content of codeine. The stability of these traits will be tested further.

Keywords: *Papaver somniferum*; thebaine content; thin-layer chromatography (TLC); genetic resources

The increasing risk of drug abuse forces the drug manufactures to produce safer drugs, which cannot be easily abused. The UN Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances was signed in 1988. Countries were forced by the convention to introduce new arrangements in poppy production including selection and production of new cultivars. BERNATH and NEMETH (1999) discuss the effectiveness and possibilities of traditional and advanced breeding methods for the obtaining of cultivars accumulating low or high alkaloid content or plant material with a special alkaloid spectrum (codeine, thebaine, narcotine). Cultivars with low or high alkaloid content are registered (SHARMA *et al.* 1999) and genotypes with different alkaloid spectrum are developed.

An important source material for the production of non-addictive analgesics is the alkaloid thebaine. Thebaine is used in the production of a number of semisynthetic morphinan derivatives – butorphanol (strong and safe analgesic) naloxone (morphine antagonist), nalmefene (morphine antagonist, which reduces the permanent damage to the central nervous system after a stroke) and buprenorphin (strong and safe analgesic). Thebaine, as

well as other morphine-type alkaloids (codeine, morphine) cannot be synthetically produced. For the needs of the pharmaceutical industry thebaine is imported. The attempts to grow poppy (*Papaver bracteatum*) as a domestic source of thebaine failed, because it is a perennial plant with slow initial development and its agronomy characteristics makes it unsuitable for commercial growing.

Since thebaine is a precursor of codeine and morphine, there might exist a genotype in which thebaine conversion to codeine and morphine is inhibited. In literature, apart from other methods (e.g. spectrophotometry, isotachopheresis) most studies employed chromatographic screening methods for thebaine content. Of gas, thin-layer, column and paper chromatography, the most suitable screening method is thin-layer chromatography (TLC) (GUPTA & VERMA 1996), and high pressure liquid chromatography (HPLC) the most accurate method of analysis (ANONYM 1996; GOMEZ-SERRANILLOS *et al.* 1995; KRENN *et al.* 1998).

The aim of the study was to develop a screening method for the preliminary determination of thebaine content in poppy capsules and to use this method to find genetic resources of poppy with high thebaine content.

MATERIAL AND METHODS

The search for genetic resources of poppy with high content of thebaine was performed on material from three localities, including Opava, Keřkov and Prague. The materials from Opava and Keřkov comprised varieties of a collection of oil crop genetic resources and poppy breeding lines at different stages of development. At Prague the materials involved poppy plants at the Czech University of Agriculture in Prague derived from the local varieties of the Bohemian-Moravian Highlands and from Turkey and India. In all localities a large number of different genotypes was grown with the aim to record the highest possible genetic variability.

Five to twenty plants of each origin were isolated immediately before blooming. Calyx and corolla petals were removed from the straightening buds prior to blooming and the remaining part of the flower was isolated using a paper bag. The isolated flowers were marked with a colored ribbon. After natural self-pollination and the stigma died off (one week later), the bags were removed. The mature plants were harvested and all capsules including the non-isolated ones were manually shelled. The isolated seeds from each plant were marked and stored separately. To ensure a sufficiently large size of samples for chemical analysis, the capsules from the whole plant (including the non-isolated ones) were used. Very hard nodes, which cannot be ground in the mills, were removed from the capsules. The remnants were ground and passed through a fine sieve so that the particles were of uniform size.

Screening method – procedure of analysis

Sample preparation: 0.5 g of finely ground capsules were added to 25 ml of an extraction mixture of chloroform, methanol and 26% ammonia at the ratio 15:9:1 and kept in a wide-mouth bottle. The bottle with the mixture was closed and allowed to stand overnight. The second day it was shaken for 8 hours, again allowed to stand overnight and finally shaken for another 2 hours. The extract was filtered and the filtrate was thickened from the volume of 5 ml to 1 ml by blowing nitrogen to the surface at increased temperature.

A 20 µl sample of the extract was spread on a thin-layer chromatography plate of Silicagel 60 F₂₅₄ and allowed in the chamber to develop by using a mixture of diethyl ether, chloroform, methanol and 26% ammonia at the ratio 30:5:3:1. After termination the chromatogram was detected using Dragendorff's reagent and visually assessed. The method is suitable for the preliminary determination of the presence or absence of thebaine. The promising materials must be further tested by an accurate method. After the first cycle of analysis it was found that this method covers the whole spectrum of alkaloids. One part of the analysis included also the determination of codeine and morphine.

The accurate method for the determination of thebaine in poppy

Procedure of the analysis: The analysis of the content and composition of alkaloids utilizes a filtered extract prepared in a way as described above. 5 ml of extract is evaporated and reconstituted in 5 ml of methanol with 4 ml/l triethylamine (TEA) in an ultrasound bath for 3 min. The sample is passed through 0.45 mm membrane filter before spraying.

Conditions of the chromatographic analysis:

column: Hypersil ODS 5 µm, 250 × 4.6 mm

column temperature: 35°C

mobile phase: mixture of acetonitrile and water 45:55 with 2 ml/l TEA

flow rate of the mobile phase: 1.5 ml/min

wavelength: 286 nm

dosing loop: 50 µl

RESULTS AND DISCUSSION

The TLC screening method (GUPTA & VERMA 1996) was successfully modified so, that the prepared extract of alkaloids could be readily analyzed by the accurate HPLC method (ANONYM 1996; GOMEZ-SERRANILLOS *et al.* 1995; KRENN *et al.* 1998).

This procedure enables routine analysis of samples preselected by the TLC method, with no extra costs for

Table1. Determination of thebaine in materials of 1998

| Number of varieties/lines | Number of analysed plants | Number of plants with high thebaine content |
|---|---------------------------|---|
| Opava – varieties of the world collection | | |
| 32 | 234 | 25 |
| Opava – breeding lines | | |
| 52 | 318 | 34 |
| Keřkov – breeding lines | | |
| 19 | 154 | 8 |

the preparation of extracts needed for HPLC. The size of poppy capsule samples is limited and in some cases it is not large enough for repeated extraction. The TLC-screening method was also calibrated using HPLC. A high content of thebaine corresponds to the value of 0,1% or even higher.

Table 1 gives the results of screening of breeding materials from Opava and Keřkov from 1998. Approximately 9% of genotypes of these provenance were high in thebaine content.

In 1999 the screening continued at Opava and Keřkov within the collection of poppy genetic resources and of breeding lines. The lines with high thebaine content, identified in 1998, were grown. Genotypes, derived from crossing local varieties from the Bohemian-Moravian Highlands with poppy plants from Turkey and India, were cultivated at the Czech Agricultural University in Prague. In Opava the poppy stands suffered severe damage by torrential rains and storms and the stands lodged completely before flowering. For this reason, only a small part of the necessary number of plants was isolated and yield assessment was not possible. The isolated plants were harvested from the breeding materials and from lines with high thebaine content identified in 1998. The varieties from genetic resources were completely damaged.

Table 2 gives the results of screening tests of genotypes from the year 1999. A more precise screening method was used to analyze the material from the Czech University of Agriculture in Prague – a total of 225 plants from 34 lines. Thirty-seven plants with high thebaine

content were found, 6 plants showed a higher codeine content. The majority of plants with high thebaine content had also high morphine content, only 2 plants combined increased thebaine content and low content of other alkaloids. In the breeding material from Opava all the 13 plants with high thebaine content had also increased morphine content. Similar results were obtained in Keřkov. A total of 193 plants from 38 blue-seeded and white-seeded varieties of poppy in the world collection and 207 plants from 18 white-seeded breeding lines were analyzed. All materials from Keřkov had high morphine content, 76 plants had high thebaine content and 38 plants had high codeine content. No plants with high thebaine or codeine content and low content of other alkaloids were found in this material.

The analysis of the progeny of plants selected in 1998 for high thebaine content showed a rather poor stability of this trait. Out of 61 analysed plants from Opava, only 10 (16.4%) had high thebaine content. All these plants had also high morphine content. In the material from Keřkov out of 120 plants from 7 lines, high in thebaine in 1998, only 12 plants had high thebaine content in 1999. These plants had also high morphine content. No progeny from both localities showed a high and stable thebaine content.

The spring of the year 2000 was exceptionally warm and dry. The emergence was poor and the poppy stands therefore very thin. Only a small number of plants could be harvested and therefore the number of analyzed plants was also small. The results are given in Table 3.

Table 2. Determination of thebaine in materials of 1999

| Number of lines/varieties | Analysed plants | Number of plants with high content of | | | |
|---|-----------------|---------------------------------------|---------|----------|---------------|
| | | thebaine | codeine | morphine | thebaine only |
| Opava – breeding lines under development | | | | | |
| 44 | 101 | 13 | 0 | 101 | 0 |
| Opava – progeny from lines with high thebaine content | | | | | |
| 24 | 61 | 10 | 3 | 60 | 0 |
| ČZU Praha – lines | | | | | |
| 34 | 225 | 37 | 6 | 207 | 2 |
| Keřkov – varieties of the world collection | | | | | |
| 38 | 193 | 46 | 13 | 193 | 0 |
| Keřkov – breeding lines | | | | | |
| 8 | 107 | 29 | 15 | 107 | 0 |
| Keřkov – progeny from lines with high content of thebaine | | | | | |
| 7 | 120 | 12 | 16 | 120 | 0 |
| Keřkov – comparative experiment | | | | | |
| 10 | 100 | 18 | 9 | 100 | 0 |

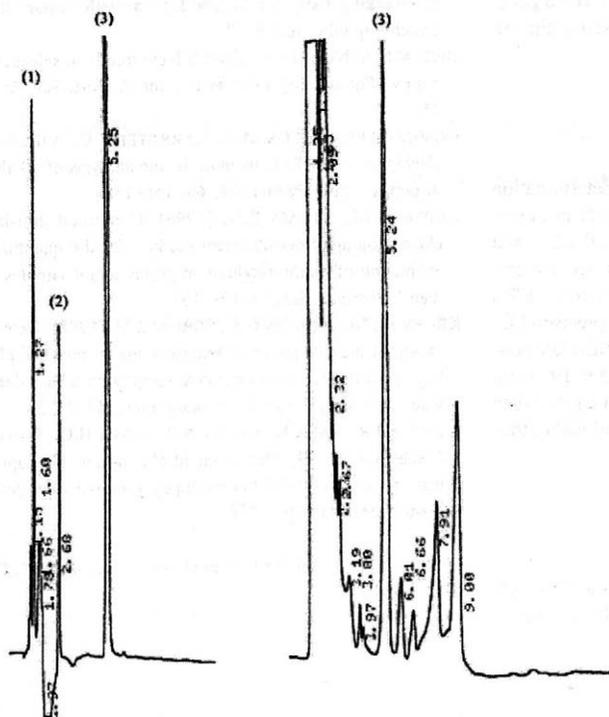


Fig. 1. The HPLC analysis of poppy alkaloids: mixture of morphine, codeine and thebaine (left) and analysed sample of alkaloid extract (right)

(1) = morphine, (2) = codeine, (3) = thebaine

Out of 532 analyzed plants only 49 (about 9%) had a high content of thebaine. Only 3 plants with high thebaine content had a low content of other alkaloids. One plant only had a high content of codeine.

The study of genetic resources of poppy revealed that in the poppy populations there were about 10% of plants with a high content of thebaine. In most of these plants, however, the high thebaine content is associated with a high level of morphine, and in some plants also of co-

deine. Types with a very low content of all alkaloids, described by SHARMA *et al.* (1999), which were also found in the studied population, are interesting as genetic resources. Five genotypes had a high content of thebaine and a low content of other alkaloids. These may represent the most valuable genetic resources for breeding. The stability of thebaine content in these genotypes has not yet been tested. BERNATH and NEMETH (1999) describe the possibilities to obtain the poppy cultivars

Table 3. Determination of thebaine in materials of 2000

| Number of lines/varieties | Analysed plants | Number of plants with high content of | | | |
|--|-----------------|---------------------------------------|---------|----------|---------------|
| | | thebaine | codeine | morphine | thebaine only |
| Opava – breeding lines under development | | | | | |
| 59 | 150 | 22 | 56 | 125 | 2 |
| Opava – varieties from a collection of genetic resources | | | | | |
| 31 | 78 | 4 | 17 | 72 | 0 |
| Opava – winter poppy | | | | | |
| 6 | 60 | 0 | 20 | 60 | 0 |
| Kefkov – breeding material under development | | | | | |
| 10 | 122 | 19 | 9 | 110 | 1 |
| ČZU – lines | | | | | |
| 5 | 122 | 4 | 4 | 110 | 0 |

exhibiting a special alkaloid spectrum. The found genotypes varying in alkaloid content can be used as the initial material for such research types.

CONCLUSION

A screening method for the preliminary determination of the composition and content of alkaloids in poppy capsules, based on TLC, and an accurate HPLC-based method was developed and successfully applied. Among varieties and breeding lines tested in 1998, there were 9% of plants with high thebaine content. A more precise TLC-based method revealed marked variability of alkaloid content in individual poppy genotypes analyzed in 1999 and 2000. The stability of thebaine content in the genotypes was investigated. A progeny with high and stable thebaine content has not yet been found.

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Abstrakt

HAVEL J., KOLOVRAT O., KAMENÍKOVÁ L., BECHYNĚ M. (2001): Vyhledávání genetických zdrojů máku setého s vysokým obsahem tebainu a vývoj screeningové metody. Czech J. Genet. Plant Breed., 37: 87–91.

Tebain je významný výchozí materiál ve farmaceutickém průmyslu pro výrobu nenávykových analgetik. Cílem projektu bylo vyvinout screeningovou metodu pro stanovení skladby alkaloidů u máku a nalézt genetické zdroje máku setého (*Papaver somniferum*) s vysokým obsahem tebainu. Jako nejvhodnější metoda pro předběžné stanovení skladby alkaloidů se ukázala modifikovaná metoda tenkovrstvé chromatografie. Tato metoda byla použita k testování šlechtitelských materiálů a odrůd z kolekce genetických zdrojů máku. V materiálech z roku 1998 bylo zhruba 9 % rostlin s vyšším obsahem tebainu. V letech 1999 a 2000 bylo zastoupení těchto rostlin kolem 15,5 a 11 %. Většina rostlin měla kromě vyššího obsahu tebainu též vyšší obsah ostatních alkaloidů, především morfinu. Jen pět rostlin mělo vysoký obsah tebainu a jedna rostlina vysoký obsah kodeinu. Stabilita těchto znaků bude dále testována.

Klíčová slova: *Papaver somniferum*; obsah tebainu; tenkovrstvá chromatografie (TLC); genetické zdroje

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Productivity and Diversity of Agronomic Characters in Selected Cultivars of Eleven Alternative Crops

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Abstract: Diversity among cultivars and ecotypes (215 accessions of eight species of alternative crops) has been studied in the years 1996 and 1997 and promising materials were selected for further evaluation. Large variability has been detected in flowering time as well as in maturity. The extent of variability in seed weight was crop-specific (CV 7.2–21.5%). The variability of characters was relatively low in crambe and false flax, large in Abyssinian mustard, rocket and very large in foxtail millet and Indian mustard. Twenty four cultivars, belonging to 11 species, were selected and evaluated in subsequent field trials at three locations in the years 1998–2000. Early flowering and maturity was found in the examined cultivars of buckwheat, false flax and crambe (up to 107 days), and also in black mustard, fiddleneck and panic millet (110–112 days). In contrast, Indian mustard, foxtail millet and Abyssinian mustard had a longer vegetation period (126–149 days). All evaluated mustard species, especially white mustard and Indian mustard, and foxtail millet produced very tall plant stands, especially under the humid and cool conditions at the location Humpolec (1.1–1.8 m). Apart of buckwheat, relatively large seeds were found in some cultivars of white mustard, crambe and Abyssinian mustard (TSW 5–7 g). White mustard and crambe had the highest mean seed yields (both 2.34 t/ha) followed by foxtail millet (2.22 t/ha) and panic millet (2.04 t/ha). The white mustard cultivar Nakielska and the ecotype SET 621/91 of foxtail millet had the highest seed yields and acceptable earliness. The three experimental locations differed in climate: Prague-Ruzyně was intermediate, Humpolec was humid and cooler, and Olomouc had a warm climate. Humid cool conditions increased the yield, plant height and vegetation period in most crops, especially in all mustards. Under warmer climatic conditions crambe was the best yielding crop.

Keywords: alternative crops; diversity; earliness; plant height; seed weight; seed yields

Maintenance and utilization of biological diversity is considered a precondition of sustainable development of agriculture. Bio-diversity relates to both broader genetic base of present cultivars of agricultural crops and to a wider spectrum of crops in agriculture. The widening of crop spectra can contribute to a better commercialization of the products, to the utilization of specific local conditions (especially in marginal regions) and reduce the input requirements (agrochemicals, energy). The increase of crop diversity can in this way help to implement principles of sustainable development in agriculture. Beside this, alternative crops can contribute to the maintenance and improvement of soil fertility (increase of organic matter, “catch effect” of soil nitrogen, protection of soil against erosion, sanitary leverage on soil or other specific impacts).

Among mustard species, which were involved in the trials, white (yellow) mustard is usually reported as a crop providing relatively high (1.4–3.0 t/ha) and stable seed yields (BRANDT 1992; AULD *et al.* 1993; NIKITCHIN *et al.* 1994; BROWN *et al.* 1999; MUSNICKI *et al.* 1997). Also Abyssinian mustard and Indian (brown) mustard are considered as crops providing relatively good seed yields. AULD *et al.* (1993) reported from Idaho (Canada) mean yields of five tested cultivars of 1.8 t/ha in Abyssinian mustard, 1.6 t/ha in black mustard and 1.2 t/ha in Indian mustard, with the highest yield of the latter reaching 10.3 t/ha with the cv. 72-525. ROHTASH SINGH *et al.* (1993) reported identical seed yields in both above-mentioned mustard species (1.8 t/ha). BUKHTIAR *et al.* (1992) found in field trials the highest yield of 1.5 t/ha in Abyssinian mustard, followed by Indian mustard, while rocket was

less productive (0.7 t/ha). GETINET *et al.* (1996) noted in some years good yield in Abyssinian mustard grown in Saskatchewan. However, the yields fluctuated between growing seasons. MUSNICKI *et al.* (1997) and TOBOLA and MUSNICKI (1999) analyzed results of long-term field experiments with spring oilseed crops in Poland. The best yielding cruciferous crop was white mustard (mean 1.54 t per ha), followed by crambe. The yield of white mustard were also very stable, while Indian mustard had moderate stability and false flax and crambe showed high yield variability.

Crambe is becoming a competitive crop in some regions of USA (JOHNSON *et al.* 1996). Crambe and false flax are also considered as convenient alternative oil crops for Central Europe (HAVEL 1995). Crambe performed well in Austria, where seed yields ranged from 1.0 to 3.3 t/ha (VOLLMANN & RUCKENBAUER 1993), in Poland 2.8–3.7 t per ha (KULIG *et al.* 1996) and Italy 0.8–3.0 t/ha (LAZZERI *et al.* 1995). STRAŠIL and SKALA (1997) reported slightly

lower yields (1.4–2.0 t/ha) in Czechia from experiments at three locations during three years. Also false flax provided relatively good seed yields, MÜLLER *et al.* (1999) reported a mean yield of 1.9 t/ha in Germany. False flax seems to be suitable also for low-input growing conditions and reduced tillage systems (PUTMAN *et al.* 1993). Rocket (*Eruca sativa*) is a rather rare oil crop in Czechia. An important trait in this crop can be considered salt-tolerant (tolerant genotypes were found e.g. by ASHRAF & NOOR 1993).

Buckwheat, proso millet and foxtail millet represent another group of alternative crops with starchy seeds, which provide relatively high yield and can be utilized for food and fodder. Proso millet yields up to 6.0 t/ha, while buckwheat and foxtail millet yield significantly less (TIMMAIAH *et al.* 1989; AUFHAMMER & KUBLER 1998; BORGHI *et al.* 1995; MICHALOVÁ *et al.* 1998). Fiddleneck is often used as green manure and can also be used as fodder (PELIKÁN 1998). Seed of fiddleneck is

Table 1. Diversity of cultivars (ecotypes) within species of selected alternative crops (variability in some characters among evaluated accessions of genetic resources)

| Crop | Number of cvs. | Characters | Mean value of characters | Coefficient of variation (%) | Earliest cultivars (days) or cultivars with high TSW (g) |
|--------------------|----------------|------------|--------------------------|------------------------------|--|
| White mustard | 48 | FLW (days) | 59 | 4 | Sabre (43), Ochre (43) |
| | | MTR (days) | 125 | 4.9 | Nakielska (107) |
| | | TSW (g) | 6.07 | 13.2 | Metex (9.4), Sabre (9.3) |
| Abyssinian mustard | 28 | FLW (days) | 83 | 5 | Canada (50) |
| | | MTR (days) | 135 | 5.1 | Canada (132) |
| | | TSW (g) | 3.47 | 16.6 | CGN 4001 (9.4) |
| Indian mustard | 35 | FLW (days) | 69 | 8.7 | VNII-MK (46) |
| | | MTR (days) | 128 | 5.9 | CGN 7151 (105) |
| | | TSW (g) | 1.8 | 21.5 | Mustard (2.9) |
| Black mustard | 27 | FLW (days) | 54 | 8.4 | RCAT 040343 (43) |
| | | MTR (days) | 121 | 7 | FRA N5A95 (103) |
| | | TSW (g) | 2.35 | 12.7 | RCAT 069393 (3.0) |
| Crambe | 11 | FLW (days) | 76 | 4.4 | Borowska (73) |
| | | MTR (days) | 116 | 2 | BGRC 32855 (115) |
| | | TSW (g) | 4.95 | 7.2 | Borowska (6.1) |
| Rocket | 19 | FLW (days) | 65 | 3.7 | BGRC (22532 (45) |
| | | MTR (days) | 129 | 5 | BGRC 30345 (119) |
| | | TSW (g) | 2.33 | 17.2 | ERU 21/84 (3.1) |
| False flax | 30 | FLW (days) | 70 | 2.4 | Hoga (52) |
| | | MTR (days) | 113 | 2.3 | Hoga (105), Pernice (105) |
| | | TSW (g) | 1.67 | 8.4 | BGR 17634 (2.2) |
| Foxtail millet | 17 | FLW (days) | 104 | 12.7 | SET 621/91 (77) |
| | | MTR (days) | 174 | 11 | SET 621/91 (125) |
| | | TSW (g) | 2.3 | 19.6 | FRA 84-40 (3.0) |

FLW = days to flowering

MTR = days to maturity

TSW = thousand seed weight

very small and has no commercial use except for crop multiplication.

MATERIAL AND METHODS

The Genetic resources of eleven alternative crops in the Research Institute of Crop Production Prague-Ruzyně comprise 240 cultivars and ecotypes. Preliminary evaluation has been carried out in eight crops where larger sets of cultivars were available (11–48 accessions – Table 1). The experiments were sown on plots of 1 m² in Prague-Ruzyně in the years 1996 and 1997 to estimate the variability of cultivars within species and to select promising material for further studies.

Twenty four cultivars and ecotypes, belonging to 11 alternative crop species, were evaluated in field trials at Prague-Ruzyně from 1998 to 2000 for productivity, earliness, plant height and seed weight. The crops were: white (yellow) mustard – *Sinapis alba*, Abyssinian (Ethiopian) mustard – *Brassica carinata*, Indian (brown) mustard – *Brassica juncea*, black mustard – *Brassica nigra*, crambe – *Crambe abyssinica*, rocket (arugula) – *Eruca sativa*, false flax – *Camelina sativa*, fiddleneck (tansy phacelia) – *Phacelia tanacetifolia*, foxtail millet – *Setaria italica*, panic millet (common millet) – *Panicum miliaceum* and buckwheat – *Fagopyrum esculentum*. The trials consisted of four randomized complete blocks of 5 m² plots. Reduced sets of selected cultivars, belonging to eight heterogamous species (one cultivar per crop), were sown also at the locations Humpolec and Olomouc

in the years 1999 and 2000 to estimate the influence of climate and soil conditions and to multiply seeds in isolation. The preceding crops were always cereals. The trials were sown in April, as soon as the soil conditions allowed it (considering also the species seed size). No fertilizer was applied before sowing or during growth. Days from emergence to flowering (about 15% of flowering plants on the plot) and to maturity (more than 50% of mature seeds) were recorded. The seed was dried after harvest to 12–13% moisture. The seed weight per plot and the thousand seed weight (TSW) were determined from dried seeds and seed yield was converted to t/ha. The yield data from replicated trials were statistically evaluated by ANOVA (Table 5) and differences between cultivars or years were tested using the Tuckey test ($P = 0.05$). Climatic characteristics of the three experimental locations are shown in Table 2.

RESULTS AND DISCUSSION

In the first approach, we studied existing diversity of important traits in the available genetic resources of alternative crops with the aim, to select most suitable materials for local conditions. Data on diversity within the selected eight species are given in Table 1. Cultivar diversity of fiddleneck, panic millet and buckwheat was not included, because some data from earlier studies could be utilized and, therefore, only limited numbers of accessions of these crops were tested in preliminary trials.

Table 2. Climatic parameters during periods of vegetation in particular localities and years: mean temperature (°C); sum of precipitation per month (mm)

| Year | Environment | | Month | | | | | | | | | | | | Sum per whole period (mm) |
|----------------------|-------------|--|-------|------|------|------|------|-------|------|-------|--------|------|-----------|------|---------------------------|
| | sowing date | | April | | May | | June | | July | | August | | September | | |
| | | | °C | mm | °C | mm | °C | mm | °C | mm | °C | mm | °C | mm | |
| Prague-Ruzyně | | | | | | | | | | | | | | | |
| 1998 | 15. 4. | | 10.6 | 7.7 | 14.7 | 29.5 | 17.6 | 97.0 | 17.3 | 57.4 | 17.6 | 35.7 | 13.3 | 78.4 | 305.7 |
| 1999 | 14. 4. | | 9.4 | 23.4 | 14.8 | 48.6 | 16.1 | 48.7 | 19.6 | 97.0 | 17.8 | 22.9 | 17.2 | 45.5 | 286.1 |
| 2000 | 20. 4. | | 11.8 | 11.9 | 17.8 | 47.2 | 20.1 | 46.3 | 17.6 | 71.5 | 20.4 | 46.6 | 15.5 | 24.4 | 247.9 |
| Humpolec | | | | | | | | | | | | | | | |
| 1999 | 22. 4. | | 5.3 | 26.3 | 10.6 | 61.8 | 12.1 | 64.1 | 16.1 | 91.6 | 14.5 | 44.0 | 13.1 | 33.7 | 351.6 |
| 2000 | 17. 4. | | 9.0 | 40.8 | 14.3 | 94.2 | 17.0 | 64.5 | 14.3 | 115.9 | 18.2 | 48.0 | 14.8 | 49.5 | 382.8 |
| Olomouc | | | | | | | | | | | | | | | |
| 1999 | 15. 4. | | 10.2 | 59.8 | 14.2 | 29.0 | 16.6 | 113.8 | 20.0 | 71.7 | 17.9 | 39.5 | 17.0 | 39.5 | 353.3 |
| 2000 | 17. 4. | | 12.8 | 12.0 | 16.2 | 21.9 | 18.8 | 37.2 | 16.9 | 131.5 | 20.0 | 29.2 | 13.2 | 14.6 | 246.4 |

Based on the experiments, carried out in Prague-Ruzyně in the years 1996 and 1997, diversity in days to flowering and days to maturity was similar in most of the

evaluated species (slightly higher differences in flowering time might be present in Indian mustard and possibly also in crambe). As indicated by the coefficients of vari-

Table 3. Mean values of agronomically important characters in selected cultivars of eleven alternative crops grown at Prague-Ruzyně in the years 1998–2000

| Crop | Cultivar (ecotype) | Days to flowering | Days of filling period | Days to maturity | Plant height (cm) | Thousand seed weight (g) |
|--|--------------------|-------------------|------------------------|------------------|-------------------|--------------------------|
| White mustard (<i>Sinapis alba</i>) | Zlata | 50.9 | 67.9 | 118.8 | 131.8 | 7.0 |
| | Carine | 50.8 | 67.5 | 118.3 | 121.5 | 6.6 |
| | Nakielska | 50.4 | 67.5 | 117.9 | 131.3 | 6.9 |
| | mean of cvs. | 50.7 | 67.6 | 118.3 | 128.2 | 6.8 |
| Abyssinian mustard (<i>Brassica carinata</i>) | Svp Sao Tome | 67.4 | 81.6 | 149.0 | 104.1 | 5.1 |
| | CGN 4001 | 70.0 | 78.5 | 148.5 | 107.9 | 5.4 |
| | mean of cvs. | 68.7 | 80.1 | 148.8 | 106.0 | 5.3 |
| Indian mustard (<i>Brassica juncea</i>) | Vittasso | 78.4 | 63.6 | 142.0 | 129.4 | 2.4 |
| | Secus | 57.8 | 68.7 | 126.5 | 124.8 | 3.2 |
| | mean of cvs. | 68.1 | 66.2 | 134.3 | 127.1 | 2.8 |
| Black mustard (<i>Brassica nigra</i>) | RCAT 069393 | 50.8 | 58.6 | 109.4 | 121.3 | 1.6 |
| | BGRC 34177 | 50.7 | 59.5 | 110.2 | 101.0 | 1.5 |
| | mean of cvs. | 50.8 | 59.0 | 109.8 | 111.2 | 1.6 |
| Crambe (<i>Crambe abyssinica</i>) | Borowska | 60.7 | 45.1 | 105.8 | 102.2 | 5.8 |
| | Voronezhskii | 62.2 | 44.5 | 106.7 | 98.8 | 5.5 |
| | mean of cvs. | 61.5 | 44.8 | 106.3 | 100.5 | 5.7 |
| Rocket (argula) (<i>Eruca sativa</i>) | K-3 | 59.0 | 65.5 | 124.5 | 86.2 | 1.7 |
| | BGRC 22532 | 53.4 | 67.6 | 121.0 | 82.6 | 1.5 |
| | mean of cvs. | 56.2 | 66.6 | 122.8 | 84.4 | 1.6 |
| False flax (<i>Camelina sativa</i>) | Hoga | 52.6 | 52.5 | 105.1 | 70.7 | 1.1 |
| | Pernice | 53.7 | 52.7 | 106.4 | 66.9 | 1.0 |
| | mean of cvs. | 53.2 | 52.6 | 105.8 | 68.8 | 1.1 |
| Fiddleneck (<i>Phacelia tanacetifolia</i>) | Větrovská | 53.4 | 58.0 | 111.4 | 69.4 | 1.6 |
| | Boratus | 53.3 | 58.4 | 111.7 | 71.1 | 1.7 |
| | Stala | 54.2 | 57.5 | 111.7 | 75.4 | 1.6 |
| | mean of cvs. | 53.6 | 58.0 | 111.6 | 73.3 | 1.6 |
| Foxtail millet (<i>Setaria italica</i>) | Urjinskaja 1 | 93.9 | 48.6 | 142.5 | 123.8 | 2.5 |
| | SET 621/91 | 78.3 | 49.2 | 127.5 | 112.2 | 1.8 |
| | mean of cvs. | 86.1 | 48.9 | 135.0 | 118.0 | 2.2 |
| Millet panic (<i>Panicum miliaceum</i>) | Hanácká mana | 62.5 | 48.5 | 111.0 | 109.4 | 4.1 |
| | Skorospeloe 66 | 62.8 | 49.2 | 112.0 | 101.9 | 4.7 |
| | mean of cvs. | 62.7 | 48.8 | 111.5 | 105.7 | 4.4 |
| Buckwheat (<i>Fagopyrum esculentum</i>) | Aelita | 43.9 | 63.8 | 107.7 | 85.2 | 24.1 |
| | Pyra | 42.2 | 64.5 | 106.7 | 79.4 | 21.9 |
| | mean of cvs. | 42.9 | 64.3 | 107.2 | 82.3 | 23.0 |

ation (CV), relatively low variability in earliness was noticed in the genetic resources of false flax (CV = 2.4%). However, early cultivars were found. Hoga and Pernice were 18 and 8 days, respectively, earlier than the mean of cultivars. In crambe, rocket, white mustard and Abyssinian mustard intra-specific CV for earliness reached 5.1% and in all these crops early cultivars (ecotypes) could be found. E.g. white mustard cv. Nakielska reached maturity in 107 days, while the mean in this species was 125 days. Rather large diversity in earliness among 42 evaluated crambe accessions has been reported by MASTEBROEK *et al.* (1994).

Still earlier maturity had the ecotype FRA N5A99 of black mustard (103 days) and CGN 7151 of Indian mustard (105 days). These crops showed CV values for earliness close to 8%. Foxtail millet showed the largest diversity in days to flowering and in days to maturity (CV = 12.7% and 11.0 %, respectively). Even though this crop had the longest mean vegetation period (174 days) and late types were not able to mature completely in our conditions, convenient early types could be found, e.g. the ecotype SET 621/91 with 125 days to maturity. ONO *et al.* (1991) also confirmed large diversity in earliness in this crop as well as low productivity of the late types.

Large diversity was found in seed weight (CV = 7.2–21.5%), which is particularly important in the breeding of small seeded species. We have found relatively lower diversity in crambe and false flax (CV = 7.7% and 8.4%, respectively). Slightly higher variation was recorded in black mustard and white mustard (CV close to 13%). The thousand seed weight in black mustard did not exceed 3.0 g (ecotype RCAT 069393) whilst in white mustard large-seeded cv. Metex (TSW = 9.4 g) exceeded remarkably the mean value of 6.07 g.

Abyssinian mustard and rocket had a CV for TSW of 16.6% and 17.2%, respectively. In the small-seeded rocket the largest seeds were found in the ecotype ERU 21/84 (TSW 3.1 g) while in Abyssinian mustard with a mean TSW of 3.5 g a few large-seeded types were found (e.g. ecotype CGN 4001 with TSW 9.4 g). Large diversity in seed weight was found in foxtail millet (CV = 19.6%) and Indian mustard (CV = 21.5%), even though the TSW of the best cultivars didn't exceed 3 g in both crops (mean values 2.3 g and 1.8 g, respectively).

Although plant height and micro-plot yield were also recorded in the preliminary trials, these traits are not discussed here, since too large environmental effects did not allow safe conclusions.

Table 4. Mean values of evaluated characters in selected cultivars (ecotypes) of eight species of alternative crops grown on localities Humpolec and Olomouc in the years 1999 and 2000

| Locality | Crop | Cultivar (ecotype) | Days to flowering | Seed filling period (days) | Days to maturity | Plant height (cm) | Thousand seed weight (g) |
|-----------------|--------------------|--------------------|-------------------|----------------------------|------------------|-------------------|--------------------------|
| Humpolec | White mustard | Nakielska | 42.0 | 87.5 | 129.5 | 135.5 | 7.4 |
| | Abyssinian mustard | Vsp Sao Tome | 62.0 | 83.0 | 145.0 | 151.9 | 5.2 |
| | Indian mustard | Vitasso | 65.5 | 72.5 | 138.0 | 190.0 | 2.2 |
| | Black mustard | BGRC 34177 | 43.0 | 82.0 | 125.0 | 110.3 | 1.7 |
| | Crambe | Borowska | 61.5 | 62.5 | 124.0 | 126.3 | 6.6 |
| | Arugula (rocket) | BGRC 22532 | 44.5 | 86.5 | 131.0 | 94.5 | 2.2 |
| | False flax | Hoga | 52.5 | 74.0 | 126.5 | 98.0 | 1.4 |
| | Fiddleneck | Boratus | 53.0 | 93.5 | 146.5 | 90.7 | 1.7 |
| Olomouc | White mustard | Carine | 44.0 | 64.5 | 108.5 | 86.6 | 6.2 |
| | Abyssinian mustard | CGN 4001 | 48.0 | 88.0 | 136.0 | 73.9 | 3.7 |
| | Indian mustard | Secus | 45.5 | 72.5 | 118.0 | 103.7 | 3.9 |
| | Black mustard | RCAT 069393 | 45.5 | 51.0 | 96.5 | 107.2 | 1.8 |
| | Crambe | Voronezhskii | 53.0 | 62.0 | 115.0 | 76.6 | 5.8 |
| | Arugula (rocket) | K-3 | 49.5 | 71.5 | 121.0 | 59.7 | 1.5 |
| | False flax | Pernice | 48.0 | 46.5 | 94.5 | 57.9 | 1.0 |
| | Fiddleneck | Stala | 49.0 | 59.0 | 108.0 | 51.7 | 2.0 |

In general, the diversity found in economically important characters in most alternative crops is sufficient for breeding and for the use in agriculture. It was also possible to find cultivars or ecotypes with parameters convenient for local conditions.

The most promising accessions from the preliminary trials in 1996 and 1997, 2–3 per crop, were further evaluated from 1998 to 2000 in replicated field trials at Prague-Ruzyně. The mean values of days to flowering, days to maturity, days of seed filling period, plant height and seed weight are summarized in Table 3. Eight of the accessions, each of a different crop, were tested in field trials during 1999 and 2000 also at Olomouc and other 8 accessions at Humpolec (supposed to be suitable for local conditions) see Table 4.

Buckwheat (Table 3) flowered very early (on average 43 days) from emergence and belonged to the earliest crops (107 days to maturity) with very small differences between the two tested cultivars. Also crambe and false flax matured very early (106 days) while flowering was later than in buckwheat (53 days in false flax and 62 days in crambe). Black mustard, fiddleneck and panic millet reached maturity in 110–112 days, early flowering was noted in black mustard (51 days) and partly also in fiddleneck (53–54 days) whereas panic millet flowered later (63 days). Differences in days to flowering between cultivars were not significant, similarly as in days to maturity in all above-mentioned crops.

A slightly longer growth period than in the previous group of crops was noted in white mustard (mean of cultivars 118 days) and rocket (121 and 125 days). However, white mustard belonged to the earliest flowering species (51 days). Rocket was slightly later (53 days in BGRC 22532 and 59 days in K-3). White mustard, besides Abyssinian mustard, had a relatively long period of seed formation (about 68 days) what could favor good seed yields in this crop (NANDA *et al.* 1994). Later maturity was noted in both ecotypes of rocket (121 and 124 days), Indian mustard, foxtail millet and especially Abyssinian mustard (mean growth period 134, 135 and 148 days, respectively). Abyssinian mustard showed a very long period of seed filling (80 days). Significant differences between cultivars in days to maturity were found in Indian mustard where cv. Secus was markedly earlier (127 days) than cv. Vitasso (142 days); an even greater difference was found in flowering date. Also the ecotype SET 621/91 of foxtail millet, the earliest among all tested accessions, reached maturity after 128 days, i.e. 15 days earlier than cv. Ukrajinskaja 1.

White mustard and Indian mustard were very tall (mean plant height 128 and 127 cm respectively). Differences between cultivars were up to 10 cm in white mustard and only 5 cm in Indian mustard. GILL AMORES *et al.* (1996) has also found very tall cultivars in Indian mustard. Plant

height of about 118 cm can be expected in foxtail millet, where the early ecotype SET 621/91 was by 12 cm shorter than cv. Ukrajinskaja 1. We have found also in black mustard a difference between the two tested ecotypes (121 cm in RCAT 069393 and 101 cm in BGRC 34177). A mean height of about 106 cm was found in Abyssinian mustard and panic millet, cv. Skorospeloe 66 of panic millet was shorter (102 cm). Other crops could be arranged in decreasing order of plant height as follows: crambe (101 cm), rocket (84 cm), buckwheat (82 cm), fiddleneck (73 cm) and false flax (69 cm); differences between cultivars did not exceed 6 cm in the particular crops.

All the evaluated alternative crops belong to small-seeded species, except buckwheat with a mean TSW of 23 g. Relatively larger seeds among the evaluated crops were noticed in white mustard (mean 6.8 g), crambe (5.7 g) and Abyssinian mustard (5.3 g). All selected cultivars belonged to those with larger seeds (see also Table 1) and, therefore, differences within crops were small. Panic millet cv. Skorospeloe 66 had slightly larger seeds (TSW 4.7 g) than the local cv. Hanácká mana (4.1 g). Low seed weight was found in Indian mustard (mean TSW 2.8 g, but cv. Secus had a TSW of 3.2 g) and foxtail millet (mean TSW 2.2 g but 2.5 g in cv. Ukrajinskaja). Very small seeds without significant differences among cultivars and ecotypes (TSW 1.5–1.7 g) had fiddleneck and rocket and even smaller (TSW 1.1 g) were seeds of false flax. It seems that seed size was not so important for the productivity of rocket and false flax, because accessions with larger seeds were not selected as superior in the preliminary evaluation (Table 1).

In general, results of field experiments at Humpolec and Olomouc confirmed the above-mentioned relations between crops in the evaluated characters. The same cultivars of most crops reached maturity at Humpolec considerably later (from 10 days in rocket to 35 days in fiddleneck) than in Prague-Ruzyně, while Abyssinian mustard and Indian mustard were at Prague-Ruzyně only by 4 days earlier than at Humpolec. Because the flowering date was at Humpolec not later than at Prague-Ruzyně (in all mustard species even earlier by 5–13 days), all crops had at Humpolec a longer seed filling period. Also the TSW was at Humpolec higher in most crops (7.4 g in white mustard, 6.6 g in crambe and 2.2 g in rocket). Plants of all species were in this cool and humid location very tall (e.g. white mustard 136 cm, Abyssinian mustard 152 cm and cv. Vitasso of Indian mustard even 190 cm).

Warmer climate and slightly lower precipitation at Olomouc have influenced mainly the plant height in all crops, which was reduced at this site by 9–35 cm, compared to Prague-Ruzyně. The tallest crop was black mustard (107 cm), while Indian mustard was shorter (104 cm). Abyssinian mustard, which was very tall in other locations, reached at Olomouc only 74 cm. All crops were

Table 5. Estimation of seed yield in 24 cultivars (ecotypes) of eleven species of alternative crops – Results of field trials (4 × 5 m²) on localities Prague-Ruzyně (1998, 1999, 2000), Olomouc (1999, 2000) and Humpolec (1999, 2000)

| Crop | Cultivar (ecotype) | Donor country | Seed yields (t/ha) | | | | |
|--|--------------------|---------------|--------------------|--------------------|---------------------|--|------|
| | | | Locality | | | mean value of | |
| | | | Praha | Olomouc | Humpolec | cultivars in all environments (3 or 5) | crop |
| White mustard (<i>Sinapis alba</i>) | Zlata | CZE | 2.13 | – | – | 2.13 | 2.34 |
| | Carine | FRA | 2.07 | 1.93 | – | 2.01 | |
| | Nakielska | POL | 2.26 | – | 3.81 | 2.88 | |
| Abyssinian mustard (<i>Brassica carinata</i>) | SVP Sao Tome | STP | 1.17 | – | 1.21 | 1.19 | 1.09 |
| | CGN 4001 | ETH | 1.27 | 0.57 | – | 0.99 | |
| Indian mustard (<i>Brassica juncea</i>) | Vittasso | DEU | 0.52 | – | 0.54 | 0.53 | 0.55 |
| | Secus | DEU | 0.57 | 0.54 | – | 0.56 | |
| Black mustard (<i>Brassica nigra</i>) | RCAT 069393 | DEU | 0.89 | 0.72 | – | 0.82 | 0.98 |
| | BGRC 34177 | GRC | 1.01 | – | 1.31 | 1.13 | |
| Crambe (<i>Crambe abyssinica</i>) | Borowska | POL | 2.63 | – | 1.88 | 2.33 | 2.34 |
| | Voronezhskij | SUN | 2.57 | 2.02 | – | 2.35 | |
| Rocket (argula) (<i>Eruca sativa</i>) | K-3 | GRC | 1.02 | 0.95 | – | 0.99 | 1.19 |
| | BGRC 22532 | DEU | 1.38 | – | 1.38 | 1.38 | |
| False flax (<i>Camelina sativa</i>) | Hoga | DNK | 1.59 | – | 3.02 | 2.16 | 1.75 |
| | Pernice | YUG | 1.46 | 1.16 | – | 1.34 | |
| Fiddleneck (<i>Phacelia tanacetifolia</i>) | Větrovská | CZE | 0.83 | – | – | 0.83 | 0.80 |
| | Boratus | DEU | 0.78 | – | 0.67 | 0.73 | |
| | Stala | POL | 0.93 | 0.68 | – | 0.83 | |
| Foxtail millet (<i>Setaria italica</i>) | SET 621/91 | DEU | 2.61 | – | – | – | 2.22 |
| | Ukrajinskaja 1 | SUN | 2.19 | – | – | – | |
| Millet panic (<i>Panicum miliaceum</i>) | Hanácká mana | CZE | 1.81 | – | – | – | 1.82 |
| | Skorospeloe 66 | SUN | 1.96 | – | – | – | |
| Buckwheat (<i>Fagopyrum esculentum</i>) | Aelita | SUN | 2.44 | – | – | – | 2.04 |
| | Pyra | CZE | 1.85 | – | – | – | |
| F-value for | cultivars (crops) | | 1.4 ⁺ | 30.9 ⁺⁺ | 545.4 ⁺⁺ | ⁺ P = 0.05 | |
| | years | | 11.7 ⁺⁺ | 19.6 ⁺⁺ | 0.06 | ⁺⁺ P = 0.01 | |

earlier in flowering (44 days in white mustard to 53 days in crambe) and also ripened earlier than at Prague-Ruzyně (by 3–12 days, except of crambe). The seed filling period was in most crops by few days longer than at Prague-Ruzyně, but much shorter than at Humpolec. The seed weight was at Olomouc in some crops different from Prague-Ruzyně. The TSW was increased in Indian mustard (3.9 g), fiddleneck (2.0 g) and crambe (5.8 g).

Concerning seed yields, white mustard and crambe were superior to other evaluated crops providing mean yields of 2.34 t/ha over all environments and cultivars.

Similar results have been reported for white mustard also in some other papers (AULD *et al.* 1993; NIKITCHIN *et al.* 1994; BROWN *et al.* 1999; MUSNICKI *et al.* 1997). Good seed yields were recorded also in crambe (VOLLMANN & RUCKENBAUER 1993; KULIG *et al.* 1996; STRAŠIL & SKALA 1997). Relatively high mean yield has been found in foxtail millet (2.22 t/ha), which was, however, sown only at Prague-Ruzyně (Table 5), whereas the former crops were tested in all three locations. The highest yielding crop at Prague-Ruzyně was crambe (2.60 t/ha), followed by foxtail millet (2.22 t/ha) and white mustard (2.15 t/ha).

This shift has been caused by different response of crambe and white mustard to environmental conditions. Whereas crambe yielded most in the warmer climate of Olomouc, white mustard was significantly better yielding in the colder and humid climate at Humpolec (located in the potato growing region). The white mustard cv. Nakielska (POL) was the best yielding accession in field experiments (mean yield 2.88 t/ha) and outyielded significantly the local cultivar Zlata by 26%. Also NOWAKOWSKI *et al.* (1996) found in trials with five cultivars of white mustard high seed and dry matter yields in cv. Nakielska. The ecotype SET 621/91 (DEU) of foxtail millet reached a mean yield of 2.61 t/ha and took the second rank in a general comparison of productivity with a significant benefit of 17% against the second tested cultivar of this species Ukrajinskaja 1 (RUS). In crambe, both tested cultivars showed only small non-significant differences in seed yield (2.33 t/ha in cv. Borowska and 2.35 t/ha in cv. Voronezhskij, respectively). Because very similar results were obtained in all environments, crambe can be considered as the most stable among the examined crops. The buckwheat cultivar Aelita had only a slightly lower seed yield than crambe (2.44 t/ha at Prague-Ruzyně), but the seed yield of another tested buckwheat cultivar, Pyra, was significantly lower by 32%, which indicates the high importance of selection of convenient cultivars in small-scale crops.

Panic millet reached a mean yield of 2.04 t/ha and the difference between both tested cultivars, Hanácká mana and Skorospeloe 66, was not significant. Similar figures reported ONO *et al.* (1991) from experiments with five millet cultivars (0.97–2.17 t/ha). However, millet can produce under favourable conditions much higher seed yields, up to 6 t/ha, as shown by AUFHAMMER and KUBLER (1998).

Among the remaining tested crops only false flax had mean seed yields above 1.5 t/ha. Differences were found between both evaluated cultivars. Cv. Hoga had high seed yield at the location Humpolec (3.02 t/ha) and slightly outyielded cv. Pernice also in Prague-Ruzyně.

Black mustard, Abyssinian mustard and rocket had a low seed production; it seems that seed yields in the best cultivars (ecotypes) can reach 1.1–1.4 t/ha. Of the two evaluated rocket ecotypes BGRC 22532 (DEU) yielded 1.38 t/ha, this is significantly more by 39% than K3. Differences were found also in black mustard, where the ecotype BGRC 34177 (GRC) had a mean yield of 1.13 t/ha, whereas the ecotype RCAT 069393 had 17.5% less. We have found similar levels of seed yield (close to 1.2 t/ha) also in Abyssinian mustard, with smaller differences between the cultivars. There was a large significant difference in yield between the localities Olomouc (0.57 t/ha) and Humpolec (1.21 t/ha), indicating most likely the importance of sufficient water supply for this crop.

Very low seed yields (probably also due to very small seeds) can be expected in fiddle neck (0.80 t/ha) and especially in Indian mustard (0.55 t/ha). Differences between cultivars were negligible in both species and seed yields were also only moderately influenced by the environments.

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Abstrakt

HERMUTH J., DOTLAČIL L., MICHALOVÁ A., ČEJKA L. (2001): **Produktivita a diverzita agronomických znaků u vybraných odrůd jedenácti druhů alternativních plodin.** *Czech J. Genet. Plant Breed.*, 37: 93–102.

U 215 položek osmi druhů alternativních plodin byla v letech 1996 a 1997 studována vnitrodruhová diverzita a byly vybrány perspektivní materiály pro podrobné hodnocení. Byla prokázána značná vnitrodruhová variabilita v ranosti kvetení a v době zralosti. Rozsah variability v hmotnosti semen byl druhově specifický (V_k 7,2–21,5 %) – nízká variabilita byla zjištěna u odrůd a ekotypů krambe a lničky, naopak vysokou variabilitu vykázaly odrůdy a ekotypy hořčice habešské, roketky a zvláště pak bėru vlašského a hořčice sareptské. V navazujících pokusech bylo v letech 1998 až 2000 hodnoceno 24 vybraných odrůd (ekotypů) jedenácti plodin. Ranost v době kvetení i zralosti prokázaly pohanka, lnička a krambe (doba vegetace do 107 dnů), také hořčici černou, svazenku a proso lze považovat za rané plodiny (vegetační doba do zralosti byla u zkoušených genotypů 110–112 dnů). Naopak hořčice sareptská, bėr vlašský a hořčice habešská měly relativně delší vegetační dobu (126–149 dnů). U všech hodnocených druhů hořčic (zvláště pak hořčice bílé a hořčice sareptské) a u bėru vlašského byly rostliny značně vysoké, zejména při dostatku vláhy (1,1–1,8 m). Vedle pohanky měly poněkud větší semena některé odrůdy hořčice bílé, krambe a hořčice habešské (HTS 5–7 g). Hořčice bílá a krambe poskytly také nejvyšší průměrné výnosy semen (oba druhy 2,34 t/ha), za nimi následoval bėr vlašský (2,22 t/ha) a proso (2,04 t/ha). Odrůda hořčice bílé

Nakielská a ekotyp béru vlašského SET 621/91 poskytly nejvyšší výnosy semen a vykázaly i dostatečnou ranost. Při srovnání tří pokusných lokalit lze podmínky v Praze-Ruzyni považovat za intermediární, chladnější a vlhčí klima v Humpolci mělo pozitivní vliv na výnosy většiny druhů, prodlužovalo vegetační dobu a výšku porostů, zvláště u všech druhů hořčic. Opačný vliv mělo teplejší a poněkud sušší klima v Olomouci, kde bylo nejvýnosnější plodinou krambe.

Klíčová slova: alternativní plodiny; diverzita; ranost; výška rostlin; hmotnost semen; výnos semen

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New Varieties

Nové odrůdy

Flax *Linum usitatissimum* L. Venica

Registered: Czech Republic, 2001

Breeder's rights: AGRITEC, Research, Breeding and Services Ltd., Šumperk, Czech Republic

Breeder and maintainer: AGRITEC, Research, Breeding and Services Ltd., Šumperk, Czech Republic

Pedigree: SL 988 × Viking. The parents used for crossing were breeding lines SL-988 from SEMPRA Praha Join Stock company, breeding station Slapy u Tábora and French variety Viking of the company Cooperative Linière Fontaine de Cany. The choice of parental lines was carried out purposefully with the aim to reach very high content of fibre inherited from both varieties and also middle vegetation period based on Viking. In addition good resistance to lodging as well as to diseases remained conserved.

Breeding method – pedigree: The parental components were crossed in 1990. F₁ generation due to shortening of breeding process was multiplied in Chile in 1991 according to the agreement between previous Research and Breeding Institute of Technical Crops and Legumes and Dutch Breeding and Seed company Van de Bilt Zaden. F₂ generation was sown the same year in the Czech Republic. Individual selection of 100 plants was carried out in F₂ generation as the base of the progenies establishment in F₃ generation. F₄ and F₅ generation were further evaluated as the progenies of the second and third year in 1993–1994. The initial progeny SU 29-2/93 was selected in F₆ generation. This line was tested in F₇ and F₈ on two locations Šumperk and Slapy u Tábora on stem and seed yield and other characters. The line SU 29-2/93 was included in F₉ generation in the Official Registration Trials (ORT) in 1998. During the first year of ORT also maintenance breeding was started. ORT were carried out during 1998–2000 resulting to registration under the name Venica in 2001.

Yielding parameters: Variety Venica belongs to the semi-early maturing group of flax. This blue flowering variety has middle stem and seed yield. The main priority of this variety is combining of semi-early duration of vegetation period with very high content in stem reaching level 38–40%. High content of fibre is also accompanied with the relative high fibre yield per ha. The variety Venica was better in the above mentioned parameters during Official Registration Trials 1998–2000. It overcame the standard varieties both in long fibre content and in total fibre content by 4.06% respectively 5.10% in absolute values. The highest performance was manifested in long and total fibre yield. It reached 120.47% long fibre yield in comparison with standard varieties on the average of all locations ORT as well as 116.30% in total fibre yield.

Other qualities: Good resistance to lodging and fungi diseases, especially to Fusarium wilt are important other characters of Venica variety.

Len setý, přadný Venica

Registrován: Česká republika, 2001

Šlechtitelská práva: AGRITEC, výzkum, šlechtění a služby s. r. o., Šumperk, Česká republika

Šlechtitel a udržovatel: AGRITEC, výzkum, šlechtění a služby s. r. o., Šumperk, Česká republika

Rodokmen: SL-988 × Viking. Základem šlechtění se stala jednoduchá hybridní kombinace novošlechtění SL-988 (Semptra Praha a. s. šlechtitelská stanice Slapy u Tábora) a francouzské odrůdy přadného lnu Viking (šlechtitelská firma Cooperative Linière Fontaine de Cany). Výběr těchto rodičovských komponent byl proveden záměrně pro získání velmi vysokého podílu vláknna ve stonku od obou odrůd a středně dlouhé vegetační doby po odrůdě Viking. Současně zůstala zachována dobrá odolnost proti poléhání a houbovým chorobám.

Metoda šlechtění – rodokmenová: Křížení rodičovských komponent SL-988 × Viking bylo provedeno v roce 1990. F₁ generace byla pro urychlení šlechtitelského cyklu množena na základě smlouvy mezi tehdejším Výzkumným a šlechtitelským ústavem technických plodin a luskovin v Šumperku a holandskou firmou Van de Bilt Zaden v Chile v roce 1991 a v tomtéž roce byla vyseta v podmínkách ČR F₂ generace. Individuálním výběrem bylo v F₂ generaci vybráno 100 výchozích rostlin, které vytvořily základ F₃ generace jako kmenů I. roku. V následujících generacích F₄ až F₅ pokračovala v letech 1993–1994 selekce a hodnocení kmenového materiálu II. a III. roku ve zkouškách výkonu. V roce 1995 byl v generaci F₆ vybrán výchozí kmen označený SU 29-2/93, který byl zařazen do mezistaničních zkoušek. Mezistaniční zkoušky probíhaly v letech 1995–1997 na dvou pokusných místech v Šumperku a ve Slapech u Tábora. V generaci F₉ v roce 1998 bylo novošlechtění pod označením SU 29-2/93 zařazeno do Státních registračních zkoušek a současně bylo v tomto roce založeno udržovací šlechtění. Státní registrační zkoušky probíhaly v letech 1998–2000. V roce 2001 bylo novošlechtění SU 29-2/93 zaregistrováno pod názvem Venica.

Výnosové ukazatele: Odrůda Venica patří do skupiny předných lnů. V sortimentu registrovaných odrůd se řadí ke středně raným odrůdám s modrou barvou květu. Výnos neroseného stonku je střední, stejně tak výnos semene. Hlavní předností odrůdy Venica je kombinace středně dlouhé vegetační doby a velmi vysokého podílu vlákna ve stonku, dosahujícího v absolutních hodnotách 38–40 %. Tento velmi vysoký podíl vlákna umožňuje současně získání relativně vysokého výnosu vlákna z 1 ha. V průběhu registračních zkoušek překonávala odrůda Venica standardní odrůdy zejména v obsahu dlouhého vlákna v průměru všech oblastí o 4,06 %, v obsahu celkového vlákna o 5,1 % v absolutních hodnotách, ve výnosu dlouhého vlákna dosáhla 120,47 % na průměr standardních odrůd v průměru všech oblastí a ve výnosu celkového vlákna 116,30 %.

Ostatní vlastnosti: Odrůda Venica disponuje dobrou odolností proti poléhání a komplexu houbových chorob, z nichž nejvýznamnější je rezistence vůči fuzarióze.

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Materiál a metody: Model pokusu musí být popsán podrobně a výstižně. Popis metod by měl umožnit, aby kdokoli z odborníků mohl práci opakovat. Zkratky jsou používány jen pokud je to nutné; první použití zkratky musí být uvedeno úplným popisem nebo vysvětlením. Používané měrové jednotky musí odpovídat soustavě měrových jednotek SI.

Výsledky: Doporučuje se nepoužívat k vyjádření kvantitativních hodnot tabulek, ale dát přednost grafům anebo tabulky shrnout v statistickém hodnocení naměřených hodnot. Tato část práce by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce. Je přípustné spojení s předchozí kapitolou (Výsledky a diskuse).

Poděkování se uvádí před přehled použité literatury.

Literatura: Měly by být citovány práce uveřejněné v lektorovaných periodikách. Odkazy na literaturu v textu se provádějí uvedením jména autora a roku vydání publikace. V části Literatura se uvádějí jen práce citované v textu. Citace se řadí abecedně podle jména prvního autora: příjmení, zkratka jména, rok vydání (v závorce), plný název práce, úřední zkratka časopisu, ročník, první–poslední stránka; u knih je uvedeno místo vydání a vydavatel.

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Tabulky a obrázky: Tabulky, obrázky a fotografie se dodávají zvlášť a všechny musí být citovány v práci. Akceptovány budou pouze obrázky, které jsou nezbytné pro dokumentaci výsledků a umožňují pochopení textu. Není přípustné dokumentovat výsledky jak v tabulkách, tak pomocí grafů. Všechny ilustrativní materiály musí mít kvalitu vhodnou pro tisk. Fotografie i grafy jsou v textu uváděny jako obrázky a musí být průběžně číslovány. Každý obrázek musí mít stručný a výstižný popis.

Separáty: Autor obdrží 10 separátních výtisků publikované práce.

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