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Examination of resistance to clubroot in accessions of Brassica oleracea using a glasshouse seedling test

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Abstract

A glasshouse test was elaborated for assessing large numbers of seedlings of *Brassica oleracea* for resistance to clubroot, a disease caused by the fungus *Plasmodiophora brassicae*. The method offers good control of inoculum density per plant, and requires 6–7 weeks from sowing. The results from the glasshouse test correlated well with field test results. With this method, 71 accessions of *B. oleracea* reported to carry resistance to clubroot, and one susceptible control cultivar were tested with a Dutch clubroot isolate. High levels of resistance were found in several accessions of cabbage, broccoli and curly kale. F_1-populations of resistant cabbage or curly kale × susceptible cabbage were fully susceptible, indicating recessive inheritance of resistance in all cases.

Additional keywords: Plasmodiophora brassicae Wor., inoculation methods, recessive inheritance.

Introduction

Plasmodiophora brassicae Wor., the causal agent of the clubroot disease of cruciferous crops is a widespread and harmful pathogen. It induces the infected roots to develop large galls, so-called clubs. The structure of vascular elements in the clubbed roots is disrupted (Ikegami and Yamashita, 1983), resulting in a reduced uptake and transport of water and nutrients. The diseased plant wilts and usually has a slightly darker, blue-grey leaf colour. Decaying clubs release large numbers of newly formed resting spores of *P. brassicae* in the soil, which can remain infectious for periods up to 15 years (Mattusch, 1977).

The clubroot disease has been the subject of numerous investigations (Colhoun, 1958; Crute et al., 1980). The occurrence of club formation has been studied in plants grown in naturally and artificially infested fields. More recently, seedling tests were devised for use under glasshouse conditions, using artificial inoculation methods. Several of these seedling test methods were compared by Dixon (1976). Buczacki et al. (1975) proposed a standardized test method and symptom grading scale, and the use of a set of differential hosts known as the European Clubroot Differential Set (ECD).

The research reported here is part of a project aimed at the characterization of genes regulating resistance to Dutch populations of *P. brassicae* and their introduction into modern cultivars. An effort was made to collect and test most of the known sources of resistance (reviewed by Crute et al., 1980), as well as other genotypes described as potential sources of resistance. The present paper reports experiments to evaluate seedling test methods, to determine the effectiveness of the resistance in collected accessions to a Dutch field population of *P. brassicae*, and to investigate its mode of inheritance.

Materials and methods

Pathogen. A field isolate of *P. brassicae* was obtained from a heavily infested field of the Experimental Station Brabant at Breda, the Netherlands. Large clubs of an unknown, highly susceptible cauliflower cultivar were collected, washed, and stored at –20 °C. This isolate is hereafter designated as the Breda isolate.

Spore suspensions were prepared by macerating frozen clubs in 4–5 volumes demineralized water using an electric blender, and filtering the crude suspensions through four layers of cheese-cloth. Suspensions generally contained 1–3 · 10^8 spores · ml^{-1} and were stored for up to 8 weeks at 4 °C before use. Prior to inoculation, they were diluted to 10^7 spores · ml^{-1}.

Plant material. The European Clubroot Differential Set (ECD; Buczacki et al., 1975) was obtained from H. Toxopeus (CPRO-DLO). Cabbage cv. Septa (Bejo Seed b.v., Warmenhuizen, the Netherlands), which is equivalent to ECD host 14 was used as a susceptible control in all tests. In some tests, susceptible Brussels sprouts cv. Leander (Asmer Seeds Ltd, Leicester, UK) was also used.

The 71 accessions screened can be subdivided in 11 groups (Table 2). Bindsachsener and Böhmerwaldkohl were German cabbage landraces of which several accessions were obtained. A number of Russian cabbage cultivars was described as carrying field resistance (Giessmann and Bauch, 1974; Crute and Pink, 1989; N.V. Krasheninnick, VNIISSOK Institute, Moscow, Russia, pers. comm.). The material labeled as 'Wisconsin' was derived from a breeding programme of Walker and Larson at the Wisconsin Agricultural Experimental Station, Madison, USA; it consisted of cv. Badger Shipper (ECD host 11), two lines derived respectively from 'Larson 8353 T' and from a cross involving cv. Resistant Detroit (Nieuwhof and Wiering, 1963), and one line derived from a cross involving line 8-41 (Chiang and Crête, 1970). The Oregon cabbage and OSU CR broccoli material were developed by Baggett (1976, 1983) and Baggett and Kean (1985). The Brussels sprouts lines were derived by Nieuwhof at the Institute for Horticultural Plant Breeding (now CPRO-DLO), Wageningen, the Netherlands from crosses with cv. Bindsachsener. The curly kale material included both open-pollinated and F_1-hybrid cvs. The Portuguese Tronchuda cvs were obtained from local suppliers.

F_1-seeds were obtained by bud-pollination of individual plants grown in insect-free glasshouse compartments.

Test methods. The standard seedling test method, hereafter referred to as 'pipette method' was as described by Lamers and Toxopeus (1977), with the following modifications. Seven sets of six 4.5 × 4.5 cm^2 square, 110 ml pots were filled with potting compost (pH 6.0, sterilized by gamma irradiation) and placed on a tray in 1 cm deep water, refilled daily. Up to 30 trays were placed on glasshouse benches warmed to 23 °C, with a minimum air temperature of 18 °C. During the winter period (October-March), daylight was supplemented by 45 $\mu E \cdot m^{-2} \cdot s^{-1}$ radiation supplied by 400 W SON-T lamps for 14 h · day^{-1}. No tests were performed in December and January.

One seed was sown per pot at a depth of 2.5 cm. Two ml of a suspension of 10^7 spores · ml^{-1} was pipetted to each pot. The pots were covered for 3 days with black plastic until the seedlings emerged. After 6 to 7 weeks, plants were washed and disease symptoms assessed on a scale of 0–3, according to Buczacki et al. (1975): 0, no swelling visible; 1, very slight swelling, usually confined to lateral roots; 2, moderate swelling on lateral and/or tap roots; 3, severe swelling on lateral and/or tap roots.

In all glasshouse experiments, each treatment or accession was tested in two replicates

of 18 and 24 pots respectively. A disease index, ranging from 0 (no symptoms) to 1 (severely affected) was calculated by dividing the mean disease rating of each accession by three. Where necessary, plants were recovered after the test by cutting off the root system, rinsing the plants in running tap water, allowing the cut ends to dry overnight, and planting the cuttings in sterile potting compost at high air humidity.

A preliminary experiment was performed with cvs Septa and Leander to test the effect of growing the seedlings for 10 days before inoculation. In one half of the experiment seeds were sown 10 days earlier than in the other half. All pots were inoculated on the same day using the pipette method.

In another experiment the pipette method was compared with the root-dip method (Johnston, 1968), using cvs Septa and Leander. The same spore suspension was diluted to 10^8 spores · ml^{-1} for the root-dip method and to 10^7 spores · ml^{-1} for the pipette method.

Resistance under field conditions was determined by growing seedlings in trays and transplanting them into an infested field. Six accessions were planted in four replicates of 16 plants each. After 4 months the symptoms on the root system were rated in four classes equivalent to those used for the classification of seedling symptoms, and a field disease index was calculated as above.

Data analysis. Most accessions in the collection were tested once, using two replicates per accession. A small number of accessions was studied in two or more tests. The evaluation data of the accessions were collected from eight separate tests, with cv. Septa and an accession of Böhmerwaldkohl used as standards.

For statistical analysis, plants were classified in two groups: 'healthy' (symptom classes 0 and 1) and 'diseased' (classes 2 and 3). To overcome problems caused by observations equal to 0% and 100%, data were transformed to $(y + 1/2)$ and $(n + 1)$, where y represents the number of healthy plants and n represents the total number of plants tested (Cox, 1970). These data were analyzed according to a generalized linear model for binomial data (McCullagh and Nelder, 1989).

Results

Development of the seedling test method. A preliminary experiment was performed to investigate the effect of a growing period of 10 days prior to inoculation, as described by Lamers and Toxopeus (1977), on the results of the pipette method. The disease indices of cvs Septa and Leander were 1.00 and 0.94 respectively when inoculated directly after sowing, and both 0.97 when inoculated 10 days later. Since elimination of the 10-day growing period resulted in a shorter test duration without affecting symptom development, in all further experiments inoculation was performed directly after sowing.

In contrast to the results obtained with the pipette method, the results of the root-dip method (Johnston, 1968) were erratic. About half of the plants treated with the root-dip method remained healthy, while all plants developed severe symptoms when inoculated using the pipette method. Although the root-dip method could possibly have been improved, the pipette method was chosen for further experiments because of its superior control of inoculum load per plant and shorter test duration.

Six accessions with seedling disease indices ranging from 0.03 to 0.98 as established using the pipette method were also tested in the heavily infested field from which the Breda isolate was obtained. Results obtained under glasshouse and field conditions were highly correlated. Spearman's rank correlation coefficient between seedling and field disease indices was 0.83 ($P \leq 0.05$).

Characterization of the P. brassicae field isolate. The Breda isolate used for the resistance tests was characterized using the ECD (Table 1). Most differential hosts showed a reasonably uniform reaction, allowing classification as resistant or susceptible. Intermediate values were however obtained for the disease indices of ECD hosts 08 and 15. Host 08 was not genetically uniform, as was indicated by the fact that some inbreds were produced which were highly resistant to the Breda isolate. This host was therefore considered to be resistant. For ECD host 15 such indications of genetic variability for resistance were not obtained. Using a cut-off value of 0.50 or lower resulted in a susceptible classification. The Breda field isolate could therefore be characterized as ECD 16/3/30 (Buczacki et al., 1975).

Resistance tests of collected accessions. Seventy-one *B. oleracea* accessions with reported or putative resistance to clubroot were screened in eight tests (Table 2). Two replicates (42 pots) each of cv. Septa and of one accession of Böhmerwaldkohl were included as controls to compare the levels of infection between tests. The disease indices of these two accessions were 0.95–1.00 and 0.60–0.80 respectively.

In 9 out of 11 groups of accessions studied, resistance to the Breda isolate was found. The Wisconsin cabbage and OSU CR broccoli groups were consistently resistant. In the Böhmerwaldkohl, Brussels sprouts and curly kale groups large differences in level of resistance were observed between accessions. All four accessions of the Oregon cabbage group, and some of the Russian, Bindsachsener and 'other cabbage' groups showed intermediate levels of resistance, while most accessions in the latter three groups were fairly

Table 1. Assessment of differential reactions of *Brassica* hosts to the Breda isolate of *Plasmodiophora brassicae.*

ECD host[1]	Symptom scale[2]				n[3]	D.I.[4]
	0	1	2	3		
01	35	0	0	0	35	0.00
02	34	0	0	0	34	0.00
03	33	0	0	0	33	0.00
04	38	0	0	0	38	0.00
05	0	0	0	37	37	1.00
06	0	0	0	40	40	1.00
07	0	1	0	32	33	0.98
08	23	1	5	6	35	0.28
09	36	0	0	0	36	0.00
10	35	0	0	0	35	0.00
11	17	5	2	0	24	0.13
12	0	0	4	32	36	0.96
13	0	0	0	36	36	1.00
14	0	0	0	21	21	1.00
15	10	3	16	7	36	0.52

[1] ECD: European Clubroot Differential set (Buczacki et al., 1975).
[2] Symptom scale: the scale ranges from 0 (no symptoms) to 3 (severely affected). The number of plants in each class is indicated.
[3] n = the total number of plants tested.
[4] D.I. = Disease Index. The index is calculated by dividing the mean symptom rating by 3.

Table 2. Proportions of plants not or slightly affected by clubroot (class 0 and 1) in groups of accessions and in the most resistant accession of each group.

Accession group	Number of accessions	Proportion in class 0 or 1[1]	
		mean	most resistant
Cabbage			
Septa (control)	8[2]	0.03[2]	
Bindsachsener	5	0.18***	0.68***
Böhmerwaldkoh	18	0.44***	0.87***
Irish 'Flat Dutch'	9	0.03	0.15
Oregon	4	0.48***	0.59***
Russian	17	0.17***	0.56***
Wisconsin	4	0.77***	0.97***
other cabbage	2	0.13*	0.40**
Broccoli			
OSU CR	8	0.85***	0.94
Brussels sprouts breeding lines	5	0.28***	0.79***
Curly kale cultivars	6	0.33***	0.93***
Tronchuda cultivars	3	0.06	0.08

[1] Proportions near 0 or 1 are over- or underestimated by approx. 0.025.
[2] Results of eight tests of cv. Septa.
*, **, and *** indicate significant differences with cv. Septa at the 5%, 1% and 0.1% confidence level, respectively.

susceptible. No resistance was found in the Irish 'Flat Dutch' cabbage landraces, nor in the Portuguese Tronchuda cvs.

Very high levels of resistance were observed in Wisconsin cabbage breeding line Dr Larson, in a line of Böhmerwaldkohl selected by Dr Crute (HRI, Wellesbourne, UK), in broccoli OSU CR-7 and in curly kale cv. Petibor. The seedling disease indices of these accessions were below 0.15, and more than 85% of the plants were scored in classes 0 and 1.

Resistance of F_1 progenies. Randomly chosen plants of 11 partially resistant accessions (three Böhmerwaldkohl, four Wisconsin and four curly kale) were self-pollinated and crossed with susceptible cv. Septa to determine the inheritance of resistance factors (Table 3). All F_1-populations were highly susceptible, while the inbreds showed moderate to high levels of resistance. These preliminary results indicated that in these accessions the resistance was expressed as a recessive character.

Three resistant cabbage accessions of the Wisconsin group, cv. Badger Shipper and breeding lines Resistant Detroit and Dr. Larson were crossed pairwise for an allelic test. As shown in Table 4, the resistance of the F_1-populations was similar to that of the inbred lines. In all cases a small number of plants was rated as class 2 or 3, while the majority was rated as class 0 or 1. In combination with the recessive inheritance of resistance observed in the crosses with cv. Septa, this indicated that the resistance genes in these accessions were allelic.

Table 3. Disease indices of progenies of plants from three groups of accessions, obtained by self-pollination (I_1) and by crossing with cv Septa (F_1).

Accession group	Number of accessions	Disease index	
		I_1	F_1
Böhmerwaldkohl	3	0.25 – 0.63	0.99 – 1.00
Wisconsin	4	0.22 – 0.40	0.96 – 1.00
Curly kale	4	0.58 – 0.69	0.94 – 1.00

Table 4. Disease indices of inbreds and F1's of three cabbage accessions in the Wisconsin group. Number of plants tested is indicated in parentheses.

Accession	Resistant Detroit	Dr. Larson	Badger Shipper
Resistant Detroit (breeding line)	0.22 (27)		
Dr. Larson (breeding line)	0.29 (14)	0.31 (79)	
cv. Badger Shipper (ECD11)	0.30 (85)	0.21 (42)	0.28 (57)

Discussion

Reliability of the seedling test method. The seedling test method used in this study proved to be suitable for the screening of resistance of relatively large numbers of plants. It has the advantage over other methods that the number of spores applied to each plant is well controlled. In the widely used root-dip (Johnston, 1968) and slurry (Toxopeus and Jansen, 1975) methods, the number of spores per plant can vary significantly due to unknown and varying amounts of spores adhering to root systems of different sizes, or to the difficulty of obtaining a uniform distribution of spores in batches of slurry. Another advantage of the pipette method is that inoculation can be made directly after sowing, thus reducing the test duration by 10–14 days. A slight drawback of this method is that the number of plants tested depends on the germination potential of the seed lot, which is not always known in advance. The results obtained with this seedling test were shown to correlate well with the symptom development found under normal field conditions.

Identification of resistant accessions. The aim of the present research was to identify accessions with resistance to Dutch populations of *P. brassicae*, for further genetic studies. Useful resistance was identified in nine groups of accessions, although a common source of resistance was involved in several cases. From these experiments it is not possible to conclude whether the occurrence of both healthy and affected plants in a specific accession is caused by genetic variation within the accession or by partial resistance which is genetically uniform. This will be investigated by studying the resistance of doubled haploid lines derived from these accessions.

The resistance found by Crisp et al. (1989) and Crute and Pink (1989) in Irish landraces was shown to be completely ineffective to the Breda isolate used in this study. The same was true for the resistance in many Russian cultivars described by Krasheninnick (pers. comm.). These accessions therefore must carry differential resistance, or the Breda isolate

used in this study is generally more aggressive than the isolates to which these accessions were found to be resistant. The Portuguese Tronchuda cvs were also susceptible in our tests, although Crisp et al. (1989) found some resistance in related material. The resistance in two accessions received from the Wisconsin Agricultural Experimental Station in the 1950's was shown to be related to that in cv. Badger Shipper.

The complete susceptibility of all resistant × susceptible crosses agrees with earlier findings in *B. oleracea* (Crute et al., 1980), where resistance appeared to be generally recessive. This has now also been shown to be true for the resistance present in curly kale.

Further work is needed to determine whether the resistance found in different groups of accessions is caused by different genes. If so, these could possibly be accumulated in commercial cultivars to achieve higher and more stable resistance. Work in progress concentrates on genetic analysis of resistance, using doubled haploids derived from selected accessions.

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References

Baggett, J.R., 1976. 'Oregon CR-1' broccoli. HortScience 11: 622–623.

Baggett, J.R., 1983. Clubroot-resistant cabbage breeding lines Oregon 100, 123, 140 and 142. HortScience 18: 112–114.

Baggett, J.R. & Kean, D., 1985. Clubroot-resistant broccoli breeding lines OSU CR-2 to OSU CR-8. HortScience 20: 784–785.

Buczacki, S.T., Toxopeus, H., Mattusch, P., Johnston, T.D., Dixon, G.R. & Hobolth, L.A., 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. Transactions of the British Mycological Society 65: 295–303.

Chiang, M.S. & Crête, R., 1970. Inheritance of clubroot resistance in cabbage (*Brassica oleracea* L. var. *capitata* L.) Canadian Journal of Genetics and Cytology 12: 253–256.

Colhoun, J., 1958. Club root disease of crucifers caused by *Plasmodiophora brassicae* Woron. A Monograph. Phytopathological Paper No.3, Commonwealth Mycological Institute. 108 pp.

Cox, D.R., 1970. The empirical logistic transform. In: The analysis of binary data. Methuen & Co Ltd, London. p. 30–42.

Crisp, P., Crute, I.R., Sutherland, R.A., Angell, S.M., Bloor, K., Burgess, H. & Gordon, P.L., 1989. The exploitation of genetic resources of *Brassica oleracea* in breeding for resistance to clubroot (*Plasmodiophora brassicae*). Euphytica 42: 215–226.

Crute, I.R., Gray, A.R., Crisp, P. & Buczacki, S.T., 1980. Variation in *Plasmodiophora brassicae* and resistance to clubroot in Brassicas and allied crops – a critical review. Plant Breeding Abstracts 50: 91–104.

Crute, I.R. & Pink, D.A.C., 1989. The characteristics and inheritance of resistance to clubroot in *Brassica oleracea*. Aspects of Applied Biology 23: 57–60.

Dixon, G.R., 1976. Methods used in western Europe and the U.S.A. for testing *Brassica* seedling resistance to club root (*Plasmodiophora brassicae*). Plant Pathology 25: 129–134.

Giessmann, H.-J. & Bauch, W., 1974. Stand und Probleme der Kohlhernieresistenzzüchtung bei Kopfkohl in der DDR. Deutsche Gartenbau 21: 194–195.

Ikegami, H. & Yamashita, Y., 1983. Changes of vessel structure in turnip roots infected with *Plasmodiophora brassicae*. Research Bulletin of the Faculty of Agronomy, Gifu University 48: 27–35.

Johnston, T.D., 1968. Club root in *Brassica*: A standard inoculation technique and the specification of races. Plant Pathology 17: 184–187.
Lamers, W. & Toxopeus, H., 1977. New 'Multipot' clubroot resistance screening method in use at the SVP. In: Buczacki, S.T. & Williams, P.H. (Eds), Woronin +100 International Conference on Clubroot, Madison, Wisconsin. p. 122–123.
Mattusch, P., 1977. Epidemiology of clubroot of crucifers caused by *Plasmodiophora brassicae*. In: Buczacki, S.T. & Williams, P.H. (Eds), Woronin +100 International Conference on Clubroot, Madison, Wisconsin. p. 24–28a.
McCullagh, P. & Nelder, J.A., 1989. Binary Data. In: Generalized linear models. Chapman and Hall, London. p. 98–148.
Nieuwhof, M. & Wiering, D., 1963. Clubroot resistance in *Brassica oleracea* L. Euphytica 11: 233–239.
Toxopeus, H. & Jansen, A.M.P., 1975. Clubroot resistance in turnip II. The 'slurry' screening method and clubroot races in the Netherlands. Euphytica 24: 751–755.