Variability in Resistance to Clubroot in European Cauliflower Cultivars

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Abstract

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Fifty genotypes of cauliflower (*Brassica oleracea* var. *botrytis*) were evaluated for resistance to clubroot disease (*Plasmodiophora brassicae* Wor.) under controlled conditions in a plant growth chamber. The cultivars with the highest resistance were Brilant, Agora, and Bora, while the most susceptible were the cultivars White Top, White Fox, and Octavian. The variation in disease index is probably due to different pathogenicity rates of clubroot pathotypes and genetic heterogeneity of European cauliflower cultivars. The obtained results will be tested in an infested and non-infested field.

Keywords: *Brassica oleracea* var. *botrytis*; germplasm; *Plasmodiophora brassicae* Wor.; disease resistance

Traditional European crops such as cauliflower (*Brassica oleracea* var. *botrytis*) and broccoli (*Brassica oleracea* var. *italica*) have become widespread in Asia during recent decades, while their cultivation in Europe has been more or less stable (Branca 2008; FAOSTAT 2011). Presently, these popular cruciferous vegetables are considered as functional foods, due to the content of health-improving compounds and important nutritious substances. In recent years, interest in cauliflower and broccoli has grown because of new breeding programmes aimed at satisfying current consumer requirements. The great genetic diversity of cauliflower and broccoli conserved in gene bank germplasm collections along with modern biotechnological approaches allow utilisation of both crops in commercial breeding (Branca 2008). Resistance breeding against diseases and insect pests has been highlighted as one of the most important objectives in Brassicas (Gupta & Pratap 2009). Clubroot disease (*Plasmodiophora brassicae* Wor.) infects most cruciferous species. Traditionally, it has been considered a major pathogen which occurs mainly in temperate regions (Dixon 2009). Clubroot causes severe losses in crops such as cauliflower and Chinese cabbage, since a substantial part of planted cultivars are highly susceptible (Tewari & Mithen 1999). Basic knowledge of *Plasmodiophora brassicae* available in current literature, its biology, distribution, control and prevention, including modern breeding methods have been summarised by Diederichsen *et al.* (2009) and

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Dixon (2009). Significant variability in resistance to clubroot was found among different cultivars of *Brassica oleracea* (Diederichsen *et al*. 2009). Based on the presence of resistance genes two systems of pathogen classification were adopted. Williams (1966) developed a system for the identification of *P. brassicae* races commonly used in North America. Buczacki *et al*. (1975) proposed a numerical system to characterise *P. brassica*e pathotypes known as the European Clubroot Differential Set (ECD) representing a set of five accessions each of *B. rapa*, *B. napus*, and *B. oleracea*. Resistance in *Brassica oleracea* has traditionally been considered to be non-differential, determined by a series of recessive resistance genes, and thus difficult to use in conventional breeding (Tewari & Mithen 1999; Diederichsen *et al*. 2009). European field isolates of clubroot disease display a great genetic variation, and frequently consist of several pathotypes. Studies focused on the testing of resistance against clubroot demonstrated that *P. brassicae* isolates have a high potency to overcome resistance of all *B. oleracea* ECD hosts (Crute *et al*. 1983; Diederichsen *et al*. 2009). Clubroot disease is controlled most effectively by cultivation of resistant cultivars. Recently, the importance of resistant landraces in cruciferous crops has grown due to the latest biotechnological approaches in breeding (Gupta 2009).

This study is a follow-up of the evaluation of resistance to clubroot disease in the germplasm collection of European kohlrabi cultivars (Kopecký *et al*. 2010a). The aims are (*i*) to test accessions of European cultivars of cauliflower germplasm for resistance to clubroot under controlled conditions in a plant growth chamber, and (*ii*) to find accessions suitable for resistance breeding.

MATERIAL AND METHODS

Plant material. Fifty genotypes (accessions) of cauliflower (*Brassica oleracea* var**.** *botrytis* L.) were evaluated for resistance to clubroot disease (*Plasmodiophora brassicae* Wor.). These accessions have been conserved in the National Collection of Genetic Resources of Vegetables at the Department of Genetic Resources for Vegetables, Medicinal and Special Plants in Olomouc, Crop Research Institute, Czech Republic. The majority of accessions included in the collection have their origin in Central European countries, whereas two of them originate from Canada and the USA. The passport data of particular accessions is documented in the Plant Genetic Resources Documentation of the Czech Republic (EVIGEZ 2011). Plants were cultivated under standard conditions (day length 14 h, temperature 23°C day/15°C night, illumination intensity 100 μE/m^2/s) in a plant growth chamber.

Pathogen isolate. The isolate represents a mixture of the fiercest races of the pathogen *P. brassicae* originating from the whole Czech Republic, which were concentrated in the former experimental area at Světlá Hora (Bruntál District, Czech Republic). The isolate was obtained from field populations of clubs of naturally infested Chinese cabbage (*B. rapa* L. subsp. *pekinensis* cv. Granaat). The pathogenicity of the isolate (ECD 16/31/31) was characterised using the ECD set (Buczacki *et al*. 1975). Clubs were harvested, washed and stored at –20°C at the Department of Genetic Resources for Vegetables, Medicinal and Special Plants of Crop Research Institute, Olomouc, Czech Republic, for no longer than two years. The inoculum was prepared as follows: clubs were mixed with distilled water, filtered, and the final spore concentration was adjusted with distilled water to 10^7 spores/ml using a haemocytometer (Chytilová & Dušek 2007).

Resistance testing on young plants. Inoculation using imbibitions was performed according to Chytilová and Dušek (2007). Eight-week old plants were assessed for the rate of infestation using a four-point scale (0–3) according to Buczacki *et al*. (1975): 0 (no symptoms), 1 (very small clubs, mainly on lateral roots and not impairing the main root), 2 (small clubs, covering the main root and a few lateral roots), 3 (large clubs on lateral and main roots, deformation of the root system) (Figure 1). Plants were evaluated in the stage of having at least three true leaves in water, in order to assess the infection degree precisely. The modified disease index (DI) following Siemens *et al*. (2002) was calculated according to the formula

$$DI = (0n_0 + 1n_1 + 2n_2 + 2n_3) \times 100/3N_t$$

where:

n_0–n_3 – number of plants in the indicated class

N_t – total number of plants tested

Accessions with a DI of less than 20 were considered as highly resistant (Chytilová & Dušek 2007). A highly susceptible cultivar of Chinese cabbage (*B. rapa* L. subsp. *pekinensis*) cv. Granaat was used as a control. The experiment was

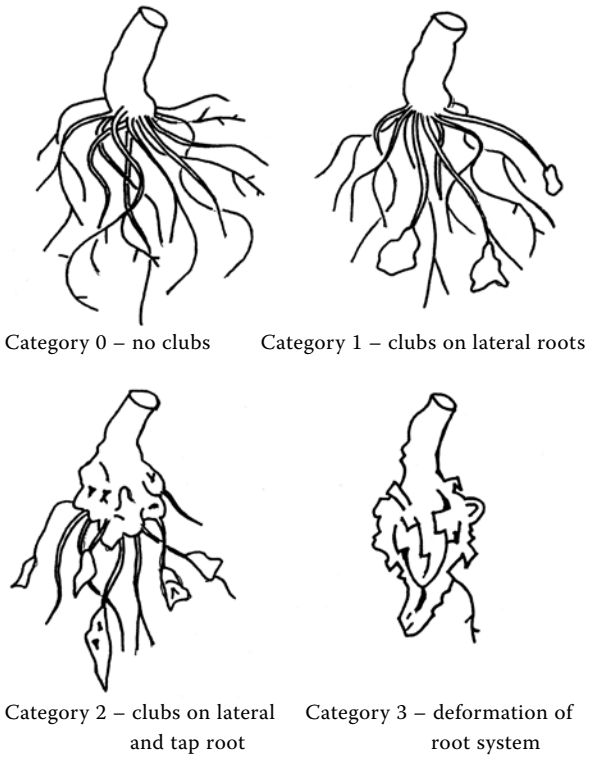

Figure 1. Four-point scale (0–3) according to Buczacki *et al.* (1975) for testing the infestation rate to clubroot disease

established in a randomised block design with 50 genotypes replicated in three blocks (trials). A number of 19 to 72 plants were evaluated per genotype (48 plants on average).

Statistical analysis. The following variables were tested: (*i*) proportion of plants with low infestation rate (classes 0 and 1 in % of replications per genotype and indicated as [% 0–1]), and (*ii*) disease index (DI). Common descriptive statistics were calculated and statistical tests were carried out using the Statistica 9.0 software (Statsoft Inc., Tulsa, USA). Data were analysed using GLM two-way ANOVA with genotype as fixed factor and block as random factor, followed by multiple-comparison Fisher *LSD*-test at $P = 0.05$. Statistically homogeneous groups are marked by the same line in Figure 2. The rate of infestation was square-root transformed before analysis to ensure normality and homogeneity of variance.

Because of the compositional character of infestation rate variable, a log-ratio analysis (Aitchison 1986) was performed in Canoco for Windows 4.5 (ter Braak & Šmilauer 2002). Genotypes in individual trials were considered as samples, proportional representation of the four classes of infestation as dependent variables, genotypes were visualised as centroids and the respective trials were considered as covariates (= blocks) in the analysis.

Table 1. Results of GLM ANOVA testing the effect of genotype on proportion of plants with low infestation rate (classes 0 and 1), and disease index (DI) in three blocks (trials). Genotype was considered as fixed factor and block as random factor

	DF	MS	*F*	*P*
Proportion of plants in classes 0 and 1				
Block	2	61.49	21.02	< 0.001
Genotype	50	4.90	1.68	0.02
Disease index				
Block	2	5103.84	36.74	< 0.001
Genotype	50	295.18	2.12	< 0.001

RESULTS

Under controlled conditions in the plant growth chamber the mean disease index for the Chinese cabbage control was 86.8 with 92.5% of the plants scoring in classes 2 and 3.

Genotype and block showed significant effects on both the proportion of plants with a low infestation level (% 0–1) and disease index (DI) (Table 1). Both the proportion of plants with a low infestation level and disease index were smaller in the first trial (% 0–1: 8.6; DI: 67.6) and increased in the second (% 0–1: 15.0; DI: 87.6) and third trial (% 0–1: 25.8; DI: 76.2). When comparing the genotypes according to DI and the percentage of plants not or slightly affected by clubroot (% 0–1; Figure 2), the cvs Brilant (% 0–1 = 52.8; DI = 50.2) and Agora (% 0–1 = 43.7; DI=51.9) turned out to be the genotypes with the highest resistance. On the contrary, the cvs Boomerang, Stupický obrovský, White Stone, and Maximus exhibited a higher susceptibility than the control cultivar Granaat, whereas the most susceptible genotypes were White Top (% 0–1 = 2.3; DI = 91.3), White Fox (% 0–1 = 1.3; DI = 95.5) and Octavian (% 0–1 = 2.4; DI = 95.9). Most genotypes included in the study showed an intermediate response to the infection against the clubroot isolate with DI ranging from 61.2 to 86.2 and % 0–1 ranging from 3% to 34%. The results of the multiple-comparison tests divided the genotypes into three homogeneous groups,

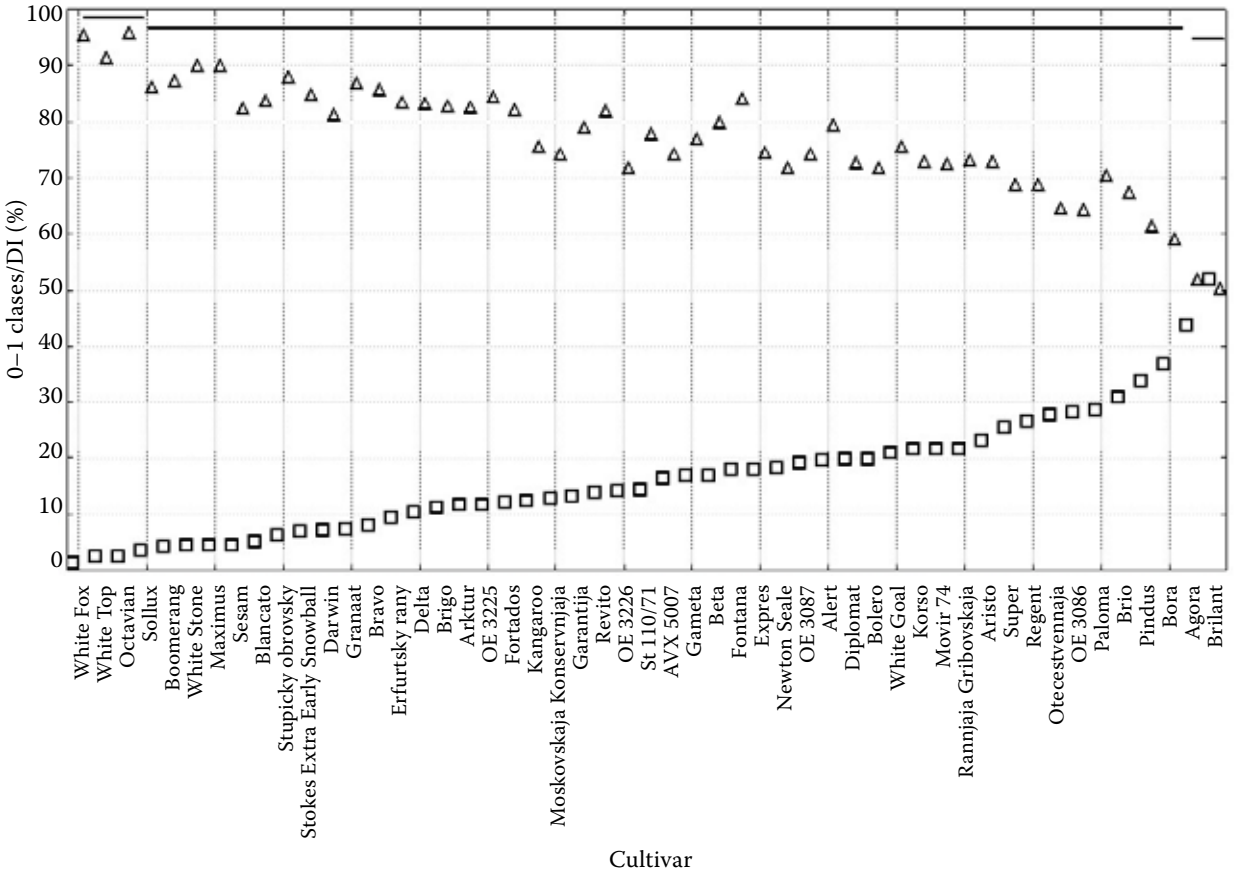

Figure 2. Mean percentage of plants not or slightly affected by clubroot disease (classes 0 and 1; % 0–1; marked by triangles) and mean disease index of cultivars (marked by squares). Cultivars are ordered by an increasing mean percentage of plants not or slightly affected by clubroot disease. Lines above the respective variables show statistically homogeneous groups ($P > 0.05$) according to the *LSD* test

but only two groups significantly differed from each other in range extremes (Figure 2).

The results of the multivariate analysis corroborated the results of univariate analyses. Except for apparently little (cvs Agora, Brillant) and highly (cvs Octavian, Fontana) infested genotypes no strong discontinuities were found among cultivars in the degree of resistance to clubroot disease. The main trends in data variability are shown in an ordination diagram (Figure 3), in which the 1st and 2nd axes explain together 91.1% of variation in the data set. The degree of resistance is evident from the position of individual accessions in relation to the direction of vectors representing respective degrees of infestation. The most resistant accessions are shown in the upper left part of diagram, while the least resistant accessions are in the right part of the diagram. Several cultivars (e.g. OE 3226 and Moskovskaja) showed intermediate infestation rates. The data also showed a strong correlation between infestation classes 0 and 1.

DISCUSSION

Since the early 20th century, breeders along with phytopathologists, genetics and biochemists have been dealing with clubroot. In Brassicas the main objectives have been resistance breeding. The efforts are however complicated by a limited amount of resistance sources. The highest probability of their occurrence is supposed to be found in geographical centres of origins of the crops. In the case of *Brassica oleracea* this is the Mediterranean and the West European seashore. Therefore the most resistant cultivars of cauliflower, which otherwise is considered to be strongly susceptible, come from Italy (Rod 2006).

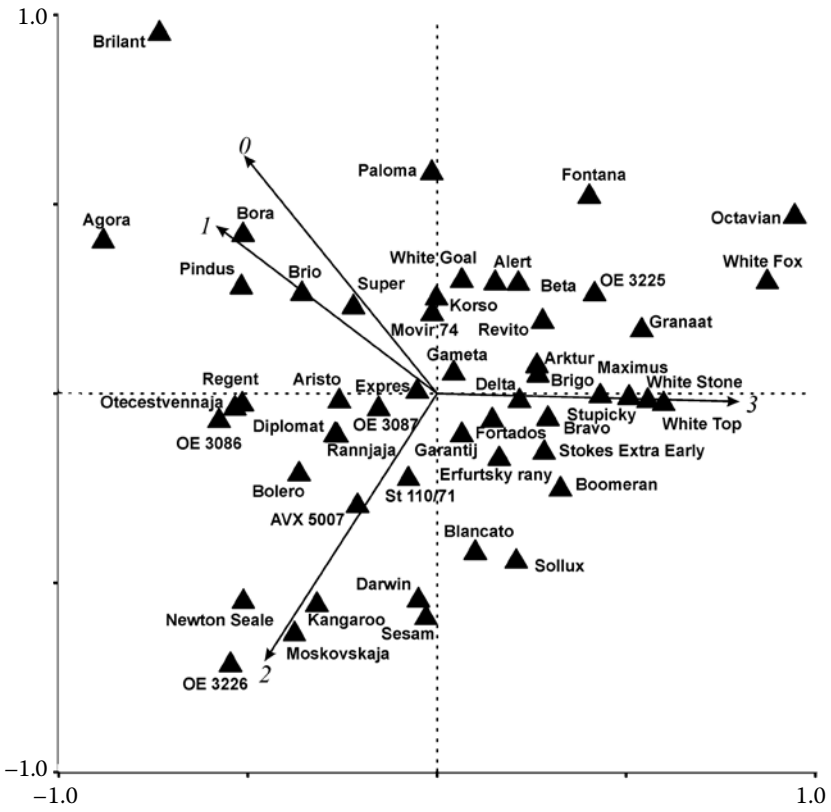

Figure 3. Ordination diagram (log-ratio analysis; the 1st and 2nd ordination axes) of variation in resistance of European cultivars of cauliflower against clubroot disease. As a control a highly susceptible cultivar of Chinese cabbage (*B. rapa* subsp. *pekinensis*), cv. Granaat, was used

Assessment of the resistance of European cauliflower cultivars against clubroot revealed a relatively wide variation, which was found to be continuous. Since *B. oleracea* is a cross-pollinating plant, the material tested is likely to be heterogeneous and reactions within plants could vary due to their being heterozygous. The variability in response to clubroot in cauliflower cultivars could also be explained by different pathogenicity rates of *P. brassicae* pathotypes. A study on the spectrum of clubroot pathotypes in the former Czechoslovakia revealed altogether 35 pathotypes (Chytilová & Dušek 2007).

It was proved that none of the accessions tested was resistant because all of them revealed a DI higher than 20 (Chytilová & Dušek 2007). The accessions of the Czech cultivars Brilant and Agora with DI values of 50.2 and 51.9, respectively, were considered to be the most resistant. Manzanares-Dauleux *et al*. (2000) reported high and moderate susceptibility of French cauliflower landraces to clubroot isolates under controlled glasshouse conditions. However, when they tested the accessions in field trials, the disease indices varied from 95 to 99 according to the life cycle of individual cauliflower accessions. Growers recommend Brilant and Agora cultivars for early and moderately late cultivation because of their short life cycle. The lower susceptibility of these cultivars observed by us under glasshouse conditions corresponds well with the results presented by Manzanares-Dauleux *et al*. (2000), whereas Dixon and Robinson (1986) reported that most summer and autumn cauliflowers were highly susceptible. More pronounced and reproducible symptoms of plant infestation could be expected under stable conditions (temperature, illumination) in a growth chamber. Manzanares-Duleux *et al*. (2000) stated that the results obtained for the cauliflower accessions under controlled conditions were correlated with the disease scores found under normal field conditions. In the field, all cauliflower accessions showed severe symptoms, but the percentage of plants scoring in classes 0 and 1 was higher (Manzanares-Dauleux *et al*. 2000).

In summary, assessing resistance to clubroot disease under controlled conditions in a plant growth chamber showed that accessions of the Brilant and Agora cultivars were more resistant. However, to confirm the obtained results and facilitate selection, plants must be cultivated in infested and non-infested fields and the yield of consumer parts in infested fields needs to be

compared with the yield in non-infested fields. This is the main objective of follow-up research.

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