Cultivation of Clubroot-Resistant Strains to Suppress Population Density of Resting Spores of *Plasmodiophora brassicae* in the Soil

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Introduction

Cruciferous crops play a very important role in human life, being used as vegetables, oil crops and forage crops etc. We can find them in many parts of the world. For cruciferous crops, clubroot caused by *Plasmodiophora brassicae* Worn. is one of the most serious diseases. The clubroot disease originated in western Europe, and many wild and economic species of cruciferae are their hosts. The clubroot organism has been spreading all over the world. Recently the infested area is not limited to the temperate zone, but is distributed in the tropical zone of the Asia, Oceania and Americas[8].

Where cruciferous crops are cultivated, resting spores of *P. brassicae* germinate in the soil and become primary zoospores.

The zoospores swim to the surface of root hair cells of an appropriate cruciferous host where they penetrate and begin to grow into the cytoplasm of the hair cells. After a few days the parasitized hair cells die and the plasmodium cleaves into a number of sporangia each of which releases several secondary zoospores[1,5,9]. The proliferation of these secondary zoospores in the roots results in the formation of typical galls of roots in susceptible strains as shown in Plate 1. The infected plants cannot transport nutrients and water to the upper parts, to wilt gradually.

The resting spores of *P. brassicae* are carried and spread by water and wind. They are highly resistant to environmental changes and to pesticides in the soil, and can survive more than several years. In the intensively cultivated fields of cruciferous crops, the density of the resting spores increases rapidly and causes serious damage, by which cruciferous crops have to be replaced by other kinds of crops. Therefore, the resistant varieties to this disease have been eagerly required. Recently several resistant varieties have been developed in Japan. These resistant varieties are known to induce germination of resting spores of *P. brassicae* just like susceptible ones, but they produce no clubs. From this fact, it was supposed that the clubroot resistant plants prohibit the proliferation of secondary zoospores in roots, resulting in the reduced number of resting spores in the soil.

In this article, results of the experiment conducted to examine effect of resistant strains on the density of resting spores in the soil[10] are presented.

Breeding for resistance in Japan and mechanism of resistance

In Japan most cruciferous vegetables are cultivated intensively, and so-called "damage caused by continuous cropping" becomes a serious problem. Clubroot is a major factor of this problem. The disease is found in almost all regions of Japan.

For the breeding of resistance, an enormous number of varieties were collected from the world and screened for the resistance. The result indicated that there are no resistant ones in Japanese varieties. On the other hand some European turnips and kales were found to be highly resistant, and some

(a)

(b)

Plate 1. (a) Clubroot disease of turnip, (b) Normal turnip

European cabbages were moderately resistant to major races of Japan[2,3,11].

Using the European turnips as breeding materials and by hybridizing them with Japanese practical varieties, resistant parental lines and F_1 varieties of Chinese cabbage, turnip and salt greens have been bred and released as shown in Table 1. However, breeding works in cabbage were difficult, because

Table 1. Clubroot-resistant varieties and parental lines bred in Japan

Kinds of vegetables	Varieties	Parental lines
Chinese cabbage	Strong CR 75 (Watanabe Seed Co.) CR Kanki (Nihon Norin Sha) Kukai 65, 70 (Takii Seed Co.)	Parental line No. 1~5 (VOCRS)
Turnip	Shinano (NPVCRS)	
Salt green		Parental line No. 1 (VOCRS)
Cabbage	Chyo-Ya Ko No. 9 (NPVCRS)	

Seed companies or research institutes where resistant varieties or parental lines were bred are shown in parentheses.
VOCRS=Vegetable and Ornamental Crops Research Station
NPVCRS=Nagano Prefectural Vegetable Crops Research Station

the resistance is controlled by polygenes. Up to now only one variety has been developed (Table 1)[12].

Along with the progress of breeding works, the mechanism of resistance was investigated. For example Kroll et al.[6] compared the primary and secondary cycle developments of the fungus in root hairs and root medullary rays, using a completely resistant, a partially resistant or a susceptible radish cultivar. Also Naiki et al.[7] studied the relationship between the frequency of root hair infection and clubroot severity using 54 cultivars of 5 cruciferous crops. In these experiments it was found that root hairs of cruciferous crops are infected irrespective of the resistance to clubroot. But resistant varieties prohibit the proliferation of the secondary zoospores and show no symptoms.

Effect of cultivation of resistant strains on the density of resting spores in the soil

In 1980 autumn, clubroot spores were inoculated into the soil at the rate of 5×10^6 spores/m*l* of soil. In this infected soil, several kinds of crops were cultivated in the spring season from 1981 to 1984 as shown in Table 2. They were two non-cruciferous and three cruciferous crops. Of the cruciferous crops, two were clubroot resistant strains and one was a clubroot susceptible strain. Of the two clubroot resistant strains, one is turnip 77b, which is a selected line from the strain used for the clubroot race testing by European Clubroot Differential (ECD) method[4]. The resistance of this turnip is controlled by a single dominant and several modifier genes[12]. This strain was used as a parent of resistant parental lines of Chinese cabbage. The other resistant strain is a kale variety, K269, which is a curled one and its resistance is controlled by a single recessive gene. After the spring season cultivation, a clubroot-susceptible Chinese cabbage (1981) or turnip (1982–1984) was cultivated in all plots in autumn.

In both seasons about two months after seed-sowing the disease incidence (percent of diseased plants and disease index) was investigated.

It is possible to estimate the relative abundance of spores among plots by comparing the disease incidence of susceptible strains in autumn season, but the estimation of actual spore density is impossible. Consequently, the soil was sampled from each plot after four years of cultivation. On the other hand artificially infested soil with the known spore density (six levels from 0 to 5×10^6/m*l*) was prepared by mixing the clubroot spores into the sterilized soil.

The soil sampled from each field plot or the soil artificially infested was placed into pots, to which a clubroot-susceptible Chinese cabbage variety New Azumasanto was planted. Two months after sowing, disease incidence was measured. By comparing the disease incidence between sampled soils and artificially infested soils, clubroot spore density in the soil of each plot was estimated.

1) Disease incidence of susceptible turnips in autumn season

Fig. 1 shows the relative degree of disease incidence of susceptible turnips cultivated in autumn in different plots shown in Table 2. The values of the disease incidence are expressed as percentage to the

Table 2. Kinds of crops cultivated in spring season to reduce the resting spores of *P. brassicae* in the soil*

Plot number	Crops cultivated in spring season	Inoculation of resting spores of clubroot**
1	Susceptible Chinese cabbage in 1981 and susceptible turnip in 1982 to 1984	+
2	Resistant kale K 269 in 1981 to 1984	+
3	Resistant turnip 77b in 1981 to 1984	+
4	Sorghum in 1981 to 1984	+
5	No cultivation in 1981 and spinach in 1982 to 1984	+
6	No cultivation in 1981 and spinach in 1982 to 1984	−

* In autumn season clubroot-susceptible Chinese cabbage was cultivated in all plots in 1981 and clubroot-susceptible turnip in 1982 to 1984 in all plots.

** +; Resting spores of clubroot were inoculated in 1980 at the rate of 5×10^6/ml soil.
−; No inoculation of clubroot spores.

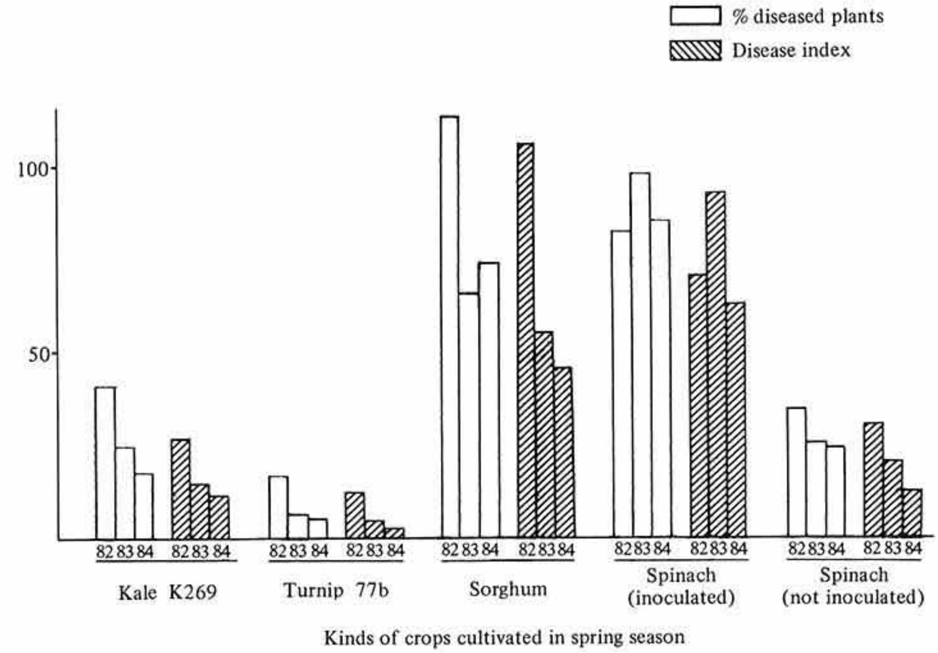

Fig. 1. Effect of different spring crops on the disease occurrence on susceptible turnip cultivated in autumn

Values of % diseased plants and disease index are expressed by taking these values in the plot where susceptible turnip was grown both in spring and autumn as 100.

values of the plot in which susceptible turnip was cultivated in both seasons.

In the plot of continuous cultivation of susceptible turnip, disease incidence was very high in every season.

There were two plots in which other kinds of crops than cruciferous, i.e., spinach and sorghum, were cultivated. Of them, the spinach plot showed a little lower disease incidence than the susceptible turnip plot, but the incidence didn't show a tendency to decrease from year to year. In the sorghum plot disease was a little more severe than the susceptible turnip plot in 1982, and then it decreased to about 50% level of the susceptible turnip plot. It suggests that sorghum has a higher effect in reducing spore density in the soil than spinach, but the effect is only

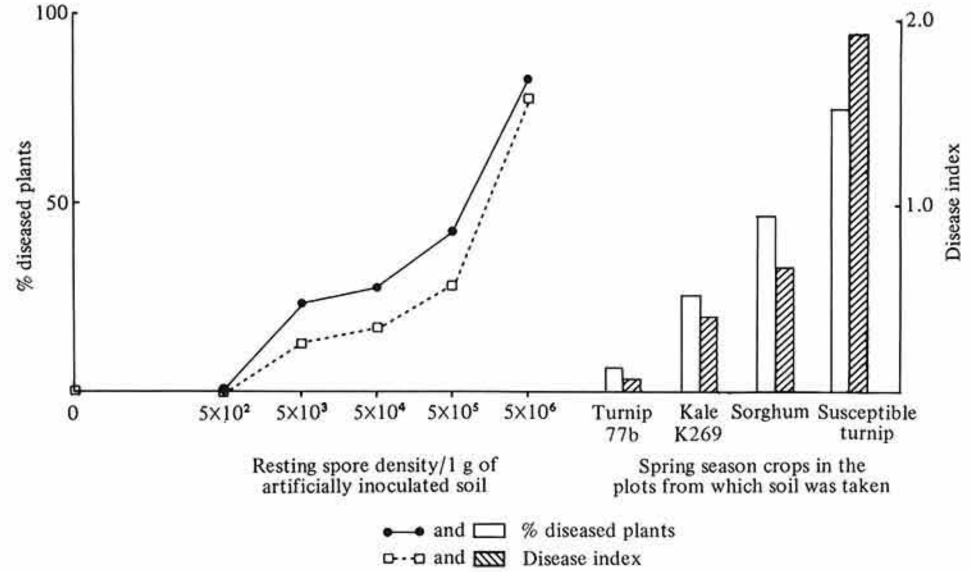

Fig. 2. Incidence of clubroot on susceptible Chinese cabbage New Azumasanto cultivated in pots containing the soil artificially inoculated or the soil taken from the experimental field plots

a partial one. It seems that positive effects to suppress resting spores may not be expected by other kinds of crops than cruciferous one.

On the contrary, the resistant strains (K269 and 77b) showed the positive effect to reduce disease incidence of susceptible autumn turnip. In the kale plot, the number of diseased plants was 40% of that of the susceptible turnip plot and the number decreased year to year, to a level as low as 17%. Similarily in the plot of resistant turnip, the disease was suppressed more markedly. The number of diseased plants was less than 20% of the susceptible turnip plot in 1982 and decreased to 5% in 1984. In these two plots, clubroot disease was of no practical problem in 1984.

2) Estimation of resting spore density in the soil

After four years of cultivation, resting spore density was estimated by the method already described in the previous section. As given in Fig. 2, in the artificially inoculated soil, disease was not found at the spore density lower than 5×10^2. When the density was higher than 5×10^2, disease symptom gradually increased in parallel with the spore density, and at the level of 5×10^6/m*l*, 83% of plants were diseased.

Disease incidence in the susceptible turnip plot was rather higher than that observed at 5×10^6 spore density, so that the spore density of this plot was estimated as a little higher than 5×10^6/m*l* of soil.

In the sorghum plot, disease incidence corresponded to nearly 5×10^5 of spore density. This density was only about 10% of the initial density, but about a half of Chinese cabbage plants were diseased.

Among the plots of clubroot-resistant cruciferous crops, 25% of plants were diseased in the kale plot. This percentage corresponded to 5×10^4 of spore density. In the turnip plot, only 5.4% of plants were diseased, corresponding to lower than 5×10^3 of density. From these results, the spore density in the kale plot and turnip plot was estimated at 1% and less than 0.1% of the initial spore density, respectively.

Prospects

For the control of disease and insect pests, various kinds of pesticides and insecticides have been developed and used world-wide. But it has been pointed out that the use of these chemicals has a risk of disturbing normal ecosystems.

To suppress the clubroot disease PCNB has been used. However, its effectiveness decreases gradually by repeated use and higher dosage becomes necessary. Besides, this chemical is known to have residual toxicity.

In place of such chemicals, the importance of biological control has been emphasized. Among many methods of biological control, the rotation with other kinds of crops or the use of resistant varieties have a long history. In addition, the use of parasites, and physiologically active substances such as pheromon and kairomon for insects, pretreatment of temperate virus for virus diseases, etc. are tried recently for practical application.

As shown in this article, the rotation with sorghum was not so effective in reducing clubroot disease. Already many scientists examined the effects of the rotation, but crops with superior effects on the clubroot were not found yet.

Compared with the rotation, cropping of clubroot-resistant cruciferous strains was found to have the positive effect of reducing clubroot spores in the soil. Thus, the resistant strains can be used for the biological control of clubroot disease.

However, the cropping of resistant strains means the continuous cultivation of cruciferous crops. It has a danger of inducing other diseases. The effective cultivation system of cruciferous crops should be established by combining the use of resistant strains and the rotation with other crops.

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