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Attempted selection for partial resistance to the sugar beet cyst nematode, *Heterodera schachtii*, in *Brassica napus* L.

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Summary – Accessions of *Brassica napus* L. were examined for (partial) resistance to the sugar beet cyst nematode, *Heterodera schachtii* (abbrev. SBCN), by assessing the number of cysts found on the root system. In addition, S_1-progenies of plants, selected for having only ten or fewer cysts on their root system, were evaluated for the number of cysts and proportion of plants with ten or fewer cysts. No indication for the existance of genetic variation in SBCN-resistance between *B. napus* L. accessions was found. The difficulties encountered in detecting genetic variation in resistance are suggested to be due to the high experimental variability, common to resistance tests with nematodes.

Résumé - ***Essai de sélection en vue de la résistance partielle de* Brassica napus *L. au nématode à kystes de la betterave à sucre,* Heterodera schachtii** - Des accessions de *Brassica napus* L. ont été testées en vue de leur résistance (partielle) au nématode à kyste de la betterave à sucre, *Heterodera schachtii* (en abrégé : SBCN), en dénombrant les kystes présents sur le système racinaire. De plus, la descendance – S_1 – des plants sélectionnés pour leur système racinaire ne comportant que six kystes ou moins, a été testée en dénombrant les kystes et la proportion de plants ne portant que dix kystes ou moins. Il n'a été observé aucune indication d'une variabilité génétique liée à la résistance au SBCN chez les accessions testées. Les difficultés rencontrées pour détecter une variabilité génétique liée à la résistance sont supposées être dues à la grande variabilité dans l'expérimentation, ce qui est fréquent dans les tests concernant les nématodes.

Key-words : Sugar beet cyst nematode, *Heterodera schachtii* Schm., *Brassica napus* L., oil-seed rape, partial resistance, selection.

Brassica napus L. ssp. *oleifera* (Metzg.) Sinsk. (oil-seed rape) is susceptible to *Heterodera schachtii,* the sugar beet cyst nematode (SBCN), and its cultivation is likely to result in an increase of the population of this nematode in the soil. Because of the risk of severe damage to sugar beet due to infestation by SBCN, oil-seed rape cannot be grown in narrow rotation with sugar beet. Hence, breeding for SBCN-resistant oil-seed rape is of importance.

It is not clear whether useful genetic variation for SBCN-resistance is available in *B. napus*. Baukloh (1976) did not find any differences in susceptibility between 215 *B. napus* accessions tested, but Bowen *et al.* (1986) found differences in root growth, in hatching and in nematode multiplication on roots, between eighteen *B. napus* cultivars. In addition, preliminary experiments carried out by Harrewijn (1987) showed that 48 out of 196 *B. napus* accessions had an average number of cysts which was significantly lower than that observed on the susceptible standard cv. Jet Neuf and might possess partial resistance to SBCN.

The aim of the present study was to verify if the results obtained by Harrewijn (1987) could be repeated and to expand on this study by testing 48 additional *B. napus* accessions for (partial) resistance. The possibilities for selection were assessed by testing inbred lines from plants, having a low number of cysts on their root system.

Materials and methods

SCREENING FOR SUGAR BEET CYST NEMATODE RESISTANCE

The resistance tests were performed according to the method described by Toxopeus and Lubberts (1979). Seeds were sown in 36 ml-PVC tubes, filled with sterilized silver sand, moistened with Steiner I (Steiner, 1968) nutrient solution and kept in a greenhouse at a 10 h light regime, a constant temperature of 18 °C and a relative humidity of 85 to 90 %. After two weeks each seedling was inoculated with 2 ml of a suspension containing approximately 300 pre-hatched second-stage ju-

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veniles (J2) of *Heterodera schachtii* Schm. using a veterinary syringe. As inoculum the population that has been the standard for the last 15 years at our institute was used, a mixture from collections carried out in 1974, 1975 and 1976 at various locations in The Netherlands, and subsequently multiplied on various susceptible plant species in the greenhouse at Wageningen. Larvae were reared on susceptible *Beta vulgaris, Brassica napus* or *Sinapis alba* plants. Cysts of *Heterodera schachtii* Schm. were collected and placed in a $ZnCl_2$ solution (1mM). Twice a week hatched juveniles were collected and stored in the refrigerator, for use within the next two days. Prior to inoculation, the number of living larvae was counted in two samples of 2 ml solution, with the use of a binocular microscope, and the inoculum density was adjusted to 150 living larvae/ml water.

After inoculation the temperature in the greenhouse was raised to 22 °C during the day. At four weeks after inoculation the root systems were washed free from sand and were evaluated for the appearance of mature females, further referred to as cysts. The level of resistance of each accession was calculated either as the mean of the absolute number of cysts per root system, further referred to as " absolute number of cysts ". In addition, to compare the results of separate tests, also the mean of the absolute number of cysts relative to that found on the susceptible standard cv. Jet Neuf was calculated and is further referred to as " relative number of cysts ". Futhermore, the proportion of plants with ten or fewer cysts was calculated, a characteristic which had also been used by Harrewijn (1987) to evaluate the level of SBCN-resistance. Differences in absolute and relative number of cysts were analysed by means of ANOVA.

PLANT MATERIAL

In total, 100 *B. napus* accessions, consisting of fodder rape, swede, spring and winter oil-seed rape accessions, were evaluated for SBCN-resistance. Fifty-two accessions had been tested earlier by Harrewijn (1987) of which twelve accessions had been found to have a number of cysts not significantly different from that on the susceptible standard ($P > 0.95$) and are further referred to as " susceptible accessions ", while 40 accessions had a significantly lower number of cysts on their root systems ($P < 0.05$) than the susceptible standard cv. Jet Neuf and are referred to as " resistant accessions ".

Three experiments (1, 2, and 3) were carried out to test the 100 accessions. In Experiment 4 a repeated evaluation of 40 accessions, which had also been evaluated in Experiment 2, was performed. The winter oil-seed rape cv. Jet Neuf was always used as susceptible control. For each accession and for the standard cv. Jet Neuf four or five replications of 20 plants each were tested.

Plants with ten or fewer cysts on the root system were selected from all accessions with an average absolute and relative number of cysts significantly lower than that found on cv. Jet Neuf, and from one accession which was as susceptible as cv. Jet Neuf. The selected plants were vernalized at 7 °C during two months, and transferred at springtime to an unheated greenhouse for flowering. Flower stalks were bagged to prevent cross-pollination. The offspring of these self-pollinated plants was tested in two experiments for SBCN-resistance in four replications of 20 plants each, together with the parental accessions and cv. Jet Neuf, of which also four replications of 20 plants each were used.

Results and discussion

EVALUATION OF *B. NAPUS* ACCESSIONS

Fifteen of the 100 *B. napus* accessions had an absolute and relative number of cysts significantly lower than thay of cv. Jet Neuf ($P < 0.05$), while fourteen of these fifteen accessions had plants with ten or fewer cysts. Three of the fourteen accessions were observed among the 48 accessions, which had not been evaluated before. The other eleven accessions were found among the 40 " resistant accessions " identified by Harrewijn (1987). The twelve " susceptible accessions " of Harrewijn (1987) were all found to be susceptible again.

Figure 1 shows the distributions of the frequency of plants with the observed absolute number of cysts of all 100 accessions tested in the Experiments 1, 2 and 3. The results for the 11, 3 and 1 accessions, having a significantly lower number of cysts than cv. Jet Neuf (group A) and the 28, 35 and 22 accessions having an equal or higher number of cysts than cv. Jet Neuf (group B) were pooled for Experiment 1, 2 and 3, respectively. The percentage of plants with ten or fewer cysts in group A was found to be higher than that for cv. Jet Neuf or group B, but only in the Experiments 1 and 2, with an overall low mean number of cysts (Fig. 1 A and 1 B *vs* 1 C). As a result, the selection pressure applied in Experiments 1 and 2 was less strong than that in Experiment 3, in which no accession containing plant with ten or fewer cysts was observed.

OFFSPRING OF SELECTED PLANTS

Of the fourteen accessions from Experiments 1 and 2, which were found to have a significantly lower number of cysts than cv. Jet Neuf, plants with ten or fewer cysts were selected. In addition, plants with ten or fewer cysts were also selected from accession 12, being as susceptible as cv. Jet Neuf (Table 1; first tests). These selected plants were selfed and 27 S_1-lines were tested in one experiment with the corresponding twelve parental accessions. In another experiment fourteen S_1-lines were evaluated jointly with their three parental accessions (Table 1; second tests).

Only three of these fourteen parental accessions (i.e. accessions 3, 9 and 10), when retested, showed again an absolute and relative number of cysts significantly lower than counted on cv. Jet Neuf ($P < 0.05$). The susceptible parental accession 12, was susceptible again

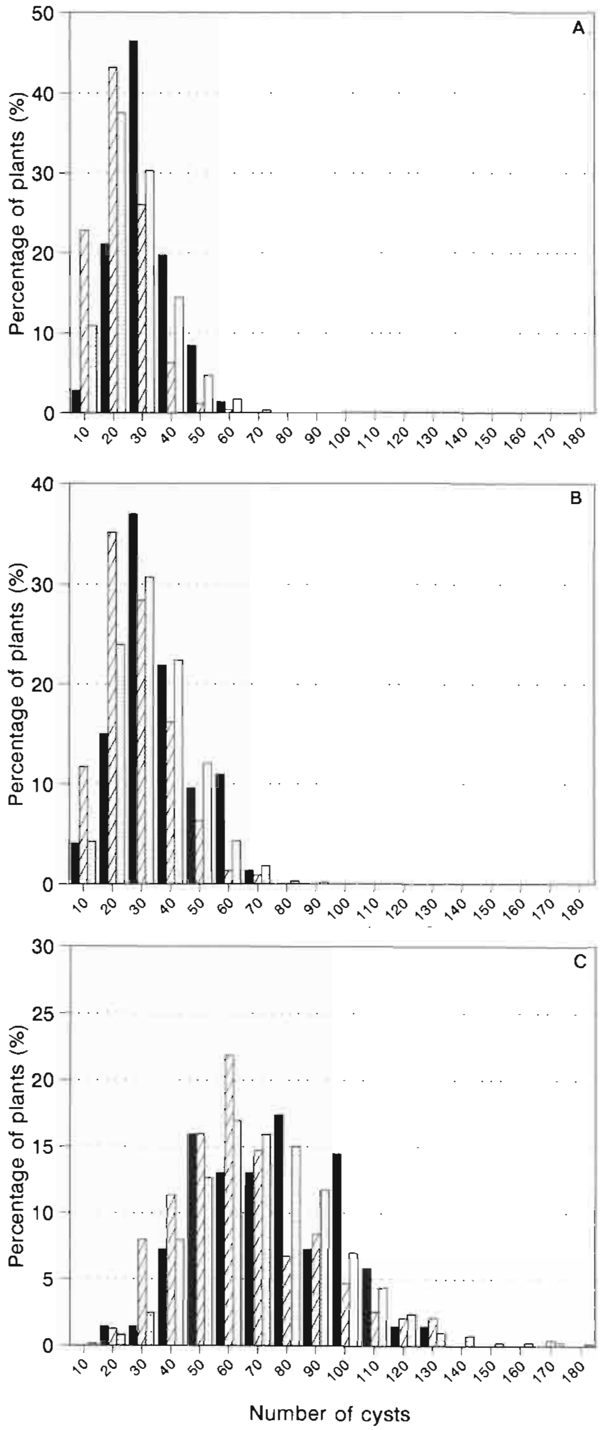

Fig. 1. *Frequency distributions for the Experiments 1, 2 and 3 of plants carrying few to many cysts among the pooled resistant accessions (group A), the susceptible accessions (group B) and the standard susceptible accession "Jet Neuf" (black : cv. Jet Neuf; striated : Group A; dotted : Group B).*

(P > 0.95). Furthermore, the percentage of plants with ten or fewer cysts of the fifteen parental accessions differed greatly between the first and the second tests (Table 1).

Only the offspring of the selected plant from accession 4 was found to carry on average fewer cysts than cv. Jet Neuf, i.e. 65 %. However, the percentage of plants with ten or fewer cysts in S_1-progenies from the selected plant of this accession was equal to that observed in the original population. The progenies of the selected plants of the other fourteen parental accessions had an equal or higher number of cysts than the parental accessions. Also, the percentage of plants with ten or fewer cysts on the progenies of selected plants was not different from that on the parental accessions (Table 1).

REPRODUCTIBILITY OF THE SBCN-RESISTANCE TESTS

Repeated testing of 40 accessions, which had been evaluated in Experiment 2, was conducted in Experiment 4. It was found that the correlation between the Experiments 4 and 2 was not significant, the correlation coefficient being 0.25. When comparing the results of all experiments, it was found that in those tests with an overall low level of infestation by the SBCN, more accessions with a significantly lower average number of cysts than that found on the cv. Jet Neuf were observed than in experiments with a generally high level of SBCN-infestation.

Harrewijn (1987) observed a range in relative number of cysts of the 40 " resistant accessions " of 14-68, while in our experiments 79-122 was observed for these accessions. Also, the absolute number of cysts found by Harrewijn (1987) on the 40 " resistant accessions » was much lower and ranged from 5 to 34 cysts, whereas 22 to 78 cysts were observed in our experiments. In addition, also the absolute number of cysts on the twelve retested " susceptible accessions " was higher in our experiments. It ranged from 24 to 72, whereas 22 to 44 cysts were observed by Harrewijn (1987). For these " susceptible accessions ", the range in relative number of cysts in our experiments, being 72-118, was similar to that found by Harrewijn (1987). The correlation coefficient between the relative number of cysts for the 52 accessions tested by Harrewijn (1987) and the experiments described was 0.14 (not significant at P > 0.95).

Conclusions

With the present method, no indication for the existence of genetic variation in SBCN-resistance between the *B. napus* accessions tested, was found. This is in accordance with some of the earlier reports (Baukloh, 1976; Bowen *et al.*, 1986), while the suggested existence of partial resistance in some *B. napus* accessions by Harrewijn (1987) could not be confirmed. The results suggest that any existing genetic variation in partial resistance may be undetectable due to high experimental variability common to resistance tests with nematodes.

Table 1. *Screening for resistance to* H. schachtii *in* B. napus. *Level of resistance of fifteen accessions of* B. napus, *of which plants were selected with ten or fewer cysts (first tests, left colums), and the level of BCN-resistance of these fifteen accessions after retesting, together with the offspring of self-pollinated plants with a number of cysts ranging from 0 to 10, selected from these fifteen accessions (second test). JN = standard susceptible* B. napus *cultivar Jet neuf.*

	First tests		Second tests			
	Parental accessions		Parental accessions		Selfed progenies from selected plants with 10 or fewer cysts	
no	Average number of cysts relative to that on *B. napus* cv. Jet Neuf (JN = 100)	Percentage of plants with 10 or fewer cysts	Average number of cysts relative to that on *B. napus* cv. Jet Neuf (JN = 100)	Percentage of plants with 10 or fewer cysts	Average number of cysts relative to that on *B. napus* cv. " Jet Neuf " (JN = 100)	Percentage of plants with 10 or fewer cysts
Experiment 1			JN 100	0		
JN	100	3	*Progeny Experiment 1*			
1	69*	8	88	4	88-91	2-5
2	65*	5	96	1	87	0
3	72*	5	58*	11	92	3
4	73*	6	87	4	65*	6
5	63*	6	93	0	99	0
6	68*	15	82	1	85	0
7	67*	16	111	0	80-109	0-6
8	51*	25	99	0	78-101	0-4
9	69*	11	76*	9	86-101	0-2
10	66*	17	73*	8	85-103	0-4
11	73*	8	109	5	89-94	1-3
12	77##	9	88##	3	84	0
LSD (5 %)	25		21		21	
Experiment 2			JN 100	2		
JN	100	4	*Progeny Experiment 2*			
13	67*	11	119	4	93-115	4-8
14	76*	12	100	13	78-122	1-16
15	64*	16	89	6	81	2-12
LSD (5 %)	21		56		56	

* Relative number of cysts significantly lower than that found on the susceptible cv. Jet neuf ($P < 0.05$).
Accession as susceptible to BCN as cv. " Jet Neuf " ($P > 0.95$).

For example, it has been shown that multiplication rates of cyst nematodes are strongly influenced by environmental factors, such as plant vigour, plant growth, plant nutrition and temperature (Kämpfe & Kerstan, 1964; Johnson & Viglierchio, 1969 *a*, *b*; Müller, 1985; Grundler *et al.*, 1991) resulting in a lower number of cysts per root system under less favourable conditions for plant growth.

The average number of cysts of the S_1-populations, with one exception was not lower than that of the parental populations. This suggests strongly that selection for resistance within *B. napus* accessions is not likely to result in improved levels of resistance.

The *B. napus* accessions tested so far (Talatschian, 1974; Baukloh, 1976; Bowen *et al.*, 1986; Harrewijn, 1987) included a wide range of genotypes, representing many countries of origin and many different plant types, such as spring, winter oil-seed rape, swedes and fodder types. All were found to be susceptible to SBCN. Therefore, other types of resistance, such as the high level of SBCN-resistance found in *Sinapis alba* (white mustard) and *Raphanus sativus* (oil-radish), should be incorporated in *B. napus* in order to obtain SBCN-resistant oilseed rape varieties.

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