Inheritance of Intermediate Resistance to Black Spot Disease in an Induced Japanese Pear Mutant, 'Gold Nijisseiki'

Testuro Sanada, Kenji Sagisaka, Junichi Soejima*, Takaya Moriguchi, Sayuri Teramoto and Kazuo Kotobuki

Division of Fruit Breeding, Fruit Tree Research Station, Tsukuba, Ibaraki 305

Summary

The intermediate resistance to black spot disease in the induced mutant, 'Gold Nijisseiki (γ -1-1)' was characterized by the appearance of necrotic symptoms only on young leaves treated with the host-specific toxin solution. To examine whether the intermediate resistance is genetically transmissible to the F_1 seedlings from crosses between 'Gold Nijisseiki' and resistant or susceptible cultivars, the degree of resistance to the disease in the F_1 seedlings was evaluated. For the identification of the seedlings with intermediate resistance in the F_1 generation, the application of toxin solutions at 0.1 and 5 ppm to the disks from the 1st and 5th leaves was a reliable method. By using this method, many seedlings with intermediate resistance were identified in the F_1 generation only when 'Gold Nijisseiki' was used as the parent. The segregation ratios in the F_1 generation suggested that an incomplete recessive mutation was induced in the L-II cell layer of the mutant, 'Gold Nijisseiki'.

Introduction

Black spot disease caused by the Japanese pear pathotype of *Alternaria alternata* (Fr.) Keissler is the most serious disease of Japanese pear (*Pyrus pyrifolia* Nakai var. *culta* Rehd.) (Tanaka, 1933; Nishimura et al., 1978). The susceptibility to the disease is controlled by a single dominant gene (Kozaki, 1973). 'Nijisseiki', which is one of the most widely grown cultivars of Japanese pear, is known to be susceptible to the disease. Several resistant mutants to black spot disease were selected from 'Nijisseiki' by chronic irradiation with gamma rays (Sanada et al., 1988; Sanada, 1988a, 1988b). However, the induced mutants exhibited intermediate resistance to the disease, either by the inoculation test using a spore suspension or the host-specific toxin solution from the pathogen. The intermediate resistance was characterized by the appearance of necrotic symptoms only on young leaves inoculated with the spore suspension or the

toxin solution (Sanada et al., 1988; Sanada, 1988a, 1988b). One cause of the intermediate resistance in the mutants was considered to be the presence of periclinal chimera based on histological observations of faint brown spot symptoms in the portion inoculated with drops of spore suspension or the toxin solution (Sanada, 1987, 1988b). The intermediate resistance to the disease was not reported in spite of repeated tests in Japanese pear. The reaction to the disease is usually characterized by complete resistance or susceptibility.

This study was undertaken to examine whether the character of intermediate resistance as the appearance of necrotic symptoms only on young leaves in the mutant, 'Gold Nijisseiki (γ -1-1)' (Sanada et al., 1988; Sanada, 1988a, 1988b; Kotobuki et al., 1992), was genetically transmissible to the F_1 generation.

Materials and Methods

F_1 seeds were obtained from combinations of the intermediate resistant mutant 'Gold Nijisseiki', resistant 'Chojuro' and 'Kumoi', as well as susceptible 'Nijisseiki' and 'Hayatama' in 1985. Three to five years old seedlings were used as materials.

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*Present address : Morioka Branch, Fruit Tree Research Station, Morioka, Iwate 020-01

Two to four years old trees of the cultivars including resistant 'Chojuro', susceptible 'Nijisseiki', and intermediate resistant mutants, 'Gold Nijisseiki' and ' γ -5-1' were used in addition to the seedlings.

1. Method of identifying intermediate resistance.

Solutions of the host-specific toxin, AK-toxin I (Nakashima et al., 1982, 1985) were prepared at concentrations of 0.1 and 5 ppm (Sanada, 1988a). Leaf disks, 7 mm in diameter, were cut with a leaf punch from the first leaf on the top to the 5th leaf towards the base of shoots in cultivars and F_1 seedlings. Ten leaf disks for each leaf positions of these cultivars and F_1 seedlings were used for the toxin treatments at the two concentrations of 0.1 and 5 ppm, respectively. The disks were placed with the adaxial surface in contact with two sheets of filter paper moistened with 5 ml of toxin solution in petri dishes, 9 cm in diameter, and were incubated at 25°C for 2 days.

2. Segregation of resistance in F_1 seedlings.

Leaf disks were cut from the 1st and 5th leaves as described above on F_1 seedlings and were treat-

ed with AK-toxin I solutions at 0.1 and 5 ppm. The treatment and method of evaluation were as same as those described above. Segregation was revealed based on the results of experiments performed repeatedly for 3 years.

Results and Discussion

1. Method of identifying intermediate resistance.

Leaf disks from leaves at different positions on the shoots were treated with different concentrations of AK-toxin I (Fig. 1). In the susceptible 'Nijisseiki', severe necrotic symptoms were observed on the leaf disks of the 1st to 5th leaves at both concentrations of 0.1 and 5 ppm of the toxin, although only slight symptoms on the leaf disks of the 1st and 2nd leaves at 5 ppm were observed in the resistant 'Chojuro'. In the mutant, 'Gold Nijisseiki' with intermediate resistance, severe necrosis was observed only on the disks from young leaves such as the 1st and the 2nd leaves treated at 5 ppm, whereas the necrosis was not so severe on the 4th and the 5th leaves treated at 0.1 ppm. Thus, the application of toxin solutions at 0.1 and 5 ppm to the disks from the 1st and 5th leaves,

Fig. 1. Severity of symptoms on leaf disks from leaves at different positions on the shoot of cultivars after treatment with AK-toxin I solution. ■ Nijisseiki; ◆ Gold Nijisseiki; ▲ γ -5-1; ● Chojuro. Necrotic areas of leaf disks were scored as follows: 0, no necrotic lesions; I, minor lesions at cut end; II, about 1 mm from the cut end; III, about 2 mm from the cut end; IV, lesions involving the whole disk. Ten leaf disks for each leaf position were treated, and the averaged results are shown. Vertical bars represent standard errors.

was considered to be a reliable method for identifying seedlings with intermediate resistance to the disease.

Some F_1 seedlings were examined by applying the toxin solutions to the leaf disks from the 1st to the 5th leaves, to determine whether the identification method of intermediate resistance was applicable to the F_1 seedlings. Leaf disks of the F_1 seedlings with intermediate resistance to which the toxin solution was applied at two concentrations gave a similar response to the degree of necrosis in the mutants, 'Gold Nijisseiki' and ' γ -5-1' with intermediate resistance (Fig. 2). Therefore, the method for identifying intermediate resistance described above could also be applied to F_1 seedlings.

2. Segregation of resistance in F_1 seedlings.

Segregation ratios of resistance to the disease in the F_1 seedlings from crosses of resistant cultivars, intermediate resistant cultivars and susceptible cultivars were examined by using the method for identifying intermediate resistance. The results shown in Table 1 indicate that no seedlings with the intermediate resistance traits were observed in the F_1 generation from crosses among resistant and susceptible cultivars. However, many seedlings with intermediate resistance were observed in

the F_1 generation only when 'Gold Nijisseiki' with the intermediate resistance was used as the parent. These results indicate that the intermediate resistance trait is genetically transmissible to these F_1 seedlings. The F_1 seedlings from crosses between the mutant and a resistant cultivar segregated in a 1 (resistance) : 1 (intermediate resistance) ratio. And the F_1 seedlings from crosses between the mutant and a susceptible cultivar segregated in a 1 (resistance) : 1 (intermediate resistance) : 2 (susceptible) ratio.

Susceptibility to this disease is controlled by a single dominant gene, and the majority of susceptible cultivars are heterozygous at this locus (Kozaki, 1973). Therefore, the segregation ratios shown in Table 1 suggest that the intermediate resistance in 'Gold Nijisseiki' was due to an incomplete recessive mutation, and that the mutant gene acted in the same way as the original allele, but with lower expression.

On the other hand, the faint brown spots were observed on the 4th leaves or young fruits inoculated with spores or toxin solution in the induced mutants. These symptoms were based by the aggregation of the cytoplasm only in epidermal cells on leaves or fruit of a mutant, 'Gold Nijisseiki', were considered to be caused by the presence of periclinal chimera (Sanada, 1987, 1988b)

Fig. 2. Severity of symptoms on leaf disks from leaves at different positions on the shoot of seedlings after treatment with AK-toxin I solution. ○ Seedlings No. 1; △ S. No. 7; ◇ S. No. 42; □ S. No. 140. Seedling No. 1, 7 and 42 originated from Gold Nijisseiki×Chojuro. Seedling No. 140 originated from Gold Nijisseiki×Hayatama. The severity of the symptoms were graded as indicated in Fig. 1.

Table 1. Segregation of resistance to black spot disease in F₁ seedlings from crosses between Gold Nijisseiki, resistant and susceptible cultivars.

Cross combination			Segregation	Ex. ratio ²	χ^2	P	
			R : I : S	R : I : S			
Chojuro	x	Gold Nijisseiki	51 : 47 : 0	1 : 1 : 0	0.16	0.5	-0.7
	x	Nijisseiki	69 : 0 : 36	1 : 0 : 1	10.37	0.001	-0.01
	x	Hayatama	25 : 0 : 16	1 : 0 : 1	1.98	0.1	-0.2
Gold Nijisseiki	x	Chojuro	62 : 43 : 0	1 : 1 : 0	3.44	0.05	-0.1
	x	Hayatama	28 : 22 : 47	1 : 1 : 2	0.51	0.7	-0.8
Kumoi	x	Hayatama	14 : 0 : 8	1 : 0 : 1	1.64	0.2	-0.3
	x	Chojuro	31 : 0 : 0	1 : 0 : 0	—		
	x	Nijisseiki	21 : 0 : 21	1 : 0 : 1	0.00	1.0	
Hayatama	x	Gold Nijisseiki	12 : 6 : 14	1 : 1 : 2	2.75	0.2	-0.3

² Expected ratio.

in the same way as many bud sports (Sanada et al., 1987). And, it is generally recognized that the gamete originates from the L-II cell layer in the meristematic zone. Therefore, it was considered that an incomplete recessive mutation was induced in the L-II cell layer of the mutant, 'Gold Nijisseiki'.

Thus, the intermediate resistance to black spot disease in the mutant, 'Gold Nijisseiki' may be associated with an intermediate resistance of genetic origin other than the presence of periclinal chimera.

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ニホンナシ黒斑病耐病性突然変異体, 'ゴールド二十世紀' における中位の耐病性の遺伝

真田哲朗・鷺坂健二・副島淳一*・森口卓哉・寺本さゆり・壽 和夫

農林水産省・果樹試験場 305 茨城県つくば市藤本

摘 要

黒斑病耐病性突然変異体品種 'ゴールド二十世紀' (γ -1-1) に見られる中位の耐病性は, 病原毒素処理が行われた時に若い葉にのみえ死を生じる特性を示す. この中位の耐病性が後代に遺伝するか否かを調べるため, 'ゴールド二十世紀' と耐病性およびり病性品種とを交雑して得られた F_1 実生の耐病性の程度を検討した. 中位の耐病性を示す実生の同定には, 病原毒素 (AK-toxin I) 5 ppm と 0.1 ppm を第1葉および第5葉

の葉片に処理する方法が最も有効な方法であることが明らかになった. この検定法を用いることにより, 'ゴールド二十世紀' の F_1 世代の中に, 多くの中位の耐病性実生が検出でき, この中間的耐病性が後代に遺伝することが明らかになった. また, F_1 世代における分離比の結果は, 耐病性変異体である 'ゴールド二十世紀' の L-II 細胞層に不完全な劣性突然変異が誘発されたことを示唆している.