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Gamma-Ray-Induced Mutation Breeding in Fruit Trees: Breeding of Mutant Cultivars Resistant to Black Spot Disease in Japanese Pear

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Abstract

Black spot disease, caused by *Alternaria alternata* Japanese pear pathotype, is the most important and serious disease of the susceptible cultivars of Japanese pear. The fungus produces a host-specific toxin (AK-toxin) which is highly toxic to the susceptible cultivars. Susceptibility to this disease is controlled by a single dominant gene, and all the susceptible cultivars are heterozygous. Mutants resistant to black spot disease were induced by irradiation of susceptible cultivars with gamma-rays. Nine resistant mutants were selected from chronically irradiated 'Nijisseiki'. One of these mutants was registered as 'Gold Nijisseiki'. A resistant mutant derived from acutely irradiated dormant scions of 'Shinsui' was registered as 'Kotobuki Shinsui'. One resistant mutant, which was induced from acutely irradiated dormant scions of 'Osanijisseiki', displayed unfavorable characteristics. Four resistant mutants were selected from chronically irradiated 'Osanijisseiki'. One of them was registered as 'Osa Gold'. A list of these resistant mutants is shown in Table 4. It was confirmed that all of the mutants showed an intermediate resistance to black spot disease and conferred various levels of resistance. Moreover, mutations with a higher level of resistance than that of 'Gold Nijisseiki' were induced from 'Gold Nijisseiki' by acute and chronic gamma-ray irradiation. The reason why various levels of resistance were induced could not be elucidated.

Discipline: Plant breeding
Additional key words: gamma-ray irradiation, *Alternaria alternata*, AK-toxin, intermediate resistance

Introduction

Black spot disease, caused by *Alternaria alternata* Japanese pear pathotype, is the most important and serious disease of the susceptible cultivars of Japanese pear (*Pyrus pyrifolia* Nakai var. *culta* Nakai), for example 'Nijisseiki', 'Shinsui' and 'Osanijisseiki'. The fungus produces a host-specific toxin (AK-toxin) which causes necrosis and permeability changes in the leaves and fruits of the susceptible cultivars[11,17].

Susceptibility to this disease is controlled by a single dominant gene[3]. It is known that the resistant cultivars are recessive homozygous and that susceptible cultivars are heterozygous for this gene. Dominant homozygous cultivars have never been detected.

In many vegetatively propagated crops or fruit trees, natural mutations have made important contributions to breed improvement. We consider that artificial mutation breeding is an effective method for improving economic agricultural species.

To achieve this objective, induced mutation breeding of temperate fruit trees has been studied in our laboratory since 1962. Gamma-ray irradiation is a promising method to induce mutants resistant to black spot disease.

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Development of selection method and selection of resistant mutants from Nijisseiki

Nijisseiki, one of the leading cultivars of Japanese pear, is known to be susceptible to black spot disease. In 1962, grafted trees of Nijisseiki were planted at a distance of 53–93 m (at intervals of 10 m) from a ^{60}Co source in a gamma-field, and were irradiated with gamma-rays chronically to induce resistant mutants.

In 1981, a twig without symptoms of the disease was detected on the tree at a distance of 53 m from the source (dose rate: 0.138 Gy/day, 20 h of irradiation). The scions from this mutated twig were grafted onto rootstocks outside the gamma-field. The resistant mutant was named 'γ-1-1'. Consequently, it was confirmed that it was possible to induce resistant mutants by irradiation with gamma-rays.

Since there was no effective method for selecting mutants, other resistant mutants could not be selected. However, it is possible to test a large number of samples by treating leaf disks with a filtrate of *A. alternata* culture solution[1,13,14]. By using this convenient selection method, 4 resistant mutants (γ-1-2, γ-2-1, γ-4-1 and γ-5-1) were selected from Nijisseiki trees planted at a distance of 53–93 m from the source in a gamma-field[1].

On the other hand, young Nijisseiki trees were planted at a distance of 40–70 m (at intervals of 10 m) from the source in a gamma-field in 1983. Selection of resistant mutants was carried out together with the cutting back pruning method[10]. Four resistant mutants (γ-A-1, γ-B-1, γ-C-1 and γ-C-2) were selected from these trees[13,14].

The leaf disks from young leaves of these mutants showed a small necrotic area by treatment with AK-toxin, although no necrosis was observed on the resistant cultivars[15] (Fig. 1). It became obvious that all of the 9 mutants showed an intermediate resistance between resistant and susceptible cultivars.

Registration of a new cultivar 'Gold Nijisseiki'

In 1986, local adaptability tests of γ-1-1, the first resistant mutant from Nijisseiki, were conducted at the National Institute of Fruit Tree Science, MAFF (Ministry of Agriculture, Forestry and Fisheries), and at 27 prefectural horticultural research stations in various pear-growing districts of Japan.

Although γ-1-1 is seldom infected with *A. alternata* in the field, very young leaves in the early growth stage and leaves on secondary growth shoots are slightly more susceptible to the disease. Many uninfected leaves remained on the tree even in late autumn, in contrast with Nijisseiki, in which all the leaves were infected at that time.

γ-1-1 bears many fruit spurs and maintains them easily, though there are relatively few axillary flower buds. The flowers are white, and most of them have 5 petals. The flowering time is medium to late. The fruit weighs about 300–350 g. The skin of the fruit is yellowish green at early maturity, and becomes yellow at late maturity. Many russets occur on the skin when the fruit is cultivated without paper bagging. All of these characteristics, except for the slightly later fruit ripening time, are the same as those of Nijisseiki[2] (Fig. 2).

Based on the results obtained, γ-1-1 was designated as cultivar Gold Nijisseiki and registered as 'Pear Norin No. 15' by MAFF on June 22, 1990*. Also it was registered as 'No. 2,932' under the Seeds and Seedlings Law of Japan on December 16, 1991.

Selection of resistant mutants from Shinsui and Osanijisseiki

Shinsui is a russet type pear cultivar whose fruit ripens earlier than that of 'Kosui' and is sweet and rich. Osanijisseiki, an excellent cultivar with a smooth skin, is a self-compatible spontaneous bud sport of Nijisseiki. Both cultivars are susceptible to black spot disease. Since 1987, the Institute of Radiation Breeding, NIAR, in collaboration with Tottori Horticultural Experiment Station, has carried out research on radiation breeding for the selection of mutants resistant to black spot disease from these 2 susceptible cultivars by using acute or chronic gamma-ray irradiation.

By using acute irradiation in a gamma-room, dormant scions were irradiated to a total exposure of 60 and 80 Gy at a dose of 2.5 Gy/h. Irradiated dormant scions were top-grafted onto mature

* The breeding staffs of Gold Nijisseiki are Teruo Nishida, Haruhiko Fujita, Fukio Ikeda, Tetsuro Sanada and Kazuo Kotobuki.

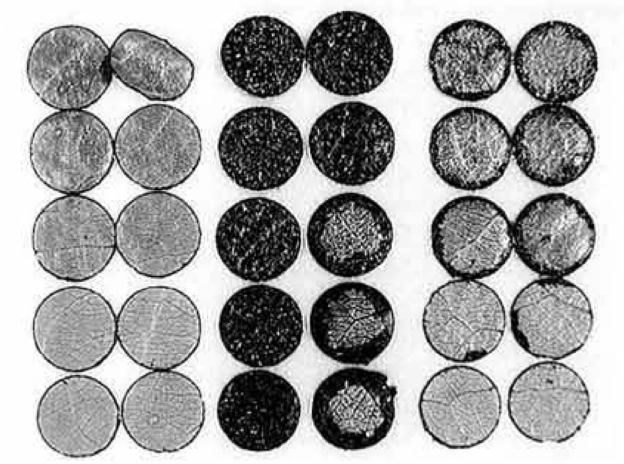

Fig. 1. Appearance of black spot symptoms on crude AK-toxin treated leaf disks from 1st to 5th leaves of the shoot tip (upper to lower)
Left to right: Chojuro, Nijisseiki and γ-1-1 (2 lanes each).

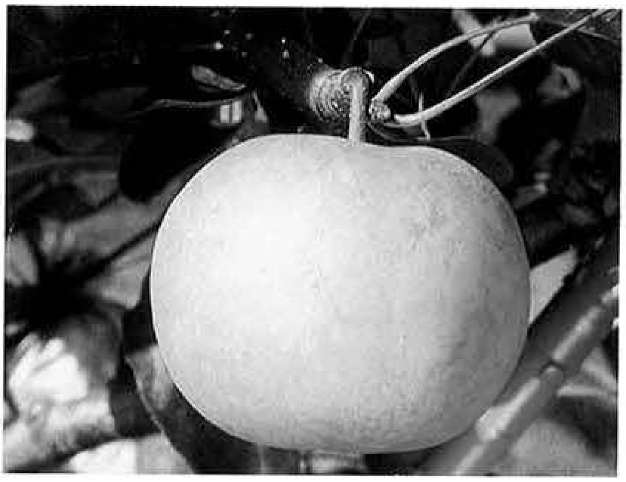

Fig. 2. Fruit-bearing shoot of Gold Nijisseiki

Fig. 3. Appearance of black spot symptoms on crude AK-toxin treated leaf disks from 1st to 5th leaves of the shoot tip (upper to lower)
Left to right: Nijisseiki, Shinsui (2 lanes), IRB 502-11T (2 lanes), Gold Nijisseiki (2 lanes) and Chojuro.

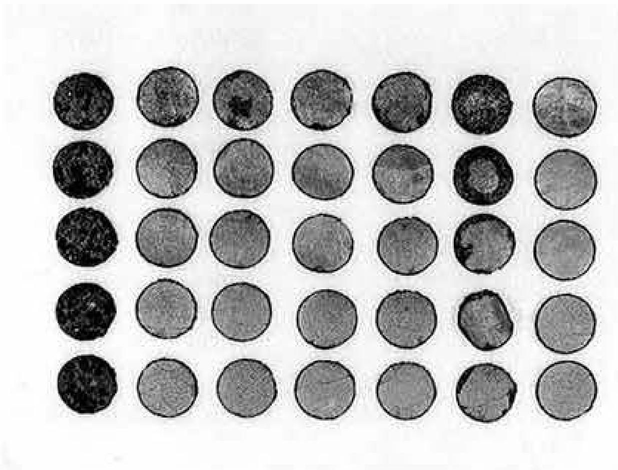

Fig. 4. Appearance of black spot symptoms on crude AK-toxin treated leaf disks from 1st to 5th leaves of the shoot tip (upper to lower)
Left to right: Osanijisseiki, IRB 502-13T, -14T, -17T, -18T, Gold Nijisseiki and Chojuro.

Fig. 5. Fruit-bearing tree of Kotobuki Shinsui

Fig. 6. Fruit-bearing shoot of Osa Gold

Table 1. Screening test using AK-toxin on gamma-ray-irradiated Shinsui[9]

	Year of grafting	No. of shoots in screening test				
		1987	1988	1989	1990	Total
60 Gy	1987	144	173	507	231	1,055
	1988		213	692	543	1,448
	1989			321	582	903
	1990				162	162
		144	386	1,520	1,518	3,568
80 Gy	1987	55	29	115	22	221
	1988		90	842[a)]	34	966
	1989			782	93	875
	1990				106	106
		55	119	1,739	255	2,168 (5,736)

a): Mutant resistant to black spot disease selected.

Table 2. Screening test using AK-toxin on gamma-ray-irradiated Osanijisseiki[9]

	Year of grafting	No. of shoots in screening test				
		1987	1988	1989	1990	Total
60 Gy	1987	94	90	272	266	722
	1988		97	473	326	896
	1989			740	717	1,457
	1990				352	352
		94	187	1,485	1,661	3,427
80 Gy	1987	22	59	210	41	332
	1988		146	575	88	809
	1989			1,012	87	1,099
	1990				95[a)]	95
		22	205	1,797	311	2,335 (5,762)

a): Mutant resistant to black spot disease selected.

trees of Japanese pear. By using a convenient selection method, one mutant resistant to black spot disease (IRB 502-11T) was selected from 5,736 leaves of Shinsui tested (Table 1, Fig. 3). Additionally, one resistant mutant (IRB 502-12T) was selected from 5,762 leaves of Osanijisseiki tested[9] (Table 2). These results demonstrate that the development of mutants resistant to black spot disease is possible not only through chronic irradiation but also through acute irradiation. In IRB 502-12T, a resistant mutant from Osanijisseiki, chlorosis of leaves and dwarfing of leaf were clearly observed[5]. These findings suggested that the frequency of unfavorable mutations induced together with desirable mutations by acute irradiation is much higher than that by chronic irradiation.

For chronic irradiation, nursery trees of Shinsui and Osanijisseiki were planted in a gamma-field at distances of 45–75 m and 40–70 m from the source, respectively, at intervals of 10 m. Screening tests using the cutting back pruning method[10] and a convenient selection method were carried out. Four buds which sprouted were selected as resistant mutants (IRB 502-13T, -14T, -17T and -18T) from a total of 12,596 shoots of Osanijisseiki tested (Table 3, Fig. 4). All of these mutants were derived from the tree planted at a distance of 40 m from the source (dose rate is about 14 m Gy/h)[5,6]. Resistant mutants from chronically irradiated Shinsui could not be selected.

All of these mutants, obtained by both acute and chronic irradiation, showed intermediate resistance to black spot disease, in the same way as 9 resistant mutants induced from Nijisseiki, including Gold Nijisseiki (Figs. 3 & 4).

Registration of a new cultivar 'Kotobuki Shinsui'

In 1993, IRB 502-11T, a mutant resistant to black spot disease induced from Shinsui, was designated

Table 3. Number of shoots tested for the selection of mutants resistant to black spot disease using crude AK-toxin on Osanijisseiki in the gamma-field[6]

Distance from ^{60}Co source	No. of shoots in screening test[a]						
	1988	1989	1990	1991	1992	1993	Total
40 m	355	462	651	513[b]	795	557[c]	3,333
50	543	973	343	539	87	1,241	3,726
60	439	253	256	400	137	804	2,289
70	653	422	235	490	215	1,233	3,248
Total	1,990	2,110	1,485	1,942	1,234	3,835	12,596

a): Nursery tree planted in the gamma-field on March 4, 1986.
b): Two mutants resistant to black spot disease selected.
c): Two mutants resistant to black spot disease selected.

Table 4. List of mutants resistant to black spot disease obtained by gamma-ray irradiation in I.R.B.

Reference number	Tentative name	Original cultivar	Irradiation			Selection year	Cultivar name	Registration
			Method	Dose rate (mGy/h)	Total dose (Gy)			
IRB 502-2	γ-1-1	Nijisseiki	Chronic	7.1	616.2	1981	Gold Nijisseiki	Pear Norin No.15 No. 2,932[a] Plant 8,529[b]
IRB 502-3	γ-1-2	Nijisseiki	Chronic	7.1	712.0	1984		
IRB 502-4	γ-5-1	Nijisseiki	Chronic	2.5	252.1	1984		
IRB 502-5	γ-2-1	Nijisseiki	Chronic	5.3	549.6	1985		
IRB 502-6	γ-C-1	Nijisseiki	Chronic	5.8	57.5	1985		
IRB 502-7	γ-4-1	Nijisseiki	Chronic	3.2	347.2	1986		
IRB 502-8	γ-B-1	Nijisseiki	Chronic	8.7	134.5	1986		
IRB 502-9	γ-C-2	Nijisseiki	Chronic	5.8	119.1	1987		
IRB 502-10	γ-A-1	Nijisseiki	Chronic	14.2	436.2	1989		
IRB 502-11T	Shinsui-R	Shinsui	Acute	2,500.0	80.0	1989	Kotobuki Shinsui	Pear Norin No.18 No. 5,436[a] Application for U.S. patent
IRB 502-12T	Osa-Tottori	Osanijisseiki	Acute	2,500.0	80.0	1990		
IRB 502-13T	Osa2111	Osanijisseiki	Chronic	13.9	238.8	1991	Osa Gold	No. 5,620[a] Application for U.S. patent
IRB 502-14T	Osa2109	Osanijisseiki	Chronic	13.9	238.8	1991		
IRB 502-17T	Osa11108	Osanijisseiki	Chronic	13.9	277.0	1993		
IRB 502-18T	Osa1313	Osanijisseiki	Chronic	13.9	277.0	1993		
IRB 502-19T	Osa10××	Osanijisseiki	Chronic	13.9	295.2	1994		
IRB 502-20	SG1602	Gold Nijisseiki	Acute	2,500.0	60.0	1994		
IRB 502-21	SG1614	Gold Nijisseiki	Acute	2,500.0	60.0	1994		
IRB 502-22	SG4706	Gold Nijisseiki	Acute	2,500.0	60.0	1994		
IRB 502-23	SG1705	Gold Nijisseiki	Acute	2,500.0	80.0	1994		
IRB 502-24	SG1439c	Gold Nijisseiki	Chronic	9.6	51.7	1994		
IRB 502-25	SG555	Gold Nijisseiki	Chronic	9.6	51.7	1994		

a): Registration number under the Seeds and Seedlings Law of Japan.
b): Patent number (application for United States patent).

as 'Pear Houiku No. 2'. Local adaptability tests of the mutant were conducted at the National Institute of Fruit Tree Science, MAFF, and at 25 prefectural horticultural research stations in various pear-growing districts of Japan.

The characteristics of Pear Houiku No. 2 are the same as those of the original cultivar Shinsui, except for the resistance to black spot disease. There were no differences between Pear Houiku No. 2 and Shinsui in the tree configuration and fruit characteristics (Fig. 5).

Based on these results, Pear Houiku No. 2 was designated as Kotobuki Shinsui and registered as 'Pear Norin No. 18' by MAFF on August 21, 1996*. Also it was registered as 'No. 5,436' under the Seeds and Seedlings Law of Japan on March 7, 1997[4].

Registration of a new cultivar 'Osa Gold'

The scions from a twig of IRB 502-13T, one of the resistant mutants induced by chronic irradiation with gamma-rays, were grafted onto rootstocks outside the gamma-field. The characteristics of IRB 502-13T were compared with those of the original Osanijisseiki. Shoot and leaf characteristics of IRB 502-13T were similar to those of Osanijisseiki. Flower characteristics, including self-compatibility, of IRB 502-13T were also the same as those of Osanijisseiki. The fruit characteristics of IRB 502-13T were similar to those of Osanijisseiki[6] (Fig. 6).

Based on these results, IRB 502-13T was designated as cultivar Osa Gold and registered as 'No. 5,620' under the Seeds and Seedlings Law of Japan on July 15, 1997[7]**.

Selection of 'Super Gold' family lines

All the 9 resistant mutants induced from Nijisseiki by gamma-ray irradiation showed an intermediate resistance between resistant and susceptible cultivars. It was obvious that this character was inherited[16]. Therefore, selection of mutants with a high level of resistance to black spot disease was performed by gamma-ray irradiation of Gold Nijisseiki. By acute irradiation in a gamma-room, 4 mutants (IRB 502-20~23) with a high level of resistance were selected. By chronic irradiation in a gamma-field, 2 mutants (IRB 502-24 and -25) were selected[8,18]. They were designated as 'Super Gold' family lines.

Investigation of the necrotic area resulting from AK-toxin treatment revealed that although these 6 mutants with a high level of resistance were clearly more resistant to black spot disease than Gold Nijisseiki, they still exhibited only an intermediate resistance[18] (Fig. 7).

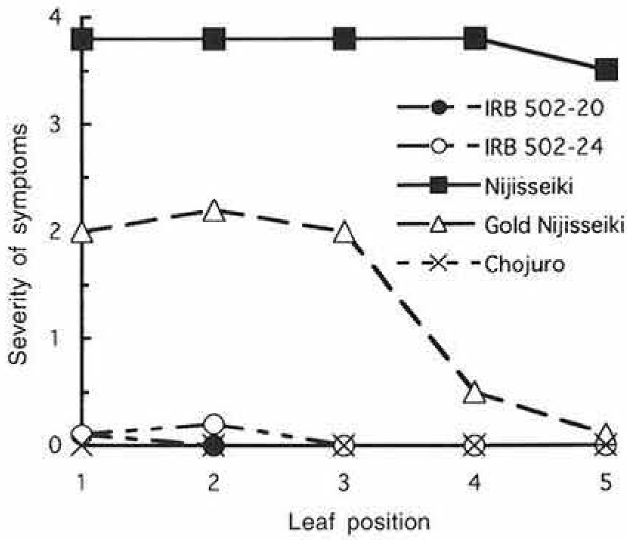

Fig. 7. Response to crude AK-toxin on the leaf disk of IRB 502-20 and -24, induced mutants with high resistance to black spot disease[18]
Leaf disks were checked at 48h after toxin treatment.

To estimate the resistance levels of these mutants to black spot disease, sensitivity to AK-toxin was tested based on the toxin-induced electrolyte loss from leaf tissues[12]. The rate of increase of the electrolyte loss derived from AK-toxin for Gold Nijisseiki compared with deionized water was considerably lower than that for Nijisseiki. Toxin-induced losses of electrolytes for 'Super Gold' family lines were clearly lower than that of Gold Nijisseiki[19]. Moreover, differences in toxin-induced loss rates were confirmed among the 'Super Gold' family lines (Fig. 8).

We could not clearly determine why the induced mutants conferred various levels of resistance to black spot disease. It will be interesting to address this problem through further investigations. The 'Super Gold' family lines, including Gold Nijisseiki, will be good materials for studies of the mechanisms of the resistance function of host-specific toxin and mutation induction by gamma-ray irradiation.

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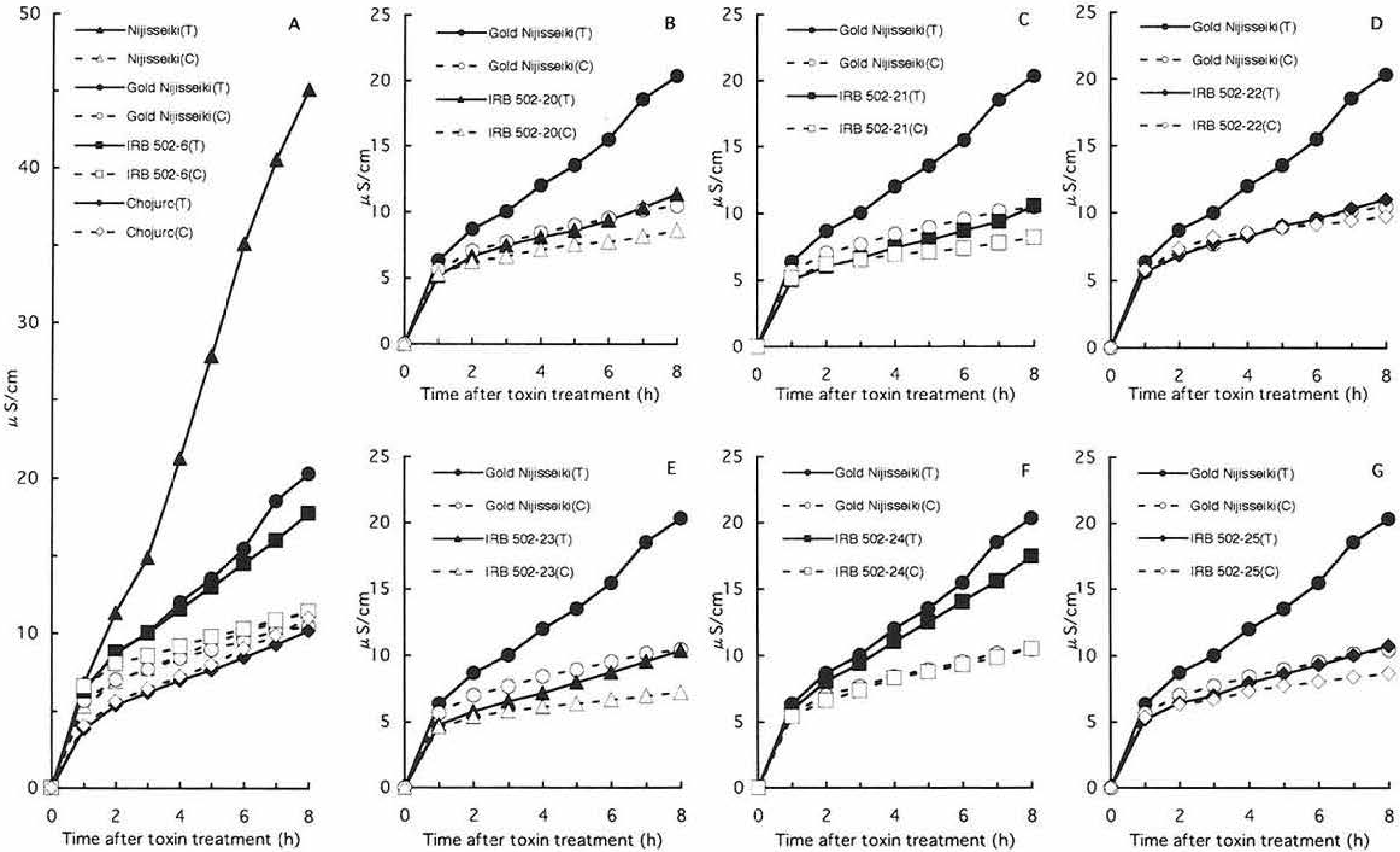

Fig. 8. AK-toxin-induced electrolyte loss from leaf tissues of Nijisseiki, Gold Nijisseiki, IRB 502-6 (γ-C-1), Chojuro and Super Gold family lines[19]

Treatments were as follows: (T); toxin, (C); deionized water.

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