Screening Techniques of Disease Resistance in *Cruciferae* Vegetables

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Cruciferae crops have very important roles for human beings. They are used not only as vegetables, but also as oil and forage crops all over the world. In Japan, *Cruciferae* crops, especially radish, Chinese cabbage, and cabbage, are the most important vegetables. These three crops occupy 25% of cultivated area and 36% of harvested amount of all vegetable production in 1983.

Recently the continuous cropping became widespread in *Cruciferae* vegetables and the problem of 'crop damage caused by continuous cropping', especially the outbreak of diseases, has become serious. Most of these diseases are soilborne and in most cases it is expensive and difficult to protect crops from these diseases by chemical pesticides or fungicides. The cheapest and most effective method to resolve the problem of diseases is the use of resistant varieties.

In the breeding work, the screening of resistance is indispensable. It is necessary not only for finding resistant materials but also for selection in progenies. In this paper we will present the screening techniques of disease resistance already established or now being exploited in *Cruciferae* vegetables.

Major diseases of *Cruciferae* vegetables and conditions necessary for screening techniques

Table 1 shows the major diseases of *Cruciferae* vegetables in Japan (from Ashizawa).[1] Nearly all diseases attack every kind of vegetables. But the degree of damage depends upon the kinds of diseases, vegetables and growing seasons. For example, clubroot causes severe damage in *B. campestris*, *B. oleracea* and *B. juncea*, and bacterial soft rot in *B. campestris*, *B. juncea* and *R. sativus*. In contrast, severe attack of black rot is restricted to *B. oleracea*.

With vegetables severely susceptible, breeding works for resistance have been undertaken and to some diseases resistant varieties have already been established. These successful breeding works are usually supported by the appropriate screening techniques of resistance.

For the techniques to be effective in the breeding work, it is necessary to fulfill several conditions as follows:

(1) The technique must detect strictly the individual difference of resistance and the detection must be stable despite the change of environments.

(2) Preparation of pathogen and inoculation must be easy with simple equipments.

(3) Many plants must be inoculated in a small area and in short time.

(4) Screening must be done in young seedling stage, and it is desirable that the selected plants can be used for the screening of other characters.

In addition to these prerequisites, it is better than resistances to 2 or 3 diseases can be tested simultaneously. Actually Williams at al.[11] proposed the method of screening for multiple disease resistance.

They showed the method of screening procedures for resistance to clubroot, downy mildew, TuMV and bacterial soft rot in Chinese cabbage. But for this method high techniques of inoculation and strict control of environment are necessary. So the method is thought not so practical for the use outside the laboratory.

Table 1. Major diseases and development of breeding works for resistance to them in Japan (from Ashizawa)[1]

Pathogen	Disease name	Severity of diseases and state of breeding			
		B. campestris	*B. oleracea*	*B. juncea*	*R. sativus*
Altenaria japonica	Altenaria leaf spot	○	○	○	○
Peronospora brassicae	Downy mildew	○	○	○	○
Sclerotinia sclerotiorum	Sclerotinia rot	○	○	○	○
Plasmodiophora brassicae	Clubroot	◉A	◉B	◉	△
Verticillium dahlie	Yellows	○	—	—	△
Fusarium oxysporum f. sp. *raphani*	Yellows	△	◉A	△	◉A
Pseudomonas syringae pv. *maculicola*	Bacterial leaf spot	○	○	○	○
Xanthomonas campestris pv. *campestris*	Black rot	○	◉B	○	○
Erwinia carotovora ssp. *Carotovora*	Bacterial soft rot	◉A	○	◉	○
TuMV, CMV	Mosaic	◉A	△	◉	◉A

—; No infestation, △; Disease occurs but damage is not significant, ○; Damage occurs, ◉;Severe damage occurs, A; Resistant varieties are established, B; Breeding works for resistance are now undertaken.

Examples of screening techniques

1) Clubroot and yellow in cabbage

As already mentioned, clubroot attacks severely all *Cruciferae* vegetables except radish. For the use in breeding of clubroot resistance, Yoshikawa et al.[7] established a screening technique named 'insertion method'. The details of this technique were introduced by Yoshikawa.[8] By the insertion method 10 plants per 8 cm diameter pot are grown, so that many plants can be tested in a small area. For cabbage it takes about 6 weeks from sowing to observation of resistance. After the observation, the resistant plants can be selected and transplanted into the field and can be tested for other general characters. But, in Chinese cabbage it is difficult to observe general characters using the selected plants, because the observation of resistance is done with washed roots which make it usually difficult to transplant.

In cabbage, yellow by *Fusarium oxysporum* is also an old and severe disease. Nomura et al.[2] tried to find an effective method of early selection. They adapted 3 inoculation methods to 20–30 day-old seedlings and described the procedure. The advantage of each method is as follows:

(1) The root-dipping method; roots of seedlings are washed and then dipped into the suspension of the pathogen before transplanting. This method is suitable to test a small number of plants within a short time.

(2) The soil-inoculation method; seedlings are transplanted in the soil previously inoculated with the pathogen or in the infested soil. This method can be done with simple equipments.

(3) The ditch-inoculation method; seeds are sown in narrow bands of the soil separated with thin plates, and afterward the suspension of pathogen is poured into the ditches made by removing those plates. This method is suitable to test a large number of plants at a time.

Among these three, they described, the ditch-inoculation method is useful for the screening of primary breeding materials or early generation progenies, while the root-dipping method is useful for the strict

Table 2. An example of dual screening for resistance to yellow and clubroot in cabbage

Varieties	Survival rate with yellow infestation	Percentage of infested plants by clubroot	Degree of disease by clubroot[a)]
YR 50 (resistant)	60.7%	65.2%	1.04
Masagosanki (susceptible)	3.6	–	–
2L16–1	67.3	45.4	0.51
2L18–1	5.4	25.0	0.25
2L18–2	37.4	62.5	0.67
1L44–1	46.3	25.9	0.26
2L31–2	42.9	76.7	0.77

a) 0: No disease, 1: 1–10% of roots were infested by clubroot, 2: 10–30% of roots infected by clubroot.

selection among a small number of hopeful plants. So we can select any methods depending upon the purpose of our screening.

We have been trying to exploit the method for screening the combined resistance to clubroot and yellow. Seeds of strains are sown in the soil blocks containing the pathogens of both the yellow (1×10^7/1 g dry soil) and the clubroot (5×10^6/1 g dry soil) in pots. Firstly the resistance to yellow can be observed, and later clubroot resistance can be observed with the survived plants.

Table 2 shows an example of screening. By the 6 weeks after sowing, nearly all plants of a susceptible variety 'Masagosanki' died by yellow, whereas 61% of a resistant variety 'YR 50' survived. Out of 2 types of resistance to yellow, Type A resistance is known to be controlled by a single dominant gene. So by comparing the rate of survived plants with those of standard varieties, we can estimate the genetic construction of tested strains. In this case '2L16-1' and '2L18-1' are estimated as a completely resistant (AA) and susceptible (aa) strain, respectively. The other 3 lines are estimated as segregating strains which involve 3 genotypes (AA, Aa, aa).

Afterward by observing the clubroot of the survived plants, we can evaluate the resistance of each strain and also can select the resistant plants. Furthermore it is possible to make selection for general characters in the field among the transplanted resistant plants.

The problem of this method is that it takes a long period (about 2 months) for the resistance screening, and the practical cultivation in the field becomes a little bit difficult.

2) Yellow of radish

Similar to the case of cabbage, several methods of inoculation are available for yellow of radish, such as root-dipping method, soil-inoculation and direct sowing method, pouring method, and ditch-inoculation method. Among them the pouring method is the easiest one but generally the disease occurrence is relatively light. Compared to this, the root-dipping method causes severe infestation but the method is laborious. The soil-inoculation and direct sowing method makes most natural infestation and can be used both in greenhouse and field.

Table 3 shows the results of soil-inoculation and direct sowing method carried out in the field. Fungi of yellow were cultured in the bran for about a month and mixed with the soil of the field. The volume of bran was about 130 l per 100 m^2. Seeds of radish were sown in this infested soil.

The symptoms of standard varieties proceeded as shown in Table 3. A very weak variety, 'Wakakoma', died completely 75 days after sowing. On the other hand, very strong variety, 'Red Prince', bred in America (Peterson et al.)[4)] showed very light symptoms.

Comparing the symptoms of the individual plants with those of standard varieties, we

Table 3. Yellow symptoms of standard varieties inoculated by the soil-inoculation and direct-sowing method

Varieties	Resitance	Symptoms after sowing[a]		
		19 days	33 days	75 days
Wakakoma	Very weak	1.4	2.1	3.0
Natsutomi	Strong	0.5	1.1	1.3
Red Prince	Very strong	0	0.3	0.4

a) 0; no disease~3; died.

can evaluate the resistance. At the same time the evaluation of morphological characters is possible.

3) Black rot of cabbage

Black rot is also one of the most widespread diseases in *B. oleracea*. Williams et al.[10] found that Japanese variety, 'Early Fuji', was resistant and it had been used as a breeding material in America, Japan etc. But the screening of resistance usually depends upon spontaneous outbreak or artificial infestation in the field (Plate 1a), and the result is not so reliable.

Ohata et al.[3] exploited the clip inoculation method as follows: They cut the uppermost leaf and subordinate leaf of cabbage seedlings of about 2 months old with scissors or nail clipper. Scissors or a nail clipper were dipped in the bacterial suspension of 10^{6-7} cells/ml before cutting, and the cutting was made at the top of leaves in parallel with the leaf margin.

Two weeks later, they evaluated the resistance by the area of lesion and by this method it was found that 'Kinpai' was highly resistant to black rot. This inoculation method is very easy and stable. But it takes a little long period from sowing to the screening. So the screening of practical characters using selected resistant plants becomes a little difficult.

We are now trying to adapt this clip method to younger seedlings. It has been found that by keeping temperature and humidity high, young seedlings with 3 or 4 leaves can be infested constantly. Plate 1b shows the symptoms on young seedlings by clip inoculation.

4) Bacterial soft rot and yellow in Chinese cabbage

Bacterial soft rot gives a very severe damage to all *Cruciferae* vegetables except *B. oleracea*. In Japan, Chinese cabbage and raddish production in hot season is strictly limited by this disease. So from old time there have been a lot of attempts of resistance breeding and artificial screening. As a resistant variety, as already referred by Ashizawa,[1] 'Hiratsuka No. 1' was established from inter-specific cross between Chinese cabbage and synthetic *B. napus*, and it has been used as a breeding material of major Chinese cabbage varieties in Japan.

Concerning the screening method, Shimizu et al.[6] tried to apply several inoculation techniques using detached leaves, such as the needle prick method, dipping method and smearing method. The reason why they used detached leaves is that living plants from which leaves are detached can be selected for other general characters and used as parents for the next generation. But until now any effective methods have not been established.

Compared to soft rot, yellow by *Verticillium dahlie* is relatively a new disease of Chinese cabbage.

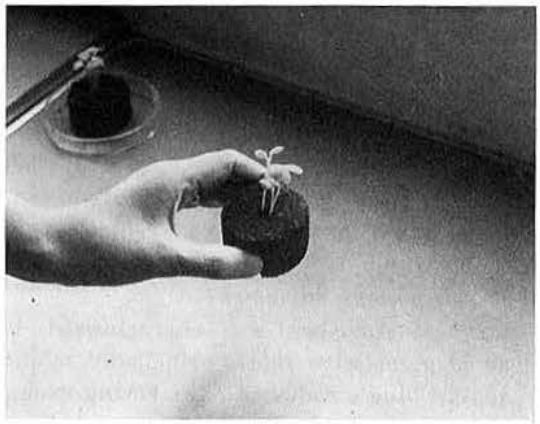
After the comparison of several methods, we[9] found that the dipping method using a special pot (Jiffy-7 or Jiffy-9) was most convenient (Plate 2). But the symptoms of this disease proceed from roots to stem and leaves slowly. Therefore, to make an early observation of resistance we must cut the crown part of plants. As a result, other characters cannot be evaluated with the plants selected for the resistance. In order to employ this method in breeding works, further improvement of screening technique will be necessary.

Prospects

In this paper we presented several prerequisite conditions and examples of screening in the breeding for disease resistance. Of course it is very difficult to establish a method which fulfills all the conditions described above. The major condition which must be fulfilled de-

Plate 1. Symptoms of black rot caused by (a) spontaneous infestation in the field and (b) artificial clip inoculation

pends upon the severity of disease and the whole purpose of breeding. For example, if the resistance is the most important character, strict and stable screening technique will be necessary, but if the resistance is not so important and it must be combined with other characters, easiness and quickness will be necessary.



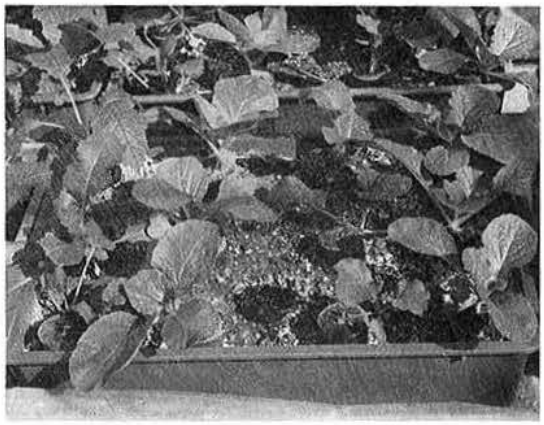


Plate 2. Preparation of Chinese cabbage seedlings in Jiffy-9 pots for the screening of resistance to yellow

As shown in this paper, there are various methods of screening. In addition, recent modern techniques such as so called *in vitro* selection by tissue or cell cultures have been under exploitation. For example Sacristán et al.[5] made it possible to infect the cultured tissue of haploid *B. napus* with the spores of clubroot. They discussed the possibility of using their system for the selection of resistance to the pathogen.

But we must not forget that the screening technique is to support the breeding work. Otherwise, it is useless even if the technique itself is excellent. The breeding work will be accelerated when it is combined well with appropriate screening techniques.

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