幼苗期におけるナシ黒星病抵抗性個体の早期選抜

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Selection of Scab-resistant Plants in *Pyrus* Seedlings at Juvenile Stage[†1]

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Synopsis

We evaluated a screening method for the detection of scab-resistant plants using one-year-old *Pyrus* seedlings in order to breed scab-resistant cultivars efficiently. One-year-old seedlings were inoculated with a conidial suspension of *Venturia nashicola* at the concentration of $2\sim3\times10^5$ conidia/ml, and kept at 20 ℃ in a humid room for 24 hr～48 hr, then transferred outdoors. Seedlings with leaves lacking sporulating lesions were evaluated as highly resistant whereas those with leaves showing sporulating lesions were evaluated as susceptible. Results from χ^2 tests indicated that the frequency of plants with a high scab resistance was not statistically different among seedlings inoculated at different growth stages. In 222 plants out of 238 plants investigated, the rating of resistance in inoculation tests was equivalent to that based on the observation of disease development in the field. When the plants were inoculated at different ages, i.e., when they were one-year-old and four-or five-year-old, the degree of scab resistance was similar in 163 plants out of 177 plants between ages, although the resistance level was different in 14 plants.

Key words : *Pyrus*, scab, disease resistance, artificial inoculation, selection

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Introduction

Scab, caused by *Venturia nashicola*, is one of the most serious diseases of Japanese pear (*Pyrus pyrifolia* NAKAI). Control of scab depends on the use of fungicides, because no scab-resistant cultivars of Japanese pear are commercially available. Scab-resistance is therefore an important objective in Japanese pear breeding.

For breeding for disease resistance, accurate evaluation of resistance at early stages is important to shorten the time requires for breeding. Screening methods of resistant plants have been developed for apple scab (SHAY and HOUGH 1952), European pear scab (STANTON 1953), Japanese pear rust (KOTOBUKI and MACHIDA 1982), black spot of Japanese pear (KOZAKI 1974, SANADA 1988), fireblight of pear (THOMPSON *et al.* 1953). A screening method for the resistance to *V. nashicola* has also been reported in Japanese pear (ABE and KURIHARA 1992, ISHII *et al.* 1992).

However, the application of the methods reported by ABE and KURIHARA (1992) or Ishii *et al.* (1992) to scab resistance breeding is difficult because the preparation of a large number of grafted plants as test materials is time-consuming. Therefore, for efficient breeding, it is necessary to develop a more convenient and rapid method using cross seedlings at the juvenile stage as test plants. In this study we evaluated a screening method of scab resistance using one-year-old *Pyrus* seedlings.

Materials and Methods

Cultivars used as parents are listed in Table 1. The F_1 seedlings obtained in the crosses made from 1987 to 1992 were used in the study. Conidia were collected from fresh scab lesions formed on the leaves of the Japanese pear cultivar 'Chojuro' which was planted at the Fruit Tree Research Station and had never been treated with fungicides. Conidial suspension was prepared at a concentration of $2\sim3\times10^5$ conidia/ml as reported before (ABE and KURIHARA 1992). One-year-old seedlings crossed with'Shinsei'and 'Hakkou','Kinchaku'and'Hosui', 'Okusankichi' and 'Kinchaku', or 'Kinchaku' and 'Hongli' as cross-parents were divided into three groups within a respective cross, and each group of seedlings was inoculated at different growth stages with the suspension using a mist sprayer. One group of seedlings was inoculated at the growth stage in which five leaves had unfolded, another group of seedlings was inoculated when ten leaves had unfolded, and another group when fifteen leaves had unfolded. Seedlings except for those listed above were inoculated when about ten leaves had unfolded. After spraying, the seedlings were kept in a humid room (nearly 100% R.H.) at 20℃ for 24hr~48hr and then transferred outdoors. The appearance of visual scab symptoms was

Table 1. Parental cultivars of *Pyrus* seedlings used as materials and evaluation of resistance to *Venturia nashicola*.

Cultivar	Species[z]	Evaluation[y]
Hakkou	*Pyrus pyrifolia* NAKAI	highly susceptible (HS)
Hosui	〃	HS
Kosui	〃	HS
Shinsei	〃	HS
Yasato	〃	HS
Hogetsu[x]	〃	susceptible (S)
Okusankichi	〃	necrotic (N)
Kinchaku	〃	highly resistant (HR)
Laiyangcili	*P. bretschneideri* REHDER	N~S
Hongli	〃	HR

[z]Classification of chinese pear cultivars followed the method of YU (1979).
[y]Evaluation was mainly performed according to the method described in the previous paper (ABE and KURIHARA 1993) with a slight modefication;
HS : densely sporulating lesions on several leaves, S : sparsely sporulating lesions on a few leaves, N : necrotic or yellow lesions but no sporulation, HR : no visible symptoms on for inoculated leaves.
[x]Same as selection, 216-9.

examined every ten days from the twentieth day after inoculation.

Responses of pear leaves to *V. nashicola* were classified into 3 types according to the methods of LANGFORD and KEITT (1942) with a slight modification as follows, no visible symptoms: 0, necrotic or chlorotic lesions without sporulation: 1, sporulating lesions: 2. Seedlings with only type-0 leaves were rated as highly resistant (HR), seedlings with type-0 and type-1, without type-2 leaves as necrotic (N), seedlings with type-2 leaves as susceptible (S).

Two-year-old plants, rated by artificial inoculation with a conidial suspension in the previous year, were transferred outdoors in late March. From mid-June to mid-July, the appearance of scab symptoms associated with natural infection was assessed. Rating of scab resistance was similar to the procedure described above.

Scions of four-or five-year-old plants, in which the resistance was rated by artificial inoculation at the age of one year, were grafted on potted rootstocks, and then reinoculated, in order to determine whether scab resistance changed along with plant age. Thirty-five days after inoculation, the degree of scab resistance was assessed. Inoculation conditions and rating of scab resistance were the same as the methods described above.

Results

Frequency of scab-susceptible plants with sporulating lesions among seedling populations at the stage of five (stage I), ten (stage II) and fifteen unfolded leaves (stage III) is shown in Fig. 1. Thirty days after inoculation, sporulation was observed in all the F_1 seedlings with 'Shinsei'and'Hakkou'as parents. In the F_1 seedlings with'Kinchaku'as a parent, the frequency of plants with sporulating lesions on leaves increased gradually until forty days after inoculation but did not change subsequently. No significant correlation was observed between the growth stage at the time of inoculation and frequency of susceptible plants, although the frequency in the plants inoculated at stage III was somewhat higher than that in the plants inoculated at stage I or II in the case of F_1 seedlings obtained from the cross between 'Kinchaku' and 'Hosui'.

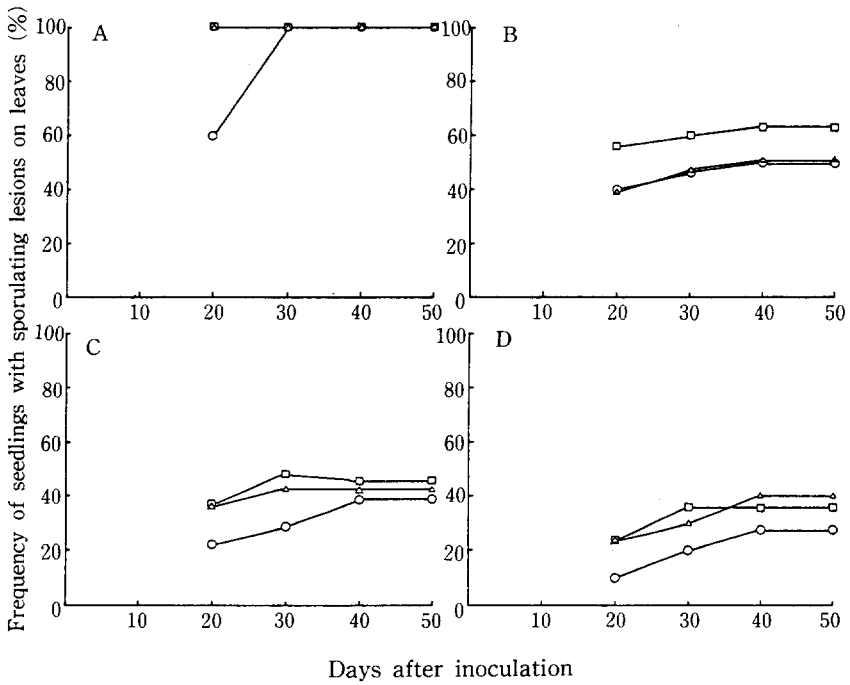

Fig.1. Changes in frequency of seedlings with sporulating lesions on leaves infected with *Venturia nashicola*.
A : Shinsei × Hakkou, B : Kinchaku × Hosui, C : Okusankichi × Kinchaku, D : Kinchaku × Hongli.
○ : five-leaf stage, △ : ten-leaf stage, □ : fifteen-leaf stage.

Highly resistant (HR) plants were observed in F_1 seedlings obtained from the crosses between 'Kinchaku' and 'Hosui' , 'Okusankichi' and 'Kinchaku', and 'Kinchaku' and 'Hongli' (Table 2). Frequencies of HR plants were 0.37~0.50 in progeny from the cross between 'Kinchaku' and 'Hosui', 0.56~0.61 in progeny between 'Okusankichi' and 'Kinchaku', and 0.60~0.73 in progeny between 'Kinchaku' and 'Hongli', respectively. Although the frequency of HR plants, inoculated at stage I , seemed to be higher than that of plants inoculated at stage II or III, results from χ^2 tests showed that the frequency of HR plants to *V. nashicola* was not statistically different among populations of seedlings inoculated at different growth stages.

Comparison was made between the evaluation of resistance by artificial inoculation with a conidial suspension (Evaluation 1) and that by natural infection in the field (Evaluation 2) (Table 3). All the progeny obtained from crosses between 'Hosui' and 'Kosui' , 'Kosui' and

Table 2. Chi-square tests of heterogeneity for the frequency of plants highly resistant to *Venturia nashicola* amang different growth stages of one-year-old seedlings.

Cross		Growth stage[z]			Average	x^2	Probability
		5	10	15			
Shinsei	× Hakkou	0[y]	0	0	0	—	—
Kinchaku	× Hosui	0.50	0.48	0.37	0.45	1.29	0.50~0.75
Okusankichi	× Kinchaku	0.61	0.57	0.56	0.58	0.16	0.90~0.95
Kinchaku	× Hongli	0.73	0.60	0.65	0.66	1.23	0.50~0.75

[z]Number of leaves that unfolded in the seedlings.
[y]Frequency of plants highly resistant to *V. nashicola.*

Table 3. Comparison of the data on resistance to *Venturia nashicola* between artificial inoculation test at juvenile stage and the test besed on natural infection in the field.

Cross		n[z]	Evaluation				(HR·S+S·HR) /n
			HR·HR[y]	HR·S[y]	S·HR[y]	S·S[y]	
Hosui	× Kosui	15	0	0	0	15	0
Kosui	× Yasato	24	0	0	0	24	0
Hosui	× Laiyangcili	28	0	0	3	25	0.107
Hogetsu	× Kosui	32	0	0	0	32	0
Okusankichi	× Kosui	32	0	0	2	30	0.063
Hosui	× Kinchaku	53	14	1	4	34	0.094
Hosui	× Hongli	54	19	4	2	29	0.111

[z]Number of plants investigated.
[y]HR·HR : highly resistant (HR) both in artificial inoculation test and in test of natural infection. HR·S : HR in artificial inoculation test but susceptible (S) in test of natural infection, S·HR : S in artificial inoculation test but HR in test of natural infection, S·S:S both in artificial inoculation test and in test of natural infection.

'Yasato', and 'Hogetsu' and 'Kosui' were rated as susceptible (S) both in Evaluation 1 and in Evaluation 2. In these progeny, no difference in the degree of resistance associated with the difference in the testing methods for evaluation was detected. On the other hand, segregation for scab resistance was observed in the progeny from the crosses between'Hosui'and 'Kinchaku' or 'Hosui' and 'Hongli'. In five plants from the cross between'Hosui'and'Kinchaku', rating of resistance in Evaluation 1 was different from that in Evaluation 2. Difference of resistance was also observed between Evaluation 1 and Evaluation 2 in six plants from the cross between 'Hosui' and 'Hongli'. As a result, rating of resistance in Evaluation 1 differed from that in Evaluation 2 for sixteen plants out of two hundred thirty-eight plants investigated, and the rate of inconsistency between the two evaluations was 0.067.

Relationship between the degree of resistance to scab and age of plants was investigated (Table 4). Segregation for resistance was observed in progeny from the crosses between 'Kinchaku' and 'Hosui' , 'Kinchaku' and 'Kosui', 'Kinchaku' and 'Hongli', and 'Hosui' and 'Hongli'. One hundred and three plants were evaluated as HR and sixty plants as S out of one hundred seventy-seven plants, regardless of the age at the time of inoculation. Frequency of plants in which the evaluation of resistance differed at different ages was 0.077 in the progeny from the cross between 'Kinchaku' and 'Hosui', 0.073 in the progeny from 'Kinchaku' and 'Kosui', 0.059 in the progeny from 'Kinchaku' and 'Hongli', and 0.109 in the progeny from 'Hosui' and 'Hongli', respectively. In fourteen plants out of one hundred seventy-seven plants, the degree of scab resistance at the age of one year differed from that at the age of four or five years, and the rate of inconsistency of resistance was 0.079.

Table 4. Relationship between age of plant and resistance to *Venturia nashicola* in cross seedlings.

Cross	n[Z]	Evaluation				(HR·S+S·HR) /n
		HR·HR[Y]	HR·S[Y]	S·HR[Y]	S·S[Y]	
Kinchaku × Hosui	39	17	0	3	19	0.077
Kinchaku × Kosui	41	23	0	3	15	0.073
Kinchaku × Hongli	51	42	1	2	6	0.059
Hosui × Hongli	46	21	0	5	20	0.109

[Z]Number of plants investigated.

[Y]HR·HR : highly resistant (HR) both at 1-year-old and at 4- or 5-year-old, HR·S : HR at 1-year old but susceptible (S) at 4- or 5-year-old, S·HR : S at 1-year-old but HR at 4- or 5-year-old, S·S:S both at 1-year-old and at 4- or 5-year-old.

Discussion

To apply screening methods for scab resistance in pear seedlings by artificial inoculation at the juvenile stage, it is necessary to confirm that 1) the rating of resistance by artificial inoculation coincides with the rating based on the observation of disease appearance by natural infection in the field, and 2) the degree of resistance at the juvenile stage coincides with that at the adult stage. When we compared the rating of scab resistance by inoculation with a conidial suspension of the fungus with the rating by natural infection in the field, we observed a difference of seven percent in the scab resistance of the plants between the ratings by artificial inoculation and those based on field observation.

Several factors such as, low accuracy of the rating by artificial inoculation, for example, uneven spray of an inoculum among seedlings planted densely in the study, or error in the records of ratings, may have affected the evaluation. Evaluation of resistance in the field is considered to vary depending on the environment, activity and density of fungi (DAYTON *et al.* 1983). As a result, low accuracy of evaluation in the field may have occured due to the short period of investigation in our study. Careful inoculation with a conidial suspension and careful evaluation in the field, in taking account of fluctuations of resistance among years, could improve the accuracy and efficiency of the screening of scab-resistant plants at the juvenile stage.

Specialization of physiological races is an another major problem for the evaluation of scab resistance in test plants. For example *V. inaequalis*, the causal pathogen of apple scab, consists of five strains that differ to varying degrees in their physiological characters (WILLIAMS and BROWN 1968, WILLIAMS and KUC 1969). Of the approximate 50 scab-resistant apple cultivars that have been released worldwide, 39 cultivars are reported to carry the *Vf* gene originating from *Malus floribunda* 821 (MERWIN *et al.* 1994). The use of scab resistance of apples encoded by the *Vf* gene in apple breeding programs is ascribed to the fact that the *Vf* resistance has been considered to be durable, and *Vf* cultivars have been free of scab for over 50 yr in the countries where they had been grown (PARISI *et al.* 1993). Recently a new race (sixth race) of *V. inaequalis* capable of overcoming the *Vf* resistance, but not the resistance of *M. floribunda* 821 itself, has been reported (PARISI *et al.* 1993). Specialization of pathogenicity has been also found in *V. nashicola* and three physiological races have been identified for Japanese pear (ISHII and ANNAMALAI 1995). It is thus possible that the presence of different races of *V. nashicola* had interfered with the evaluation of scab resistance.

We observed plants with different ratings for the resistance between the two evaluations. Some of the plants were rated as HR in the field but rated as S by artificial inoculation, and

the others were rated as S in the field but as HR by artificial inoculation. Such inconsistency in scab resistance in some plants is considered to be affected by the factors mentioned above. If we observe the plants which are rated as S in the field but rated as HR by artificial inoculation at a high frequency under artificial inoculation, we can not apply the screening method to actual breeding for scab resistance, because such plants are susceptible to scab in the field. Since the frequency of such plants was relatively low, about two percent in this study, the screening method for scab-resistant plants by artificial inoculation at the juvenile stage could be applied to the breeding, although the screening method shows some uncertainty for the evaluation of scab resistance.

As for the relationship between the disease resistance and age of plants, Populer (1978) pointed out that many authors had observed a seasonal change of resistance or change of resistance during the growth stages in plant organs or tissues. In fruit trees, the number of scab lesions was the largest from one day to five days after leaf unfolding at the time of inoculation in apple leaves (SCHWABE 1979), and the younger the Japanese pear leaves, the higher the susceptibility of leaves to black spot (KOZAKI 1974). In the case of scab in Japanese pear, the development of symptoms is also severe in younger leaves (YAMAMOTO and TANAKA 1963, ABE and KURIHARA 1992).

When we examined a difference for the frequency of scab-resistant plants among different growth stages in one-year-old seedlings, we did not detect statistically significant difference. It was considered that there was no difference in the frequency, since the inoculation was performed mainly onto young, recently unfolded leaves which were highly susceptible to *V. nashicola*. This observation indicates that it is possible to evaluate scab resistance accurately, irrespective of growth stage of seedlings, using seedlings with young, recently unfolded leaves. In apple, inoculation with a conidial suspension has been also carried out onto young leaves of cross seedlings for the evaluation of scab resistance (e.g. KELLERHALS *et al.* 1993).

In our investigation, we observed eight percent of inconsistency in the evaluation by artificial inoculation between one-year-old and four-or five-year-old plants. The discrepancy described above may be ascribed to the following factors: 1) failure of inoculation in a few plants, 2) error in records of rating, and 3) change of disease resistance with plant age. Changes of disease resistance with plant age have been reported in annual plants, and are refered to as "adult plant resistance" (POPULER 1978). Difference in the development of symptoms caused by *Phytophthora cactorum* was investigated using young and mature trees of the apple variety Cox's Orange Pippin (SEWELL and WILSON 1973), although it was difficult to determine whether "adult plant resistance" was present in fruit trees. In our experiment, in most of the plants in which the evaluation of scab resistance showed a discrepancy between

one-year-old and four-or five-year-old plants, disease resistance changed from S to HR with plant age, suggesting that"adult plant resistance"may occur in *Pyrus* plants. To confirm this assumption, accurate re-examination is needed.

Except for the possibility of change in the disease resistance with age, failure of inoculation or error in the rating still remains plausible. Two types of plants, with different ratings between one-year-old and four-or five-year-old, were observed. One type was rated as HR at the younger stage and it changed to S later (Type- I), the other was rated as S at first, then HR at a later stage (Type-II). We can not apply the screening method at the juvenile stage, if there is a high frequency of type- I plants in the evaluation by artificial inoculation, since type- I plants could be selected as scab-resistant plants through artificial inoculation in spite of scab susceptibility in the productive age. Since in our study the frequency of type- I plants was low, 0.006, the authors consider that selection of scab-resistant plants by artificial inoculation at the juvenile stage can be applied to Japanese pear breeding.

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幼苗期におけるナシ黒星病抵抗性個体の早期選抜

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摘　要

ニホンナシの黒星病抵抗性育種を効率的に進めることを目的として，'巾着'，'紅梨' の高度抵抗性品種や '幸水'，'豊水' のり病性品種等を交配親とする1年生の交雑実生集団を供試して抵抗性個体の早期選抜方法を検討した．交雑実生集団に $2\sim3\times10^5$ 胞子/ml の懸濁液を噴霧接種し，湿室内に24～48時間保持した後，屋外に置いて黒星病の発病状況を調査した．接種葉に胞子形成が認められた個体をり病性，肉眼的症状が認められなかった個体を高度抵抗性と評価した．その結果，各実生集団における黒星病発病個体の出現頻度は接種40日後以降変化せず，接種40日後までには各個体の抵抗性の判定が可能であった．また，生育ステージの違いと発病個体の出現頻度に明らかな関連性は見い出されなかった．'巾着'，'紅梨' の高度抵抗性品種の後代実生には高度抵抗性個体が出現した．接種時の実生集団の生育ステージの差異による高度抵抗性個体出現頻度の異質性を検定した結果，有意な偏りは認められず，抵抗性個体の選抜に及ぼす供試実生個体の生育ステージの差異の影響は少ないと考えられた．人工接種による幼苗検定を行ったときの抵抗性の評価と屋外での自然感染による発病状況の調査をもとにした抵抗性の評価を比較したところ，238個体中222個体で抵抗性の評価が一致したが，16個体では評価が異なり，抵抗性の検定結果の不一致の割合は0.067であった．幼苗検定により抵抗性の評価を行った各実生個体の，4年生もしくは5年生の段階での抵抗性を再度接種試験により評価して，異なる樹齢における黒星病抵抗性の検定結果を比較したところ，177個体中163個体において接種時の樹齢にかかわらず抵抗性の評価が一致した．

以上の結果から，多少の誤差を伴うものの，ほ場条件下で黒星病に抵抗性を示す個体を1年生の時点で幼苗検定により早期選抜することが可能と考えられた．