AN ARTIFICIAL INOCULATION METHOD TO EVALUATE SUGAR BEET BREEDING LINE FOR RESISTANCE TO *APHANOMYCES* ROOT ROT

OKAZAKI, KAZUYUKI*, NAOKI OGATA, HIROYUKI TAKAHASHI, KAZUNORI TAGUCHI, and KEIJI NAKATSUKA, National Agricultural Research Organization(NARO), Shinsei, Memuro, Hokkaido, 082-0071, Japan.

Introduction

Root rot, which can cause severe yield loss in sugar beet (*Beta vulgaris* L.), is caused by the soil-borne fungus *Aphanomyces cochlioides* Drechsler (Ui and Nakamura, 1963). A worldwide pest, *A. cochlioides* has been detected in about 33% of sugar beet fields in England (Payne et al., 1994), in close to 50% of fields in a warm, wet year in southern Minnesota, U.S.A. (Windels and Lamey, 1998), annually in about 20% of Japanese sugar beet fields, and is also prevalent in Australia (Martin, 2003). The problem in Japan has been particularly acute since 1999, when disease damage was very serious. While, effective chemical controls have not been available, different sugar beet lines are known to exhibit varying degrees of resistance to root rot (Chikuo et al., 1982). The

cultivation of resistant cultivars is considered to be a promising method of control, and breeding programs have been instituted.

The resistance, however, is thought to be controlled by several genes (Mukhopadhyay, 1987), so it is necessary to evaluate resistance accurately and to repeat selections over several years. We have conducted an evaluation and selection for root rot resistance in infested fields since 1998 (Taguchi et al., 2000). However, field tests rely on natural conditions, and as disease severity differ from year to year, resistance of lines could not be evaluated when climatic conditions were unfavorable to the fungus growth and disease progression (Watanabe et al., 2000).

The infective form of *A. cochlioides* is a zoospore, which is believed to move in free water toward the root surface, germinate and then infect the host tissue (Islam et al., 2001). Therefore, free water in the soil is necessary for infection to occur. In addition to soil moisture, this fungus is known to be temperature-sensitive, the progression of the disease being greatly slowed at soil temperatures below 14°C (Chikuo et al., 1986).

This study sought to develop a zoospore inoculation method for *Aphanomyces* root rot adapted to greenhouse pot-culture of sugar beet, thus allowing a greater control over growth conditions than is possible in the field. A second goal was to test the resistance of F1 hybrid lines, bred at the National Agricultural

Research Organization (NARO) in Japan, to root rot.

Materials and Methods

Inoculum preparation

Aphanomyces cochlioides AP-16, isolated from a field in Hokkaido Prefecture, Japan, was grown on Difco potato dextrose agar (PDA) for 7 days at 25°C, in a 90 mm Petri dish. Seven 8-mm diameter disks were then aseptically removed from the margin of the mycelium and placed in a 90 mm Petri dish, flooded with 20 ml of sterile deionized water. After a 24 hr incubation at 25°C, in the dark, released zoospores were counted with a haemacytometer.

Development of the resistance screening procedure

In 2003, a zoospore inoculation method for *Aphanomyces* root rot, adapted to greenhouse pot-culture of sugar beet was developed. Three treatments regimes were tested: (i) "inoculated-overwatered": plants were inoculated with zoospores and overwatered, (ii) "non-inoculated-overwatered": plants were overwatered but received no inoculation and (iii) "control": plants received neither excessive watering nor inoculation. A split-plot-design with two replicates (10 plants per plot) was employed. The full experimental protocol is shown in Fig 1. Three

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sugar beet cultivars (F1 hybrids) of known resistance were tested: one strongly resistant (Yukihinode), one weakly resistant (Kabutomoru), and one moderately resistant (Monohomare). The different hybrids were sown in paper pots (19 mm diameter and 13 cm height, Nippon Beet Sugar Mfg. Co., Ltd.) on April 15. One month after, a portion of the seedling-bearing pots were soaked continuously for a week with a zoospore suspension (3.0×10^4 zoospores per plant; "inoculated-overwatered"), while others received only water ("non-inoculated-overwatered" and "control"). After treatment (May 22), seedlings were transferred to 1/5000-are pots (1 plant per pot). Plants were grown in a greenhouse (15°C for the 30 days after inoculation, thereafter >20°C). Excessive watering (300 ml water/pot/day), where applicable, occurred for 10 day periods, one beginning immediately after transplanting and the second beginning 30 days after transplanting. Roots were harvested roughly 90 days after transplanting (August 21), and ranked for disease severity according to the criteria shown in Table 1. The disease index (DI) for a given hybrid × treatment combination was calculated as the mean rank of plants of that hybrid subjected to the particular treatment.

Screening of breeding lines for resistance to *Aphanomyces* root rot.

In 2004, eight lines (one O-type line and seven F1 lines) bred at NARO were evaluated for resistance to *A. cochlioides* root rot, in both field tests and greenhouse-based inoculation tests. For the inoculation tests the lines were sown (April 7), transplanted (May 22) and treated exactly as in the inoculated-overwatered treatment imposed in 2003 (Fig. 1), with two replicate plots (10 plants per plots) for each line.

In the case of field tests, non-inoculated seedlings were transplanted into an infested field on May 21. Three randomly placed replicate plots (each with 26 plants per plot) were used for each line.

Roots were harvested, weighed and ranked for disease symptoms for both the field test (September 4), and the inoculation test (August 9).

Results

Development of the resistance screening procedure

The results of pot-culture tests are shown in Table 2. Root < Table 2
mass in all plots averaged over 50 g and root hypertrophy was evident. Mean root mass for the inoculated-overwatered roots was 73 g, significantly lower than that of the untreated control (104 g). Mean root mass for the non-inoculated-overwatered roots tended to be slightly greater than that of the untreated control. Within the

inoculated-overwatered treatment, inter-cultivar differences in root mass were noted. The roots of Yukihinode were heavier than those of the other cultivars (P<0.05), but no significant differences occurred between the other treatments.

With respect to the disease index, virtually no symptoms were observed in the untreated control (DI= 0.1), the lowest value among the three treatments. The DI of the non-inoculated-overwatered roots was slight higher than that of the control (P<0.05), but at 0.4 it was still quite low, indicating that overwatering alone had little effect on the DI. Under this treatment the DI of all individual cultivars remained under 0.5, and showed no significant differences. In contrast, for the inoculated-overwatered treatment, clear disease symptoms were observed and at 2.1 the DI was significantly greater (P<0.05) than that of other treatments. Moreover, inter-cultivar differences in DI were significant (P<0.05), with Kabutomaru, Monohomare, and Yukihinode cultivars showing DI values of 2.9, 2.0 and 1.3, respectively, following the order of their known resistance.

Evaluation of resistance to *Aphanomyces* root rot.

The results of field tests are shown in Table 3. Since temperatures in the summer of 2004 were high but rainfall very low, conditions were unfavorable for the development of the disease.

Consequently, roots of Kabutomaru showed little rot and a low DI of 1.7. In contrast, in the inoculation test, roots of Kabutomaru exhibited numerous symptoms of the disease (Table 4). At 3.5 its DI was clearly superior to that obtained in the field test (Table4). Inter-cultivar differences in DI were highly significant (P 0.01) in both field and inoculation tests. The relative order of cultivars resistance, based on their DI values, corresponded closely between the two tests, indicating that our newly-developed inoculation method allowed an accurate evaluation of resistance in the field.

Of lines bred in NARO, the DI of Hokkai90 and Hokkai 91 (0.3 and 0.9, respectively) were lower than that of Yukihinode (1.4), the most resistant cultivar currently cultivated in Japan. It was thus evident that these two lines had considerable resistance to *Aphanomyces* root rot. Given that the DI of Hokkai90 was the lowest in both field and inoculation tests, it was considered to be the most promising of our breeding lines.

Discussion

Numerous studies have been conducted on seedling damping-off by *A. cochlioides*, but reports on root rot are much fewer. Methods for artificially inoculating sugar beet roots with *A. cochlioides* had not been developed. It was known that in the field, in Japan, root rot symptoms began to develop at the end of June,

when the root began to enlarge (Watanabe, 2002). Therefore, if one were to reproduce root rot symptoms artificially, it would be necessary to perform the inoculation at a stage of crop development when root enlargement had occurred (i.e., root mass □ 50 g), rather that at the seedling stage when plants would simply be killed by damping-off.

The inoculation method we developed to screen for resistance to root rot closely paralleled results obtained in field tests, and had the added advantage of presenting greater disease severity, allowing for greater inter-cultivar/line discrimination compared with the field test. Furthermore, this technique only required 120 days, 60 days less than the field test. It would therefore be possible to test for resistance two or three times a year. This method, useful in the breeding of resistant lines, would also be expected to be useful in the study of resistance mechanisms.

Regarding the resistance of lines bred at NARO, Hokkai90 and Hokkai91 were shown to be promising new resistant lines. In particular, roots of inoculated Hokkai90 remained healthy, with almost no symptom of root rot, suggesting considerable resistance. The cultivation of Hokkai90 would be expected to greatly reduce yield losses associated with the disease.

Literature Cited

Chikuo, Y., Naito, S., and Sugimoto, T. 1982. *Aphanomyces* black root disease of sugar beet occurring after transplanting into field. Proc. Japan. Soc. Sugar Beet Technol. 24:157-162.

Chikuo, Y., Naito, S., and Sugimoto, T. 1986. Effects of soil temperature and soil moisture on *Aphanomyces* black root. Japanese Journal of Phytopathology. 52:142

Islam, Md. T., Ito, T., and Tahara, S. 2001. Morphological studies on zoospore of *Aphanomyces cochlioides* and changes during interaction with host materials. J. Gen. Plant Pathol. 67:255-261

Martin, H.L. 2003. Management of soilborne diseases of beetroot in Australia: a review. Australian Journal of Experimental Agriculture. 43:1281-1293.

Mukhopadhyay, A.N. 1987. *Aphanomyces* Root Rot. *In* "CRC hand book on disease of sugar beet volume 1" A.N. Mukhopadhyay, CRC Press, Inc. Florida, 119－125.

Payne, P.A., Asher, M.J.C., and Kershaw, C.D. 1994. The incidence of *Pythium* spp. and *Aphanomyces cochlioides* associated with the sugar beet growing soils of Britain. *Plant Pathology*. 43:300–308.

Taguchi, K., Ogata, N., Takahashi, H., Kuranouchi, T., Kawakatsu, M., and Tanaka, M. 2000. Breeding *Aphanomyces* root rot resistance in sugar beet 1. On site screening for resistance to *Aphanomyces* root rot in disease-infested field. Proc. Japan. Soc. Sugar Beet Technol. 42:52-58.

Ui, T., and Nakamura, S. 1963. Sugar beet black root and its pathogen *Aphanomyces cochlioides*: Pathogenicity and host specificity. Tensai-Kenkyukai-Houkoku 3:78-95.

Watanabe, H., Uchino, H., and Hayasaka, M. 2000. Development of artificial inoculation method of *Aphanomyces* root rot for sugar beet. Proc. Japan. Soc. Sugar Beet Technol. 42:47-51.

Watanabe, H. 2002. Relation between occurrences of *Aphanomyces* root rot and weather condition. Proc. Japan. Soc. Sugar Beet Technol. 44:82-88.

Windels, C.E., and Lamey, H.A 1998. Identification and control of seedling diseases, root rot, and *Rhizomania* on sugar beet. North Dakota State Univ/Univ. of Minnesota Extension Services. 1142, http://www.sbreb.org/brochures/rhizoman/rhizoman.htm

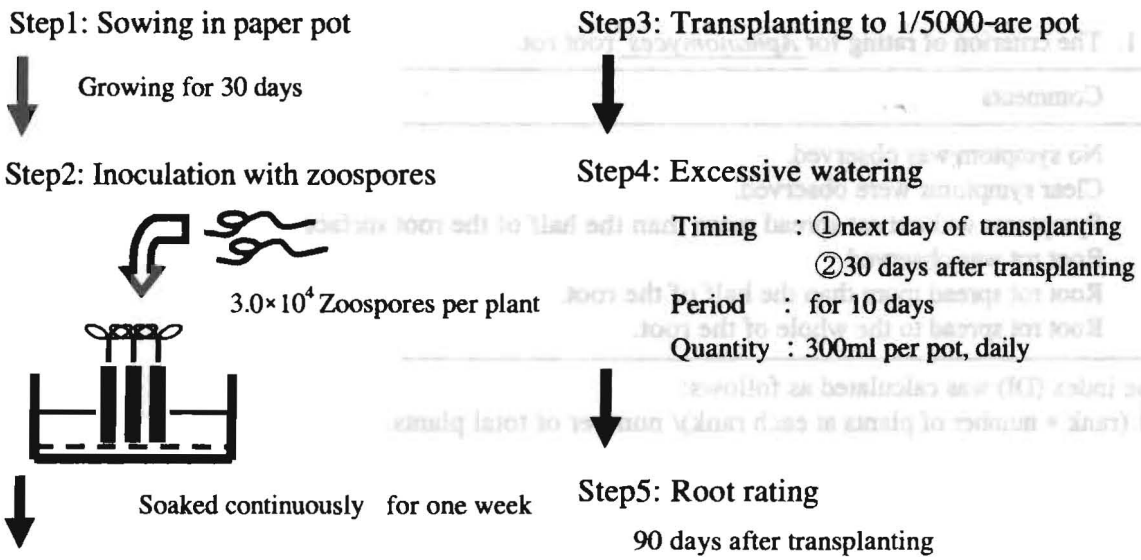

Fig. 1. Procedure of artificial inoculation method for *Aphanomyces* root rot.

Okazaki et al, Fig. 1(100/100)

Table 1. The criterion of rating for *Aphanomyces* root rot.

Rank	Comments
0	No symptom was observed.
1	Clear symptoms were observed.
2	Symptoms without rot spread more than the half of the root surface.
3	Root rot was observed.
4	Root rot spread more than the half of the root.
5	Root rot spread to the whole of the root.

Disease index (DI) was calculated as follows:
DI = □ (rank × number of plants at each rank)/ number of total plants.

Okazaki et al, Table 1(100/100)

Table 2. Influence of treatments on root weight and disease index.

Treatments	Cultivars	Root weight [v] (g)		Disease index [w] (DI)	
Control	Monohomare	105[x]	a[y]	0.0	a
	Kabutomoru	95	a	0.2	a
	Yukihinode	111	a	0.1	a
Non-inoculated-overwatered	Monohomare	118	a	0.5	a
	Kabutomoru	107	a	0.5	a
	Yukihinode	111	a	0.2	a
Inoculated-overwatered	Monohomare	69	a	2.0	b
	Kabutomoru	56	a	2.9	a
	Yukihinode	93	b	1.3	c
Average of Treatment	Control	104		0.1	
	Non-inoculated-overwater	112		0.4	
	Inoculated-overwatered	73		2.1	
Average of Cultivar	Monohomare	97		0.8	
	Kabutomoru	86		1.2	
	Yukihinode	105		0.5	
F-test	Treatment	** [z]		**	
	Cultivar	N.S		**	
	Interaction	N.S		**	

[v] Includes root and crown.

[w] Represents a scale of 0-5, 5 being most sever.

[x] Values reprensent the mean of 2 replications (10 plants per plot).

[y] Means followed by the same letter in each column are not significantly different according to Turkey test at P=0.05.

[z] Means were statistically significant at P=0.01.

Okazaki et al, Table 2(100/100)

Table 3. Evaluation of resistance of 8 sugar beet lines to *Aphanomyces* root rot in *Aphanomyces* infested field.

Lines [w]	Disease index [x] (DI)	
NK-184	4.2[y]	a[z]
Kabutomaru	1.7	b
Hokkai 88	1.3	c
Monohomare	1.1	c
Steut	1.0	c
Yukihinode	0.7	d
Hokkai 91	0.5	d e
Hokkai 90	0.2	e
L.S.D.(5%)	0.3	
L.S.D.(1%)	0.4	

[w] All lines were F1 hybrid cxcept for O-type line NK-
[x] Represents a scale of 0-5, 5 being most sever.
[y] Values reprensent the mean of 2 replications (26 plants
[z] Means followed by the same letter are not significantly different according to Duncan's multiple range test

Okazaki et al, Table 3(100/100)

Table 4. Evaluation of resistance of 8 sugar beet lines to *Aphanomyces* root rot with inoculation method.

Lines [v]	Root weight[w] (g)		Disease index [x] (DI)	
NK-184	3[y]	d[z]	4.9	a
Kabutomaru	50	c	3.5	b
Monohomare	72	b	2.7	c
Hokkai 88	71	b	2.5	c
Steut	83	ab	1.5	d
Yukihinode	72	b	1.4	de
Hokkai 91	95	a	0.9	e
Hokkai 90	90	ab	0.3	f
L.S.D.(5%)	20		0.5	
L.S.D.(1%)	29		0.7	

[v] All lines were F1 hybrid cxcept for O-type line NK-184.

[w] Includes root and crown.

[x] Represents a scale of 0-5, 5 being most sever.

[y] Value reprensents the mean of 2 replications (10 plants per

[z] Means followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

Okazaki et al, Table 4(100/100)