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Evaluation of 277 Carnation Cultivars for Resistance to Bacterial Wilt (*Pseudomonas caryophylli*)

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Summary

Bacterial wilt (*Pseudomonas caryophylli*) is one of the most important and damaging disease of carnations (*Dianthus caryophyllus*) in Japan. It causes serious crop losses in carnations grown in the warm districts. However, breeding for resistance to this disease in carnation has been rarely carried out. Therefore, 277 carnation cultivars were screened for resistance to bacterial wilt by using the cut-root soaking method with an inoculum concentration of 10^7 cfu (colony-forming units) / ml. Two hundred seven cultivars (74.7%) were highly susceptible, whereas 3 cultivars, 'Wiko', 'Nocto', and 'Sandrosa' possessed adequate resistance.

Key Words: bacterial wilt, *Pseudomonas caryophylli*, cultivar evaluation, *Dianthus caryophyllus*, disease resistance.

Introduction

Carnation bacterial wilt caused by *Pseudomonas caryophylli* (Burkholder) Starr and Burkholder commonly occurs in the warm districts of Japan during summer and causes serious crop losses. Bacterial wilt was first found in Spokane, Washington, United States in 1941 (Jones, 1941); the occurrence of this disease in Japan was first recorded in 1964 in Kanagawa Prefecture (Tsuchiya et al., 1965). The predominant symptoms are sudden wilting, vascular discoloration, and rotting of roots. When the discolored area is exposed, it is sticky to the touch, and in some instances there are slimy masses of bacteria. The wilting symptoms are severe in summer but not in winter. Under cool conditions deep longitudinal cracks often form between the nodes on the basal portion of infected plants. This symptom is called stem-crack (Dickey and Nelson, 1963). *P. caryophylli* also infects *Limonium sinuatum* Mill. (Jones and Engelhard, 1984) and *Gypsophila paniculata* L. (Liu, 1990).

Concerning resistance to bacterial wilt in carnation cultivars, an initial study in the United States reported differences in susceptibility among 5 old carnation cultivars and that 'Durango' was immune (Thomas, 1954). However, 'Durango' is now not available because of very old cultivar. Subsequently, Nelson and Dickey (1963) tested 21 cultivars and found all cultivars to be highly susceptible except 'Elegance', 'Starlite', and 'Northland'. These three cultivars are somewhat resistant as less than 50% became infected. Recently, when Uematsu et al. (1991) tested 126 commercial carnation cultivars to *P. caryophylli* in Japan, all proved to be susceptible.

There are many reports on the breeding for resistance to *Fusarium* wilt in carnation caused by *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. and Delarc.) Snyd. and Hans.. Although most breeders consider a high degree of *Fusarium* resistance an essential requirement, breeding for resistance to bacterial wilt, which is the most serious disease in Japanese carnation production, has scarcely been carried out. The control of bacterial wilt is difficult because the pathogen infects the roots and colonizes the vascular system of plants which exhibited no external symptoms. Because the practices of using pathogen-free cuttings, isolated bench culture and soil disinfection with steam or chemicals were introduced in the 1970s, the occurrence of this disease has decreased. However, in warm weather the disease often breaks out widely with plants displaying severe symptoms.

Therefore, we, at the National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT), initiated a breeding program for resistance to bacterial wilt (*P. caryophylli*) in 1988. In this paper, we report on the reaction of 277 carnation cultivars to bacterial wilt, including commercially important new accessions.

Materials and Methods

Two hundred seventy seven carnation (*Dianthus caryophyllus* L.) cultivars from the genetic resources collection at NIVOT were tested (Table 1). The screening was carried out in two experiments, one with 269 cultivars and the other with 8 cultivars. The first experiment was conducted in the summer of 1990; that 'Lena',

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Table 1. Classification of 277 carnation cultivars indexed for their resistances to bacterial wilt (*Pseudomonas caryophylli*).

Disease index	Percentage of wilted plants	Cultivars
5 highly resistant	no symptoms	Total 0 cultivars 0%
4 resistant	0<~ ≦ 20	Wiko, Nocto, Sandrosa, Total 3 cultivars 1.1%
3 moderately resistant	20<~ ≦ 40	Akebono, Embar Rose, Kolivetta, Sarisprit, Juanito, Swan, Telstar, ***Novada***, Barbara, Vanessa, Pink Hirisal, Pink Mamie, Florence, Maj Britt, Linda S64, ***Revada***, Rodeo, Total 17 cultivars 6.1%
2 susceptible	40<~ ≦ 70	P. S. N. Pink Sim, Anne Marie, Yellow Stone, Evening Red, Evening Red sp. orange, William Sim, Etna, Ooita No. 8, Orange Elf, Orange Beauty, Crown, Kurenainotubasa, Koranja, Samantha, Sam's White Scania, Sarome, Chinera, Jolivette, Sweet Heart, Scarlet Bell, Sonata, Solvik's White, Tanga, Tango Bambi, Dark Lena, Dusty, Totem, Domietta, Don Sierra, Butter Scotch, Pablo, Peterson's Red Sim, Peach Blossom, Pink Sim, Pink Barbi, Persian Pink, Honoo, White Lilli Ann, Boston, Portrait, Marina, Ministar, Myan, Mirna, Yufu, Yosooi, Linda, Luna, Regina, Royalette, Total 50 cultivars 18.1%
1 highly susceptible	70<~ ≦ 100	C-7, Calibe, CSU, CSU Red, G-G, Arthur Sim, Aoji Peter, Akane, Asaka, Anniversary, Anon, Apollo, ***Alice***, Alicetta, Albivette, Angie, Yellow No. 3, Yellow Improve, Yellow Sim, Yellow Smiling, Yellow Dusty, Yellow Beauty, Exquisite, Izu Rose, Evening Glow, Illuminator, Improved White Sim, Edith, Ehigasa, ***Elsy***, Erufego, Elegance, Angel, Ooita No. 9, Orchid Royal, Aurora, Orange Smiling, Cardinal Sim, Casino, Capri, Kaly, Carina, California, California White, Calypso, Cantaloupe, Keefers Cherry Sim, Xandra, Kito, Killer, Galaxy, Candy, Quinto, Christmas Fire, Kleopatra, Kurenai, Crowley's Sim, Glory, Geothe, Coral, Corise, Colorado White Pikes Peak, Salmon Pink Sim, ***Sacha***, Sam, ***Sam's Pride***, Sunset, Sunbeam, Zamora, Sissi, Shimada Peter, Shamrock Sp., Sirio, Shin Yosooi, Jumbo Cardinal, Super Gold, Scarlet King, Skyline, Scania, Susanna, Starlite, Stephany, Snow Crown Pink, Snow Fall, Sparkle, Smarty, Smiling, Setonohinode, Setonohatushimo, Setonohana, Setonohanayome, Setonohinode, Cerise Royal, Cerise Royalette, Select White Royalette, Zecckino, Soana, Solvic, Solvit Sydney, Takuma, Tangerine, Dark Pink Ministar, Dark Red Sim, Dusty Pink Sim, Dannebu Rope, Tikushi, Tip Top, Tetra Red, Dizeel, Tortosa, Tony, Tobia, Dooka Pink, Donna Lee Supreme, Doria, ***Niky***, New Red, Northland, Nora, Hollywood, Harunoka, Harunoyosooi, Pearl Lake, Pamera, Pallas, Paradiso, Bianca, Beauty Star, Peter Fisher OT, Peter Fisher Nanbukei, Peter Fisher Beikokukei, Pirana, Pink Ice, Pink Calypso, Pink Smiling, Pink Mist, Pink Ministar, Fantasia, Fidelio, Fuji, Flamingo Sim, Francesco, Pride of Woburn, Princess Irene, Pulcino, Hellas, Besper sim, Variegated Sim, Bellona, Page, Peppermint Lace, Pepito, Percian Pink Sim, Honome, White Christmas, White Sim, White Peter Fisher, White Benon, White Wonder, Magestic, Manon, Mamie, Manmail Myriam, Monroe Romina, Miss Kokura, Muller's Yellow, Myriam, Miltian Maid, Monako, Yufunokagayaki, Yuubae, U Conn, U Conn NR68, Yukigesyou, Yurise, Light Pink Barbi, Raggio di Sole, Rasberry Ice, La Rebe, Lilli Ann, Ruby Red, Le Reve, Red Ivette, Red Gayety, Red Cross, Red Sun, Red Smiling, Red Diamond, Red Baron, Red Ministar, Red Rum, Red River, Red Lena, ***Lena***, Lena Super, Romeo, Lolita, Ronkaruto, Lontagu, Ronda, Rondragel, Izu No. 8, Izu Coral, Izunoodoriko, Kibou, Kibounohikari, Hinotukasa, Total 207 cultivars 74.7%

Two hundred sixty nine cultivars were evaluated in the summer of 1990, the other 8 cultivars (in ***bold italic***) were tested in the summer of 1996.

'Alice', 'Sacha', 'Elsy', 'Niky', 'Novada', 'Revada' and 'Sam's Pride' was made in the summer of 1996.

Pathogenic bacteria, isolated from naturally infected carnations in Japan, (isolate 1) were maintained at about -18 ℃ in a skim-milk medium in screw-cap test tubes for preservation. The defrosted *P. caryophylli* multiplied on potato sucrose agar (PSA) medium for 3 days at 27 ℃, was further cultured in potato sucrose (PS) liquid medium on a reciprocal shaker for 3 days at 27 ℃. The reaction to *P. caryophylli* was tested by using a cut-root soaking method. Ten to eighteen fresh cuttings of each cultivar were rooted under mist in the rooting medium (sand:perlite=1:1, v/v). After about 30 days, roots of cuttings were washed free of the rooting medium and the

tips of the roots were trimmed. Then, roots of cuttings were dipped for 30 minutes in the bacterial suspension adjusted to 10^7 cfu/ml with distilled water in plastic cases. Inoculum concentration was measured by the dilution plate method. Inoculated cuttings were planted in sterilized soil beds in the greenhouse and grown at 24 - 38℃. The number of wilted plants in each cultivar was counted weekly for 13 weeks after inoculation. Finally, the percentage of wilted plants was calculated and indexed for the degree of resistance: 5 (highly resistant), no visible symptoms; 4 (resistant), 0<~ ≦ 20 % wilted; 3 (moderately resistant), 20<~ ≦ 40 % wilted; 2 (susceptible), 40<~ ≦ 70 % wilted; 1 (highly susceptible), 70<~ ≦ 100 % wilted.

To verify the resistance of the screened cultivars, 7 cultivars were retested in the summers of 1991 and 1992 using 4 different isolates of *P. caryophylli* at a high inoculum concentration. Cuttings were inoculated with a concentration of 10^8 cfu/ml. Isolates 2 and 3 were provided by Chiba Horticultural Experiment Station, Japan; isolate 4 was obtained from the collection held at Hyogo Prefectural Awaji Agricultural Institute, Japan. All procedures were as described in the screening experiments except the inoculum concentration.

Results and Discussion

The responses of the 277 carnation cultivars to the bacterial wilt (*Pseudomonas caryophylli*) are presented in Table 1. Large differences in resistance occurred: 207 cultivars (74.7%) were indexed as highly susceptible; 50 cultivars (18.1%) were classified as susceptible; 17 cultivars (6.1%) were moderately resistant; 'Wiko', 'Nocto' and 'Sandrosa' were resistant. No cultivar was indexed as 5 with no symptoms.

Plantlets of 'Coral', one of the highly susceptible cultivars, which became wilted about 2 weeks after inoculation, were completely wilted 5 weeks later (Fig. 1). Most of the highly susceptible cultivars, likewise, rapidly developed the disease symptoms; over 70% wilted within 63 days after inoculation. Contrarily, 'Wiko', a resistant cultivar, which showed no disease symptoms 49 days after inoculation slowly became infected toward the end of the experiment. 'Tanga', which is classified as susceptible, had a delayed incidence compared to 'Coral'.

The responses of the 3 resistant cultivars and 4 susceptible control cultivars to the 4 different isolates of *P. caryophylli* are presented in Table 2. The resistance level of the retested 7 cultivars varied between years and isolates. For instance, disease incidence of 'Wiko' ranged from 0% (isolate 2, 1991) to 30% (isolate 3, 1992). However, relative rankings of resistance between 7 cultivars were consistent over 4 isolates. 'Wiko', 'Nocto', and 'Sandrosa' showed a low infection frequency regardless of isolate, whereas almost all 'Coral', 'Scania', 'White Sim', and 'Kibou' plants wilted and died. Furthermore, appreciable differences in disease virulence were observed among the 4 isolates; isolate 2 was least virulent. Retesting with 3 cultivars using 4 isolates confirmed that 'Wiko' had the highest level of resistance, followed by 'Sandrosa' and 'Nocto' (Table 2).

Our results (Table 1) which show that 'Elegance', 'Starlite', and 'Northland' were highly susceptible, differ from those of Nelson and Dickey (1963) who reported that these three cultivars were somewhat resistant. The reason for the disagreement may be that roots of our tested cuttings were trimmed before inoculation. In our inoculating technique, pathogenic bacteria may have allowed to invade the plants more easily than that of Nelson and Dickey (1963). Moreover, environmental conditions (that is inoculum concentration, temperature, virulence of isolate, etc.) in our experiment may have been more suitable for infection than those of Nelson and Dickey (1963). Race differentiation in *P. caryophylli* has not been reported as yet.

On the screening experiment conducted in 1996, moderate resistance to *P. caryophylli* was observed in 'Novada' and 'Revada'; whereas 'Alice', 'Elsy', 'Sacha', 'Sam's Pride', 'Niky', and 'Lena' were highly

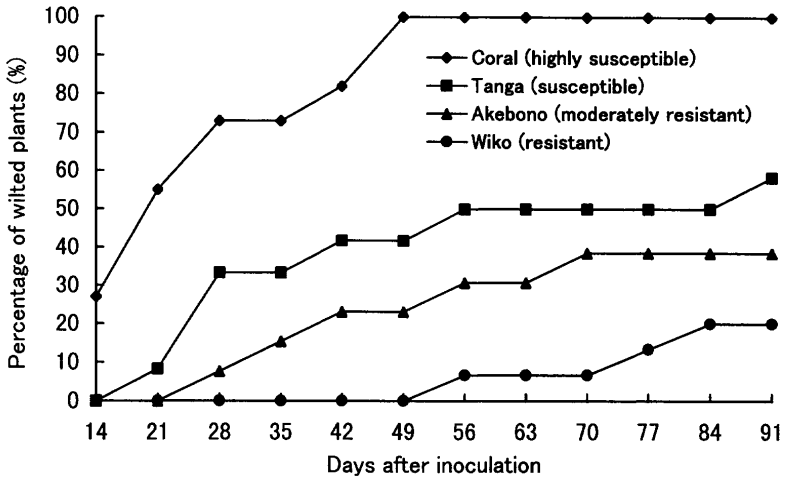

Fig. 1. Disease progression curves of 4 carnation cultivars inoculated with *Pseudomonas caryophylli*.

Table 2. Disease incidence of 7 carnation cultivars indexed 91 days after inoculation using 4 different isolates of *Pseudomonas caryophylli*.

Cultivars	Percentage of wilted plants						
	1991			1992			
	isolate 1	isolate 2	isolate 3	isolate 1	isolate 2	isolate 3	isolate 4
Wiko	20%	0%	7%	n.t.	n.t.	30%	10%
Sandorosa	n.t.	n.t.	n.t.	31%	8%	15	15
Nocto	7	0	38	38	n.t.	n.t.	38
Coral	100	67	72	100	100	100	100
Scania	100	67	75	100	100	100	91
White Sim	n.t.	n.t.	80	100	100	91	100
Kibou	n.t.	n.t.	100	100	100	100	n.t.

n.t.: not tasted

susceptible (Table 1). 'Novada' and 'Revada' are reported as resistant to races 1, 2 and 4 of *Fusarium oxysporum* f. sp. *dianthi* (Demmink et al., 1989). The use of these *Fusarium* resistant cultivars as a parental material for breeding resistance to both bacterial wilt and *Fusarium* wilt is being considered.

'Nocto', a purple red, medium flower-sized cultivar, was bred by a Dutch nursery company in the 1980s. 'Wiko', a white, small flower-sized cultivar, was bred by the P. Kooij & Zonen nursery company in Holland. 'Sandrosa', a pink, normal standard type cultivar, was introduced by the Selecta Company of Israel. 'Sandrosa', known for its long vase life, is a mutant that lacks a climacteric ethylene response (Mayak and Tirosh, 1993).

'Wiko' has a resistance that is difficult to exploit for carnation breeding, because it produces no pollen and lacks the ability to set seeds. 'Wiko' and 'Nocto' are not standard carnations, but small compact types presumably a wild species used for breeding. We crossed several susceptible carnations with 'Sandrosa' and tested the progenies for their reactions to *P. caryophylli* in 1994. No resistant seedlings were obtained from 156 F_1 plants (data not shown) which indicate that resistant carnation cultivars are unsuitable as parents for resistance.

Thus we conclude that most carnation cultivars are susceptible to bacterial wilt and that the search for resistant material to bacterial wilt among wild *Dianthus* species should continue.

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カーネーション萎ちょう細菌病 (*Pseudomonas caryophylli*) に対するカーネーション277品種の抵抗性の評価

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摘　要

Pseudomonas caryophylli により発生するカーネーション萎ちょう細菌病は，夏の高温期に多発する立ち枯れ性の土壌伝染病害であり，日本でのカーネーション栽培上最も重要で問題となっている病害であるが，その抵抗性育種は国際的に未着手の状態である．このため，抵抗性育種素材の選抜と抵抗性品種の育成が，緊急の課題となっている．

本報では，カーネーション277品種の萎ちょう細菌病に対する抵抗性を，浸根接種法による検定により評価した．接種から91日後の発病率によって，抵抗性を極強（発病率：0%），強（発病率：0<~≦20%），中（発病率：20<~≦40%），弱（発病率：40<~≦70%），極弱（発病率：70<~≦100%）の5つに分類した．検定試験の結果，供試品種のほとんどはり病性で，207品種（全体の74.7%）は抵抗性が極弱に分類された．萎ちょう細菌病に対する抵抗性が強（発病率:0<~≦20%）の品種は，‘ウィコ’，‘ノクト’，‘サンドローサ’の3品種のみであった．

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