



**HAL**  
open science

# Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassicae oleracea* ssp. *acephala*)

Francois Laurens, Grégoire Thomas

## ► To cite this version:

Francois Laurens, Grégoire Thomas. Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassicae oleracea* ssp. *acephala*). *Hereditas*, 1993, 119, pp.253-262. hal-01602284

HAL Id: hal-01602284

<https://hal.science/hal-01602284>

Submitted on 1 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

## Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *acephala*)

FRANCOIS LAURENS<sup>1,2</sup> and GREGOIRE THOMAS<sup>1</sup>

<sup>1</sup>Institut National de la Recherche Agronomique, Station d'Amélioration des Plantes, le Rheu, France

<sup>2</sup>Institut National de la Recherche Agronomique, Station d'Amélioration des Espèces Fruitières et Ornementales, Beaucouze, France

LAURENS, F. and THOMAS, G. 1993. Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *acephala*). — *Hereditas* 119: 253–262. Lund, Sweden. ISSN 0018-0661. Received April 27, 1993. Accepted August 12, 1993

For kale (*Brassica oleracea* ssp. *acephala* L.), the inheritance of resistance and that of tolerance to clubroot (*Plasmodiophora brassicae*) were studied. Six homozygous lines belonging to the half marrow-stem and green curly kale groups were intercrossed in a diallel  $F_1$  mating design. Inheritance of resistance was assessed from a glasshouse test on young plants and from a field trial on adult plants. A Disease Index was used to estimate the behaviour of each progeny. Tolerance to clubroot was defined as the ratio of yield produced by a progeny on a clubroot-infected part of the field compared with its yield on an uninfected soil. Resistance to clubroot on young and adult kales is mainly controlled by genetic effects. The broad sense heritability assessments are greater than 0.8 for the 2 trials. Resistance inheritance is characterized by a predominance of additive genetic effects with incomplete dominance. Yet, the additive components have less relative importance compared to dominance effects in the inheritance of resistance of adult kales ( $h^2_{ns} = 0.55$  compared to 0.78 for young kales). Many dominant alleles control resistance. The phenotypic and genetic values calculated for resistance are not correlated with those of tolerance. The dominance genetic effects control a great part of the genetic variation for tolerance ( $h^2_{ns} = 0.16$ ). Breeding programmes are proposed to increase clubroot resistance level in the kale group. A maternal individual recurrent selection on progenies could be undertaken.

Francois Laurens, Institut National de la Recherche Agronomique, Station d'Amélioration des Espèces Fruitières et Ornementales, B.P. 57, 49071 Beaucouze Cedex, France

Clubroot, one of the most severe diseases affecting Cruciferous crops, is provoked by *Plasmodiophora brassicae*, an obligate pathogen which remains in the heterogeneous soil pathogen populations. Cultural or phytosanitary control methods are not efficient enough to fight it, so that genetic control appears to be the most interesting approach. Few convincing genetic studies have attempted to analyze resistance to clubroot in the *Brassica* genus.

Inheritance of resistance to clubroot varies according to the species. Many studies, reviewed by CRUTE (1986), consider the relationship between *Brassica rapa* and *P. brassicae* in terms of a gene-for-gene relationship. According to the hosts studied, 2, 3, or 4 genes with major effects are involved (JAMES and WILLIAMS 1980; CRUTE 1986).

All the studies on genetic control of resistance to clubroot in *B. napus* point to an oligogenic inheritance (CRUTE et al. 1980). Some disagreements remain about the exact number of genes involved (BROKENSHIRE 1982; CRUTE et al. 1983; GUSTAFSSON and FÄLT 1986). Of interest are the results of WILLIAMSON (1984, 1987), who worked

on the same *B. napus* plants with many pathogen populations, and revealed the existence of non-differential interactions.

Precise genetic control of resistance to clubroot in the various types of *B. oleracea* group is still unclear. The studies are based on a small number of genotypes and are often inconsistent; the most widely studied sources of resistance are 3 cabbage cultivars (*B. oleracea* ssp. *capitata*): Badger Shipper, Bindsachsener, Böhmerwaldkohl, and their self-progenies. The authors usually conclude that the inheritance of resistance to clubroot in *B. oleracea* is quantitative and involves recessive alleles (SEAMAN et al. 1963; WEISAETH 1974; CRUTE et al. 1983; VOORIPS and VISSER 1991). CHIANG and CRETE (1976) analyzed a  $4 \times 4$  diallel and demonstrated a high level for broad sense heritability with mostly additive genetic effects.

Several workers succeeded in proving that the relationship between the cultivar Badger Shipper and *P. brassicae* is differential (CHIANG and CRETE 1970; CRUTE et al. 1983). While most authors think that many genes are involved in the

genetic control of resistance in other genotypes, YOSHIKAWA (1983), who worked on progenies of crosses between cultivar Böhmerwaldkohl and a susceptible cabbage, demonstrated that 1 to 4 genes control this character.

Such contradictory conclusions may be explained by the diversity of methodological approaches (inoculation methods, inoculum concentrations), by the environmental conditions, by the analyses, and the interpretations (different Disease Index and Cut Off Point calculations). The genetic types of the plant material and of the pathogen were also often unknown. Furthermore, inheritance studies were based on a few genotypes, mostly coming from the European Clubroot Differential (E.C.D.) set, among which genetic homozygosity of some entries is doubtful.

HANSEN (1989), one of the first to work on inheritance of tolerance to clubroot, analysed a  $10 \times 10$  diallel which involved the cultivar Badger Shipper and 9 Norwegian genotypes coming from the cultivar Bindsachsener, and revealed that the character is under the control of incomplete dominance.

Despite the lack of precise knowledge, some breeding programmes have been undertaken to obtain cultivars with a better level of resistance to clubroot. Most of the time, they involved intraspecific sources of resistance. The cultivar Badger Shipper has often been used to improve resistance to clubroot in many *B. oleracea* groups: cabbage, Brussels sprouts, broccoli, cauliflower (CRUTE et al. 1980). The cultivars Böhmerwaldkohl and Bindsachsener, which are also good sources of resistance, have often been included in breeding schemes (WEISAETH 1974; CRUTE and PINK 1989). Breeding for resistance has often been performed with classical methods based on successive backcrosses (BAGGETT 1983, for cabbage; BAGGETT and KEAN 1985, for broccoli). For 15 years, the introduction of genes of major effects coming from other *Brassica* species has been developed. For instance, the Canadian cultivar Richelain (CHIANG and CRETE 1989) comes from the original interspecific cross between a clubroot-resistant rutabaga (*B. napus*) and a colchicine-induced tetraploid genotype cabbage *B. oleracea* ssp. *capitata*. WILLIAMSON and BRADSHAW (1989) have also undertaken a recurrent breeding programme in the kale group with genotypes collected in different parts of the world. After 4 selection cycles, resistance level was increased significantly.

The present study, concerning the analysis of a diallel crossing design including homozygous lines

of *Brassica oleracea* ssp. *acephala*, was aimed at assessing the inheritance of resistance to clubroot from field trials and glasshouse experiments. An analysis of tolerance in the field was also performed, and a breeding programme for increasing the level of resistance in the kale group will be proposed.

In a further paper, we shall be studying inheritance in both kale and cauliflower to propose new breeding schemes for resistance in the cauliflower group.

## Materials and methods

Six homozygous lines of kale were intercrossed in a complete diallel  $F_1$  mating design. Each parent was self-fertilized. The origin of the parental lines is given in Table 1. All the homozygous lines came from kale breeding programmes of the breeding Station I.N.R.A Rennes-Le Rheu (France). The plants had not been selected for resistance to clubroot before. The six lines were chosen according to a large screening of kale accessions for resistance to clubroot and according to their wide range of behaviour.

Two trials were performed; one, conducted under glasshouse conditions on young plants for the complete diallel set; the other, in the field, for adult plants on a half diallel set.

### Test on young plants

The experiments were undertaken in a greenhouse during the summer of 1989. Maximum temperature was fixed at 20°C. The glasshouse was fitted out with cooling and shade equipments. Five 4-day-old seedlings were planted in 8-cm-diameter plastic pots filled with steamed moist peat (pH = 6). Four pots were grouped in a water-filled tray, which represented one statistical replication. Two trays were studied for each genotype.

*The parasite; source and preparation of inoculum.* — Clubroot galls were first extracted from infected cauliflower roots collected in a Brittany field; then they were washed and stored at -18°C until needed (BUCZACKI et al. 1975). To prepare spore suspensions, galls were thawed slowly, then mixed in a blender, and finally filtered through three decreasing mesh-diameter sifters (500  $\mu$ , 250  $\mu$ , 100  $\mu$ ). The spore concentration was determined with a Mallassez cell. The final inoculum density for this

Table 1. Origin of the kale lines

| Code | Type <sup>1</sup> | Origin   | Number of generations of self-fertilizing |
|------|-------------------|--|---|
| A    | HMS               | Lines coming from recurrent basic population of cv. 'Proteor' <sup>2</sup> | 11  |
| B    | HMS               | id   | 13  |
| I    | HMS               | id   | 17  |
| J    | HMS               | id   | 9   |
| K    | HMS               | id   | 14  |
| L    | CVF               | unknown <sup>3</sup>   | 5   |

<sup>1</sup> Morphological type of kale: HMS = Half Marrow stem; CVF = green curly kale

<sup>2</sup> 'Proteor' = Half Marrow stem population cultivar

<sup>3</sup> Complex genetic origin; inbred material but number of generations of inbreeding unknown

experiment reached  $10^7$  spores/ml of suspension. This inoculum mixture gives a reading of 17/31/31 according to the European Clubroot Differential Set (BUCZACKI et al. 1975).

*Inoculation method.* — 20 ml of the resting spore suspension was poured on the five plantlets of each pot ( $2 \times 10^6$  spores for 1 cm<sup>3</sup> of moist soil) according to the inoculation method used at I.N.R.A. Le Rheu (LEMOULEC 1986). Plantlets were inoculated on 20 July 1989.

*Disease assessment.* — After 47 days, the plants were uprooted and assessed for clubroot, using the 0–3 scale suggested by BUCZACKI et al. (1975). A Disease Index (D.I.) was calculated by summation of the coefficients (0; 25; 50; 100) affecting each plant class frequency. The D.I. calculation seems well related to symptom evaluation (DOUBLET et al. 1988).

#### *Test on adult plants in field conditions*

Seeds were sown in moist peat, and after one-month growth in the greenhouse, the plantlets were planted out, at the beginning of July 1989 in a field of Brittany (PLOGOULM I.N.R.A. Station). Chinese cabbage plants had been scattered in the field. Symptom evaluation of these susceptible plants confirmed the distinction between two parts in the field. So, two blocks of 20 plants were planted on the part of the field naturally strongly infected with clubroot, while two other blocks were planted on the uninfected part.

No irrigation equipment could be installed in the trial field, and 1989 was a very dry year in Brittany. The conditions were therefore not favorable for the development of the epidemics.

After 68 days of growth, the plants were uprooted. Symptoms were assessed with the same scale as above using the same Disease Index. On the other hand, as an indication of tolerance, the ratio between yield (fresh weight of the aerial parts) from the two blocks on infected soil and the mean yield of the two non-infected replications was calculated.

#### *Statistical analysis*

Diallel analysis was carried out according to two methods: GRIFFING's random effects model (1956a,b) and Hayman's graphic analysis (HAYMAN 1954).

Griffing's analysis was performed on the greenhouse test with method 3 (with reciprocal progenies) and on the field test, with method 4 (without reciprocals). Because of the allogamous biology of *Brassica oleracea*, self-fertilized genotypes were not included in the analysis. They were only used for parental-phenotypic-value analysis.

General combining ability (GCA) and specific combining ability (SCA) variances were calculated. They allowed to evaluate the genetic variance components described in Table 2.

The relative importance of additive and dominance genetic components was estimated with the ratio  $s^2_A/s^2_D$ . Heritability was evaluated from Griffing's genetic variance component estimations.

Individual GCA effects ( $g_i$ ) for each parent were also calculated (GRIFFING 1956b). Means of the parental lines were ranked and separated by Duncan's New Multiple Range Test at the 0.05 probability level.

Hayman's analysis (HAYMAN 1954) is based on the analysis of a complete diallel mating set

Table 2. Assessment of genetic variance components and heritability

- 
- $s^2_A = 2s^2_{GCA}$  = additive genetic variance
  - $s^2_D = s^2_{SCA}$  = dominance genetic variance
  - $s^2_R$  = reciprocal effect variance
  - $s^2_G = s^2_A + s^2_D$
  - $s^2_P = s^2_G + s^2_{res}$
- 

where  $s^2_{res}$  is the residual variance estimation, based on the mean of the 2 replications

- $h^2_{ns}$  = narrow sense heritability =  $s^2_A/s^2_P$
  - $h^2_{bs}$  = broad sense heritability =  $s^2_G/s^2_P$
- 

according to a fixed model. Although the graphic method we used does not give as exact an estimation as Griffing's method, it can confirm the genetic control of resistance and the importance of dominant and recessive alleles in inheritance, and it will be used to illustrate the potential genetic progress for resistance breeding. The variables  $V_p$  (variance of the parents),  $V_r$  (variance of rth array) and  $W_r$  (covariance between the parents and their offsprings in rth array) are calculated. On the graph  $W_r = f(V_r)$ , the statistical inequality  $W^2_r < V_r \times V_p$  means that points remain inside the parabola  $W^2_r = V_p \times V_r$ . The points ( $V_r$ ,  $W_r$ ) are distributed along a corresponding straight line ( $D_r$ ) of unit slope inside the limiting parabola  $W^2_r = V_p \times V_r$ . The position of the line ( $D_r$ ) compared with the parallel tangent of the parabola gives information about the genetic control of the character. The position of ( $V_r$ ,  $W_r$ ) points on the line gives the relative proportions of dominant and recessive alleles in each parent. The six hypotheses postulated by HAYMAN (1954) are required to use this diallel analysis theory. Some of them are tested in this study: the uniformity of the variable ( $W_r - V_r$ ) indicates the validity of the assumptions in the genetic model (HAYMAN 1954) for the material used in this experiment; the Bartlett test permits to check the independent gene distribution among the parents. Some assumptions could not be checked (epistasy, biallelism, ...).

## Results

### Analysis of the resistance

This section will compare field tests with glasshouse experiments.

Plants in the field trial were, on average, slightly more damaged on the basis of Disease Index ( $DI_{adult} = 56\%$ ;  $DI_{young} = 31\%$ ). Yet the isolate

inoculated in the glasshouse was very aggressive, i.e., the ECD hosts inoculated at the same time were heavily attacked ( $DI_{Chinese\ cabbage} = 100\%$ ;  $DI_{ECDmean} = 72\%$ ).

Correlations for Disease Index were made on the phenotypic values between the 21 genotypes common to the two experiments (half diallel). They were found to be highly significant ( $p < 0.001$ ; Fig. 1). Despite a high mean resistance level in the kale group, analyses of variance indicate that genotype effects are highly significant (Table 3) for the 2 experiments. Besides, field conditions are responsible for a slight block effect ( $p < 0.05$ ). Because the 1989 summer was dry, results obtained in the field experiments must be analysed cautiously.

In the early test assessment, general combining ability (G.C.A.) effects represent the major genetic component of variation of resistance to clubroot among crosses and are highly significant ( $p < 0.001$ ; Table 3). Specific combining ability effects have a minor importance but are still significant at a 0.05 level, while no reciprocal effects are observed. Thus, the genetic results obtained in the glasshouse test can be compared with those of the field trial analysed only on half diallel crossing set.

Estimations of the genetic variance components are presented in Table 4. The  $s^2_G/s^2_{res}$  (= 7.7) ratio shows the preponderance of the genetic variance over the residual error variance. The additive genetic variance ( $s^2_A = 312.4$ ) is 10 times higher than the dominance component ( $s^2_D = 30.04$ ).

Genetical analysis of resistance to clubroot is slightly different for adult kale. Table 3 shows that GCA effect level is lower and, yet, highly significant ( $p < 0.01$ ). Despite their lesser importance, SCA effects are significantly detected ( $p < 0.05$ ). The  $s^2_G/s^2_{res}$  ratio (= 6) is high but lower than for early testing. Additive variance is slightly greater than dominance component (Table 4). Compared to that obtained in the trial on young plants, the  $s^2_A/s^2_D$  ratio is weak and close to 1 ( $s^2_A/s^2_D = 1.4$ ).

Heritability estimates confirm the results (Table 5). In the 2 trials, the broad sense heritability is very high ( $h^2_{bs\ glasshouse} = 0.86$ ;  $h^2_{bs\ field} = 0.82$ ), showing the great importance of genetic variance in the inheritance of resistance to clubroot for the kale plants. The high narrow sense heritability estimated in the glasshouse trial ( $h^2_{ns\ glasshouse} = 0.78$ ) reveals the main influence of additive effects in the inheritance. In comparison, the value obtained in the field experiment is lower ( $h^2_{ns\ field} = 0.55$ ). The importance of genetic effects being similar in both trials, the latter assessment implies that additive

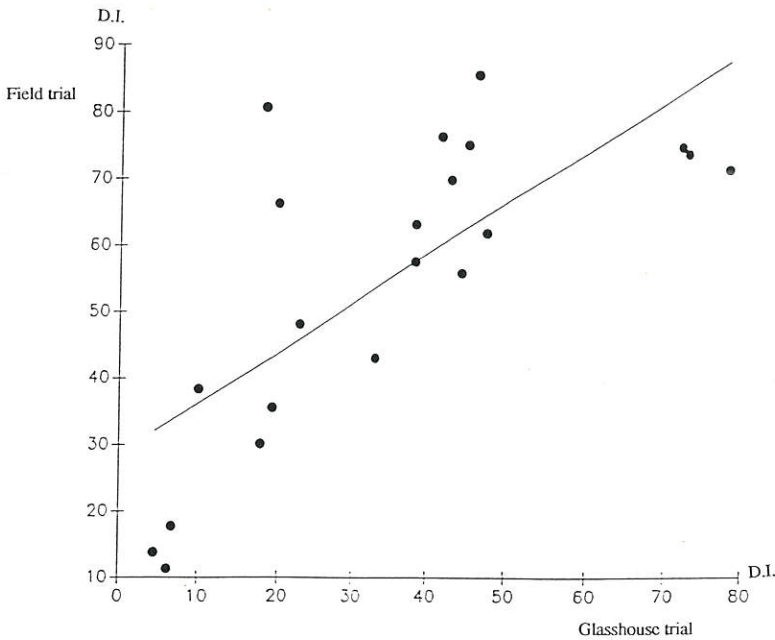


Fig. 1. Behaviour (Disease Index) of genotypes (half diallel) in the field and glasshouse trials.

Table 3. Diallel analysis of variance for the variable Disease Index (GRIFFING 1954)

| Source             | Glasshouse trial<br>(GRIFFING's method 3) |             |                                       | Field trial<br>(GRIFFING's method 4) |             |                        |
|--------------------|---|-------------|---------------------------------------|--------------------------------------|-------------|------------------------|
|                    | df  | Mean square | Degree of <sup>(1)</sup> significance | df                                   | Mean square | Degree of significance |
| Genotype           | 29  | 601.86      | ***                                   | 14                                   | 962.47      | ***                    |
| Block              | 1   | 104.69      | NS                                    | 1                                    | 876.86      | *                      |
| G.C.A              | 5   | 1353.05     | ***                                   | 5                                    | 838.85      | **                     |
| S.C.A              | 9   | 103.27      | *                                     | 9                                    | 282.52      | *                      |
| Reciprocal         | 15  | 68.83       | NS                                    | —                                    | —           | —                      |
| Res <sup>(2)</sup> | 29  | 43.19       |                                       | 14                                   | 80.32       |                        |

<sup>(1)</sup>\*\*\*: Significant at the 0.001 level of probability

\*\* : Significant at the 0.01 level of probability

\* : Significant at the 0.05 level of probability

NS: Non significant

<sup>(2)</sup> Residual error calculated from the mean of the 2 replications

Table 4. Assessment of genetic variance components for Disease Index on young and adult plant trials

|             | Young  | Adult  |
|-------------|--------|--------|
| $s^2_G$     | 332.44 | 480.36 |
| $s^2_A$     | 312.40 | 278.16 |
| $s^2_D$     | 30.04  | 202.20 |
| $s^2_{res}$ | 43.19  | 80.32  |

effects are still higher than dominance but much less than in young plant trial.

Hayman's analysis was performed on the greenhouse trial. The graph  $W_r = f(V_r)$  (Fig. 2) confirms and adds information about inheritance of resistance in this group of kale parents. The  $D_r$  line cuts the  $W_r$  axis at (0,53) level, and is situated between the tangent to the parabola and

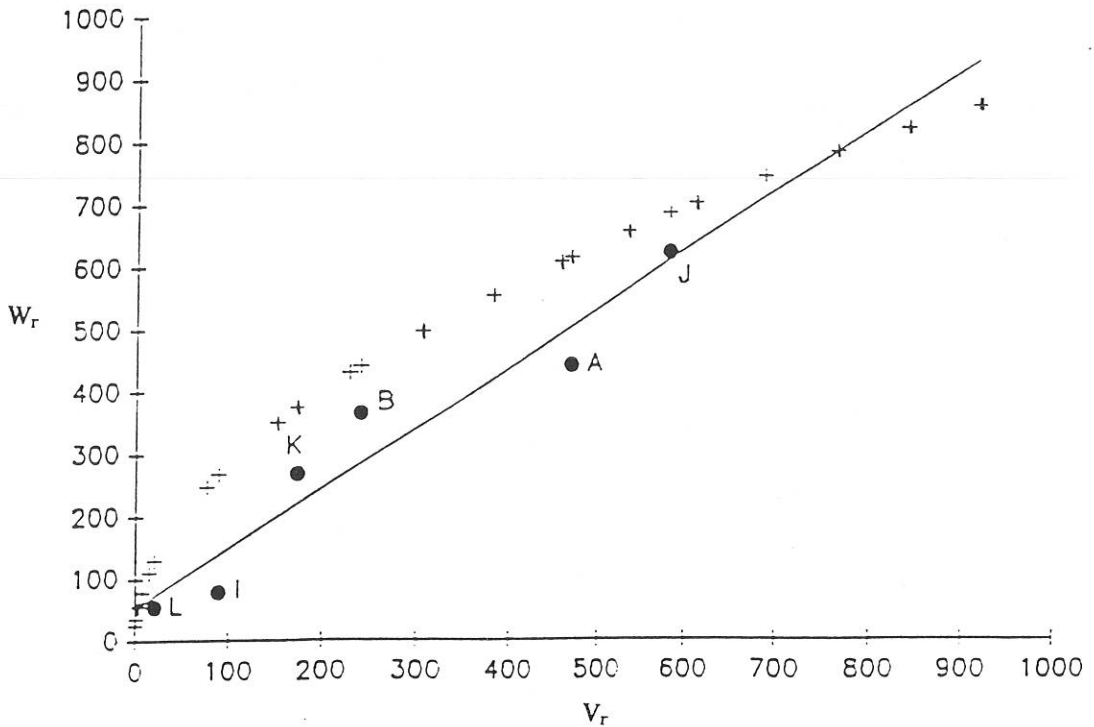


Fig. 2. Hayman's graphic analysis for the variable D.I. in the glasshouse trial.  $W_r = f(V_r)$  graph.

Table 5. Assessment of heritability for resistance (D.I.) and tolerance (L.Y.) for the 2 trials

|            | Resistance (D.I.)         |                      | Tolerance (L.Y.)   |
|------------|---------------------------|----------------------|--------------------|
|            | Glasshouse <sup>(1)</sup> | Field <sup>(2)</sup> | Field <sup>2</sup> |
| $h^2_{bs}$ | 0.86                      | 0.82                 | 0.75               |
| $h^2_{ns}$ | 0.78                      | 0.55                 | 0.16               |

<sup>(1)</sup> Assessed by GRIFFING'S method no. 3

<sup>(2)</sup> Assessed by GRIFFING'S method no. 4

the origin (0,0) line, a typical position of partial dominance.

Parents behave differently in terms of resistance to clubroot: D.I. values range from 6 to 78 % in the early trial and from 17 to 76 % in the field trial. The same parent ranking is observed on both trials (Fig. 3) even though the level of differentiation is weaker for the field experiment because of a higher residual error variance. Two parents may be distinguished for their higher level of resistance: L and I. According to the young plant trial, J and A genotypes are the most susceptible.

GCA values give information about parental ability to transmit resistance (Fig. 4). It must be

noted that the variable is Disease Index which marks for susceptibility; hence, negative GCA values mean good resistance. For both trials, the ranking of GCA values is almost the same as that of phenotypic values. Only the J genotype, which is among the most susceptible parents, seems to transmit its resistance efficiently (GCA value = -0.21 for the glasshouse trial), with a better behaviour in the field experiment (GCA = -7.41). From both trials, 3 groups of plant material could be distinguished (Fig. 4): the L line, with its very good ability to transmit resistance to its progeny; I, J, K lines, whose GCA values are close to 0, and the parents A and B, which have significant positive values.

On the graph  $W_r = f(V_r)$  plotted from Hayman's analysis (Fig. 2), the parents remain along the line  $D_r$ . The parents L and I have lower values of  $V_r$  and  $W_r$ . Hence, they have many dominant alleles. J, the most susceptible line, has the higher values of  $V_r$  and  $W_r$ . Thus, according to Hayman, it possesses mostly recessive alleles.

So, the most resistant parents have many dominant alleles and the most susceptible, less. For these genotypes, the resistance character is controlled by dominant alleles.

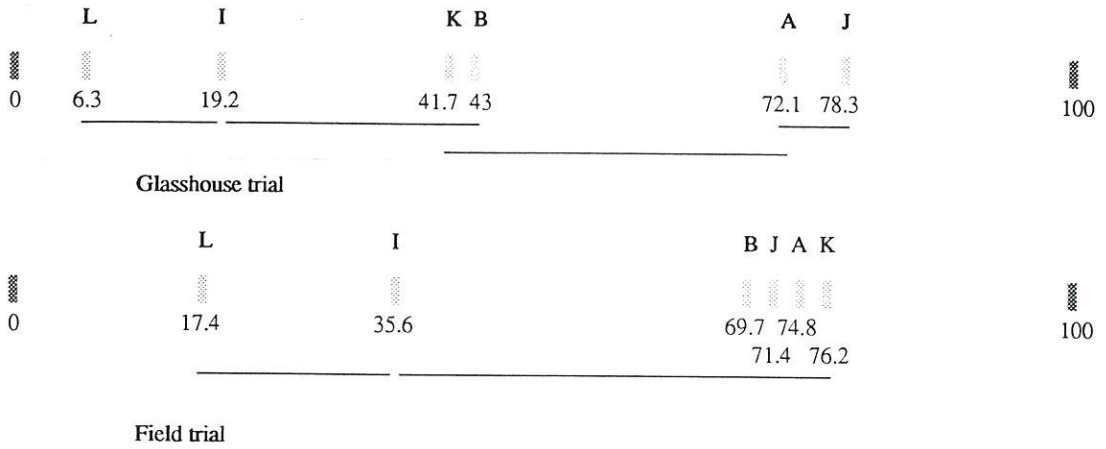


Fig. 3. Disease Index values of the 6 parents for the 2 trials (test Duncan 5 %).

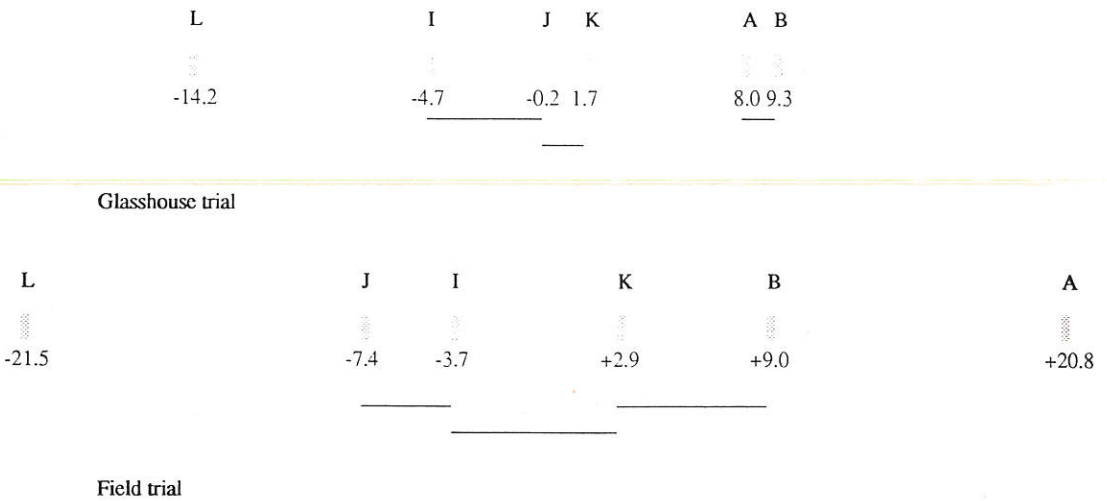


Fig. 4. G.C.A. values of the 6 parents for the 2 trials (test Duncan 5 %).

**Analysis of tolerance**

In the glasshouse trial conditions, no difference of yield was found between plants which had been inoculated and plants which had not. So, the analysis of tolerance was made only on adult plants in the field trial. Fig. 5 shows the behaviour of hybrids and parents for resistance and tolerance. Homozygous lines are the most susceptible to yield losses following a clubroot attack, while the hybrids' behaviour is very variable. The number of genotypes showing strong damage (high Disease Index) but with high yield (L.Y. > 100) is large. These values could be explained by a weak mean

attack level and by an undervaluation of the yield of the plants which were in another part of the field and were not injured. Furthermore, Fig. 5 (D.I. = f(L.Y.)) reveals clearly the dispersion of the points. Disease Index and LY are not significantly correlated.

The analysis of variance for the tolerance variable presents a significant genotype effect ( $p < 0.01$ ; Table 6). As for the Disease Index, replication effect is significant at the 0.05 level of probability, but the residual error is large. Despite the limits, diallel analysis with Griffing's method 4 (model I) could still be performed, as listed in Table 6. Although they are only slightly significant, SCA



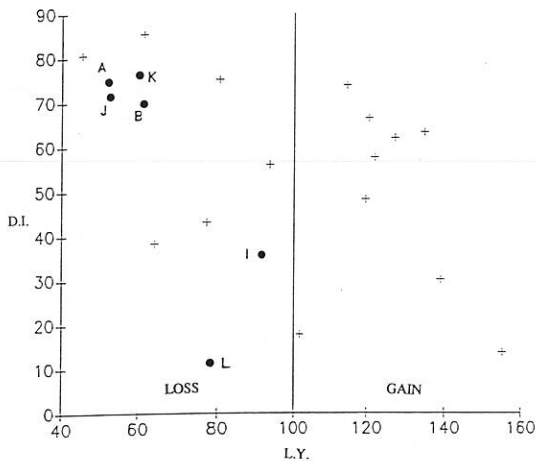


Fig. 5. Disease Index (D.I.) and Loss of Yield (L.Y.) values for all the genotypes in the field trial.

● homozygous line, + hybrids

Table 6. Diallel analysis of variance on the variable "Loss of Yield" (L.Y.) for the field trial

| Source             | df | Mean square | Degree of <sup>(1)</sup> significance |
|--------------------|----|-------------|---------------------------------------|
| Genotype           | 14 | 1900.76     | **                                    |
| Block              | 1  | 2461.6      | *                                     |
| G.C.A.             | 5  | 1158.95     | NS                                    |
| S.C.A              | 9  | 834.44      | *                                     |
| Res <sup>(2)</sup> | 14 | 252.13      |                                       |

<sup>(1)</sup>\*\* : Significant at the 0.01 level of probability

\* : Significant at the 0.05 level of probability

NS: Non significant

<sup>(2)</sup> Residual error calculated from the mean of the 2 replications

effects are the main components of genetic variation, while GCA effects are not significant.

The genetic effects are not as clearly revealed as in the resistance study ( $s^2_G/s^2_{res} = 2.95$ , for tolerance; 6 for resistance) and still the broad sense heritability assessment ( $h^2_{bs} = 0.75$ ) accounts for their major role in the inheritance of tolerance. However, contrasting data for the inheritance of resistance, the inheritance of tolerance is mainly controlled by dominance effects. The  $s^2_A/s^2_D$  ratio ( $= 0.28$ ) is inferior to 1 and the value of narrow sense heritability is very low ( $h^2_{ns} = 0.16$ ).

The study of parent GCA value ranking provides further evidence of the difference between the variables describing resistance and tolerance, i.e., Spearman's correlation coefficient between genotype ranking for D.I. and L.Y. is negative, very weak ( $\rho_{Spearman} = -0.39$ ) and not significant.

## Discussion — Conclusion

This study confirms some of the limits of field trials, such as the duration and the location of the experiments, the influence of climatic conditions that give high residual error, and significant replication effects which limit the expression of genetic variance. Confronted with these problems, the breeder aims to perform reliable early tests. Many inoculation methods have been tested for screening *Brassica* plants against *P. brassicae*, including the "root-dip method" (JOHNSTON 1968), the "germinated-seed-dip test" (LEWIS and BROKENSHIRE 1977), the "slurry method" (TOXOPEUS and JANSEN 1975; CRETE and CHIANG 1980; YOSHIKAWA 1983) and the "spraying method" which we used or the "localized-watering method" (LAMMERS and TOXOPEUS 1977; SCHOELLER and GRUNEWALD 1987; ROD 1988). The present study confirms the accuracy of the "spraying inoculation method" in glasshouse conditions, where the high correlation coefficient for the D.I. variable confirms that young plant behaviour is similar to adult plant behaviour. While conducting the present study, we compared the different methods (Laurens, unpublished), and could therefore verify the differences in efficiency that many authors had shown before (DIXON 1976; LEWIS and BROKENSHIRE 1977; SCHOELLER and GRUNEWALD 1987; ROD 1988). The "spraying method" and the "localized-watering method" are the most efficient. Both methods rank genotypes for their clubroot behaviour in the same order, but, the latter giving stronger attacks on the plants than the former for a fixed inoculum concentration, it can be used to screen for highly resistant plants.

According to the genetic approach, the resistance character on young and adult kales is mainly controlled by genetic effects, and is characterized by a predominance of additive effects with incomplete dominance. However, the narrow sense heritability is lower for the field trial, stressing that additive effects have less relative importance than dominance components in the resistance inheritance of adult plants. The results could be explained by several factors:

- (1) the 2 trials were not performed on the same genotypes (complete and half diallel);
- (2) the plant development was larger in the field than in glasshouse. So, in the field trials, the attack level assessed with D.I. values resulted largely in resistance character and also in plant vigor.

(3) although we showed that no reciprocal effects were present, methods 3 and 4 of Griffing's diallel analysis could give some variable assessments (Laurens, unpublished);

(4) higher residual error and significant reciprocal effects in the field trial could also bias the comparison.

Nevertheless, as for both trials the  $s^2_A/s^2_D$  ratio is always superior to 1, it accounts for higher additivity effects in the genetic control of resistance to clubroot in the kale group. This analysis also shows that many dominant alleles control resistance. Our conclusion contrasts most of the previous studies involving recessive alleles (WALKER and LARSEN 1951; GALLEGLY 1956; AYERS 1957; SEAMAN et al. 1963; CHIANG and CRETE 1976; WEISAETH 1974; YOSHIKAWA 1983; VOORIPS and VISSER 1991). Such contradictions may be explained by the choice of plant material; the cultivars Badger Shipper, Böhmerwaldkohl, Bind-sachsener, or their progenies are almost always at the basis of all genetic studies. The originality of our conclusions comes from the fact that the kale group represented here is of a very different origin: half marrow stem and curly green kale populations. This study confirms the various assessments of the inheritance of resistance to clubroot: involved alleles are not the same even inside the *B. oleracea* ssp. *acephala*.

Conversely, for tolerance, the conclusions are very different as, for most of the genotypes, an increase of susceptibility results in a loss of yield but the correlation on phenotypic values for D.I. and L.Y. is not significant.

According to the genetic approach, the heritability of tolerance is very weak compared to that of resistance. The dominance component controls a great part of the genetic variation. Furthermore, the ranking of GCA values is very different: the most resistant genotypes are not the most tolerant. The results on tolerance heritability are consistent with those obtained by HANSEN (1989), who worked on kales whose origins were very different from those of ours.

Several complex characters are included in the calculation of L.Y., i.e., weight of leaves, foliar morphology, yield, ... So, vigor plays a key role in the expression of the character, as also does the environmental effect between 2 plants.

Breeding programmes could be undertaken on the basis of our results. They must concern only kale belonging to half marrow stem and curly

green populations. If we take into account the genetic control of resistance to *P. brassicae* in the kale group, we can propose a maternal individual recurrent selection on progenies. The basis population should be built by intercrossing homozygous genotypes which have the highest level of resistance to clubroot. Among the few parents studied, a great variability of behaviour for clubroot resistance was found; some resistance sources will soon be available. In the basis population, the resistant reproductive plants should be chosen for their phenotypic values and also after analysis of half-sib progenies. Intercrosses between heads of each family build up the improved population. For this average resistant material the "localized-watering method" must be used with increasing inoculum concentrations as the population resistance level progresses. After a few breeding cycles, it could be possible to add selection for tolerance which could occur only on the field. Taking into account the importance of dominant effects, the breeding strategy should be very different from that proposed for resistance. A recurrent selection on specific ability may be developed, but it would be very long and very difficult to undertake because all the trials would have to be performed in the field.

Among *Brassica oleracea*, cauliflower has a bigger economical importance than kale. It is affected by clubroot which is one of the main diseases of the crop. While resistance sources are available for kales, none are available in the "*Botrytis*" group. One solution for breeding resistance is to transfer resistance from kale to cauliflower. Because of the great variability in the genetic control of resistance, specific studies are indispensable before a breeding programme can be developed. Such studies were already underway and will be presented separately.

## References

- AYERS, G. W. 1957. Races of *Plasmidiophora brassicae*. — *Can. J. Bot.* 35: 923–932
- BAGGETT, J. R. 1983. Clubroot-resistant cabbage breeding lines Oregon 100, 123, 140 and 142. — *Hortscience* 18: 112–114
- BAGGETT, J. R. and KEAN, D. 1985. Clubroot-resistant Broccoli breeding lines OSU CT-2 to OSU CR-8. — *Hortscience* 20: 784–785
- BROKENSHERE, T. 1982. The reaction of forage Brassicas to nine populations of *Plasmidiophora brassicae* Woron. from S.E. Scotland. — *Ann. Appl. Biol.* 100 (supplement). *Tests of Agrochemicals and cultivars* 3: 92–93
- BU CZACKI, S. T., TOXOPEUS, T., MATTUSCH, P., JOHNSTON, T. D., DIXON, G. R. and HOBOLTH, L. A. 1975. Study of physiologic specialization in *Plasmidiophora brassicae*: proposals for attempted rationalization through an international approach. — *Trans. Br. Mycol. Soc.* 65: 295–303
- CHIANG, M. S. and CRETE, R. 1970. Inheritance of clubroot resistance in cabbage (*Brassica oleracea* L. var. *capitata* L.). — *Can. J. Genet. Cytol.* 12: 253–256

- CHIANG, M. S. and CRETE, R. 1976. Diallel analysis of the inheritance of resistance to race 6 of *Plasmodiophora brassicae* in cabbage. — *Can. J. Plant Sci.* 56: 865–868
- CHIANG, M. S. and CRETE, R. 1989. Richelain: a clubroot-resistant cabbage cultivar. — *Can. J. Plant Sci.* 69: 337–340
- CRETE, R. and CHIANG, M. S. 1980. Screening Brassicas for resistance to clubroot, *Plasmodiophora brassicae* Wor. — *Can. Plant Dis. Surv.* 60: 17–19
- CRUTE, I. R. 1986. The relationship between *Plasmodiophora brassicae* and its hosts. The application of concepts relating to variation in interorganismal associations. — *Adv. Plant Pathol.* 5: 1–52
- CRUTE, I. R. and PINK, D. A. C. 1989. The characteristics and inheritance of resistance to clubroot in *B. oleracea*. — *Proceedings of the XIIIth Eucarpia Congress, Göttingen, Germany*, chp 17-13
- CRUTE, I. R., GRAY, A. R., CRISP, P. and BUCZACKI, S. T. 1980. Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in Brassicas and allied crops. A critical review. — *Plant Breed. Abstr.* 50: 91–104
- CRUTE, I. R., PHELPS, K., BARNES, A., BUCZACKI, S. T. and CRISP, P. 1983. The relationship between genotypes of three *Brassica* species and collections of *Plasmodiophora brassicae*. — *Plant Pathol.* 32: 405–420
- DIXON, G. R. 1976. Methods used in western Europe and the U.S.A. for testing *Brassica* seedling resistance to clubroot (*Plasmodiophora brassicae*). — *Plant Pathol.* 25: 129–134
- DOUBLET, L., ROUXEL, F. and MASSON, J. P. 1988. Réceptivité des sols à la hernie des crucifères: analyse statistique de résultats. — *Agronomie* 8: 459–464
- GALLEGLY, M. E. 1956. Progress in breeding for resistance to clubroot of broccoli and cauliflower. — *Phytopathology* 46: 467
- GRIFFING, B. 1956a. A generalised treatment of the use of diallel crosses in quantitative inheritance. — *Heredity* 10: 31–50
- GRIFFING, B. 1956b. Concept of general and specific combining ability in relation to diallel crossing systems. — *Aust. J. Biol. Sci.* 9: 463–493
- GUSTAFSSON, M. and FÄLT, A. S. 1986. Genetic studies on resistance to clubroot in *Brassica napus*. — *Ann. Appl. Biol.* 108: 409–415
- HANSEN, M. 1989. Genetic variation and inheritance of tolerance to clubroot (*Plasmodiophora brassicae* Wor.) and other quantitative characters in cabbage (*Brassica oleracea* L.). — *Hereditas* 110: 13–22
- HAYMAN, B. I. 1954. The theory and analysis of diallel crosses. — *Genetics* 39: 789–809
- JAMES, R. V. and WILLIAMS, P. H. 1980. Clubroot resistance and linkage in *Brassica campestris*. — *Phytopathology* 70: 776–779
- JOHNSTON, T. D. 1968. Clubroot in *Brassica*: a standard inoculation technique and the specification of races. — *Plant Pathol.* 17: 184–187
- LAMMERS, W. and TOXOPEUS, H. 1977. New "multipot" clubroot resistance screening method in use at the SVP. — *Proceeding of the Woronin + 100 International Conference on Clubroot, University of Wisconsin (USA)*, p. 122–123
- LEMOULEC, J. 1986. Contribution à la mise au point de tests de résistance à la hernie des crucifères, *Plasmodiophora brassicae* Woron. — *Mémoire de D.E.A. Université de RENNES I*
- LEWIS, S. J. and BROKENSHERE, T. 1977. A comparison of plant inoculation techniques for the determination of *Plasmodiophora* population. — *Proceeding of the Woronin + 100 International Conference on Clubroot, University of Wisconsin (USA)*, p. 122–123
- ROD, J. 1988. New inoculation method for the testing of *Brassica* for resistance to *Plasmodiophora brassicae* Wor. — *Cruciferae Newsl.* 13: 102–103
- SCHOELLER, M. and GRUNEWALD, J. 1987. Comparison of inoculum techniques for testing *Brassica* seedling resistance against *Plasmodiophora brassicae* Wor. — *Cruciferae Newsl.* 12: 86–87
- SEAMAN, W. L., WALKER, J. C. and LARSEN, R. H. 1963. A new race of *Plasmodiophora brassicae* affecting Badger Shipper cabbage. — *Phytopathology* 53: 1426–1429
- TOXOPEUS, H. and JANSEN, A. M. P. 1975. Clubroot resistance in turnip. II. The slurry screening method and clubroot races in the Netherlands. — *Euphytica* 24: 751–755
- VOORIPS, R. E. and VISSER, D. L. 1991. Recessive inheritance of resistance to clubroot in *Brassica oleracea*. — *Cruciferae Newsl.* 14/15: 138–139
- WALKER, J. C. and LARSEN, R. H. 1951. Progress in the development of clubroot resistant cabbage. — *Phytopathology* 41: 37 (Abstract)
- WEISAETH, G. 1974. Some problems and results in breeding for clubroot resistance in cole crops. — *Proceedings of Eucarpia Meeting*, p. 101–107
- WILLIAMSON, C. J. 1984. Resistance to *Plasmodiophora brassicae* in *Brassica napus*. — *Proceedings of Better Brassicas '84 conference, St. Andrew, Scotland*, p. 123–132
- WILLIAMSON, C. J. 1987. Assessment of resistance to *Plasmodiophora brassicae* in swedes. — *Plant Pathol.* 36: 264–275
- WILLIAMSON, C. J. and BRADSHAW, J. E. 1989. Sources of resistance to *Plasmodiophora brassicae* used in breeding programmes at S.C.R.I. — *Proceedings of the XIIIth Eucarpia Congress, Göttingen, Germany*, chp 17-12
- YOSHIKAWA, H. 1983. Breeding for clubroot resistance of crucifer crops in Japan. — *Jpn. Agric. Res. Q.* 17: 6–11