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Genetic variation of resistance of the cultivated strawberry to crown rot caused by *Phytophthora cactorum*

Sadia Schafleitner[a,b], Alain Bonnet[a,b], Nicole Pedeprat[a,b], Daniel Rocca[a,b], Philippe Chartier[c] and Béatrice Denoyes[a,b,*]

[a]*INRA, UMR 1332 de Biologie du fruit et Pathologie, Villenave d'Ornon, France*
[b]*Université de Bordeaux, UMR 1332 de Biologie du fruit et Pathologie, Villenave d'Ornon, France*
[c]*Ciref Création Variétale Fraises Fruits Rouges, Douville, France*

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Abstract. Evaluation of strawberry resistance to crown rot caused by *Phytophthora cactorum* is usually performed on a limited number of genotypes. The major objective of this study was to screen large genetic resources to identify potential parents that can be further used in breeding programs. Plants were inoculated by wounding the crown and placing a mycelium disk on the wound. Firstly, plug and cold stored plants were evaluated for their susceptibility to *P. cactorum*. Secondly, a total of 107 genotypes was evaluated using cold stored plants. Plug plants were very low affected by the wounding inoculation with *P. cactorum* whatever the genotype, whereas results obtained using cold stored plants consistently reflected the susceptibility of the genotype to crown rot. By using cold stored plants, we evaluated the susceptibility of 70 varieties and 37 advanced lines. Among the genotypes, we identified varieties such as Cirafine or Cireine with high level of resistance similar to the one of Senga Sengana. These data will be useful for choosing parents in breeding programs and for validation of markers linked to the resistance to *P. cactorum*.

Keywords: *Phytophthora cactorum*, strawberry, resistance, type of plants

1. Introduction

Strawberry plant diseases caused by viruses, bacteria, phytoplasmas, fungi, and nematodes result in severe economic losses in plant and fruit productions. Control of plant diseases using methods that are both economically and environmentally sustainable is essential to provide a regular production. More particularly, management of soilborn pathogens is a challenge when looking for alternatives to methyl bromide, which became banned in 2005 following the Montreal Protocol [1].

One of the major soilborn pathogens in Europe is *Phytohthora cactorum* (Lebert and Cohn) J. Schröt. This pathogen causes both crown rot and leather rot of fruits [2]. In fields, the first symptoms observed are a stunting of plants or wilting of young leaves and a dissection of the crown reveals a brown necrosis. According to the extension of this necrosis, partial or entire collapse of the plant is observed and plants die when all vascular vessels are disintegrated. In most cases, symptoms of wilt appear first at the upper end of the crown and spread basipetally [3] and roots often are brown discolored. When fruits are infected, they do not ripen and turn leathery in texture. Crown rot symptoms can appear similar to those caused by anthracnose, but crown tissue infected by the anthracnose pathogen usually

*Corresponding author: Béatrice Denoyes. Tel.: +33 557122460; Fax: +33 557122369; E-mail: denoyes@bordeaux.inra.fr.

takes on a darker cinnamon discoloration, which is more dark brown in case of crown rot. Diagnostic clinics of root surface scrapings under a microscope might reveal *P. cactorum* oospores in the tissues, furthermore, culturing samples of the pathogen isolated at the border of the necrosis would definitely confirm the presence of this fungus.

In Europe, strawberry crown rot was first reported in Germany in 1950 [4], then in France in 1960' [5]. It was more recently reported in Northern countries of Europe such as Finland in 1991 [6] and Norway in 1992 [7]. Until 2005, the control of diseases was achieved by soil disinfection using methyl bromide. Today, new cultural practices such as soilless culture or production of plug or tray plants under a scheme of certification [8] limit the development of diseases including crown rot caused by *P. cactorum*. As these methods do not allow a full control of crown rot, particularly in nurseries of plug or tray plants where recirculating irrigation system may be used, strawberry cultivars resistant to this disease could represent a promising step forwards in reducing plant losses.

Few studies report analyses of susceptibility variability of strawberry to *P. cactorum*. Results of inoculation performed on diploid *Fragaria* species showed no indication of any of these species being more resistant or susceptible than others to *P. cactorum* and no systematic differences resulting from geographic origin [9]. In the cultivated strawberry, *F. x ananassa*, variability of susceptibility of genotypes or cultivars was mainly reported in studies comparing different methods of inoculation, [10–14]. A single large study showed that resistance varied greatly between the 31 genotypes that were tested and offering high levels of resistance for further breeding programs [15]. Inheritance of resistance to *P. cactorum* was reported using 50 bi-parental crosses among 20 elite genotypes [16]. Results suggested additive, polygenically inherited resistance, which agreed with several QTLs found in a segregating population [17] and that offspring from crosses could display transgressive segregation with phenotypes covering the scale from highly resistant to very susceptible [15].

This paper describes firstly the effect of the type of plants, tray plants or cold stored plants, on the level of susceptibility to *P. cactorum* after artificial inoculation. Secondly, using cold stored plants, a large collection of 107 cultivated strawberry genotypes including 70 varieties and 37 advanced lines from the breeding program of Ciref was evaluated against crown rot caused by *P. cactorum*.

2. Materials and methods

2.1. Plant material

A total of 70 varieties and 37 advanced lines from the breeding program of Ciref was evaluated (Table 1) in the GenBerry project (https://www.bordeaux.inra.fr/genberry/pages/sum.htm). Their description was included in the database released by GenBerry (http://www.bordeaux.inra.fr/eustrawberrydb). Inoculation tests were performed over three successive years.

Cold stored plants were obtained from field nurseries while plug plants were raised under plastic tunnel. Both types of plants had no treatment against *P. cactorum*. After their harvest in December for plug plants, or early January for cold stored plants, they were stored at minus_1.5°C and 80% humidity until their inoculation in February or in March.

2.2. Inoculum production

The strain S22 of *Phytophthora cactorum* isolated by J.C. Navatel (Ctifl) was maintained in liquid nitrogen since 2004 at the UREF-INRA-Bordeaux. Cultures were initiated by transferring mycelium loaded potato dextrose agar (PDA)-disks maintained at −80°C from stock cultures to PARB selective medium. For this step, the disks were quickly thawed at room temperature, briefly dipped in sterile water, drained on sterile absorbent paper, and transferred on PDA medium at 23°C. About 5 days later, when the diameter of the culture had reached 1 to 2 cm, tips of hyphae were transferred on a selective medium (e.g. PARB medium). For the inoculum production, cultures were transferred on V8-oatflakes-agar medium grown in 90 mm diameter Petri dishes and kept at 23°C in a darkroom. Inoculum consisted of 5 mm diameter mycelium disks sampled from the leading edge of 5–10 days old cultures. Before the first inoculation test of this study, purity, viability and pathogenicity of the strain were checked.

Table 1
List of genotypes tested for their resistance to *Phytophtora cacotorum*

Genotypes	Ancestral data[a]	Country of Origin[a]	Year of Release[a]
Addie	Pantagruella × MDUS 3816	IT	1982
Belle de Mai	–	FR	-[b]
Belle et Bonne	Madame Moutôt × Souvenir de Charles Machiroux	FR	1958
Betty	Pajaro × CF206	FR	2007
Camarosa	Douglas × Cal 85.218 605	USA	1992
Cambridge Favourite	F. chiloensis × Blakemore or (Etter Seedling × Avant Tout) × Blakemore	GBR	1947
Candonga	-[b]	SP	2003
Capitola	CN 25 [= Cal 75.121-101] × Parker	USA,Calif.	1992
Capron royale	–	FR	-[b]
Chandler	Douglas × Klon C 55 or Douglas × CAL 72 361 105	USA,Calif.	1980
Charlotte	Mara des bois × Cal 19	FR	2001
Chili Manzanar	-[b]	-[b]	-[b]
Ciclade	-[b]	FR	1997
Ciflorette	Mamie × Earliglow	FR	1998
Cifrance	Scott × Chandler	FR	1996
Cigaline	Gariguette × Earliglow	FR	1996
Cigoulette	Belrubi × Pajaro	FR	1996
Cijosée	Mara Des Bois × Cal. 18	FR	1997
Cilady	Scoot × Chandler	FR	1996
Ciloe	Belrubi × Allstar	FR	1998
Cirafine	Cla 18 × Mara des bois	FR	1997
Cirano	Mara Des Bois × Muir	FR	1997
Cireine	Scott × Chandler	FR	1996
Clery	Sweet Charlie × Marmolada®Onebor	IT	2002
Darselect	Elsanta × Parker	FR	1996
Docteur Morère	Duc Malakoff × Palmyre Berger	FR	1871
Donner	US-634 × Blakemore	USA	1945
Dover	Florida Belle × selection USFL-71-1965E	USA	1980
Dr. Morère	Duc de Malakoff × Palmyre Berger	FR	1871
Earlyglow	MDUS 2359 [Fairland × Midland] × MDUS 2713 [Redglow × Surecrop]	USA, MD	1975
Elsanta	Gorella × Holiday	NL	1981
Favette	(Surprise des halles × Regina) × (Aliso × Pocahontas)	FR	1976
Gariguette	Belrubi × Favette	FR	1976
Georg Soltwedel	Hansa × Rotkäppchen	GE	1941
Gorella	Juspa × american selection US 3763	NL	1960
Hative de Caen	Aurore × OP	FR	1928
Havelland	Münchenberger Frühe × Georg Soltwedel	GE	1971
Ile de France	Aurore × OP	FR	-[b]
Josif Mahomet	-[b]	UKR	1955
Karmen	Georg Soltwedel × Sparkle	CZ	1971
Libération d'Orléans	Everbearer selection from F. × ananassa	FR	1899
Louis Gauthier	Belle de Meaux × Marguerite Lebreton	FR	1896
Madame Moutot	Docteur Morère × Royal Sovereign	FR	1906
Mamie	Harvester × Gariguette	FR	1988
Mara des bois	[(Humi Gento × Ostara) × (Red Gauntler × Korona)]	FR	1992
Marie France	probably an open pollinated seedling	FR	1955

Table 1
(*Continued*)

Genotypes	Ancestral data[a]	Country of Origin[a]	Year of Release[a]
Merton Ruby	USDA 3378 × Early Cambridge	GBR	1965
Mme Lefèvbre	-[b]	FR	1900
Mme Leroy-Ladurie	-[b]	FR	-[b]
Mme Moutot	Docteur Morère × Royal Sovereign	FR	1906
Mysowka	-[b]	RUS	1958
Naiad	Oso Grande × Eris®Civero	IT	2000
Orléans	Acadie × Joliette	CND	1996
Pajaro	Cal 63.7-101 × Sequoia	USA,Calif.	1973
Parker	Douglas × (Tufts × 63.7.101)	USA,Calif	1983
Senga Precosa	Regina × (Sparkle × Eva Macherauch)	GE	1953
Red Gauntlet	Auschincruive Climax × New Jersey 1051 [Royal sovereign × Howard 17]	GBR	1957
Revada	Auschincruive Climax × Ada Herzberg	NL	1956
Royal Sovereign	King of the Earliest × Laxtons Noble	UK	1892
Saint Josef	-[b]	FR	1892
Sannié	Docteur Morère × Vicomtesse Héricart de Thury or offsping	FR	1800
Ségaline	-[b]	FR	-[b]
Senga Sengana	Markee × Sieger	GE	1942
Sequoia	Cal 52.16-15 × Cal 51 s 1-1	USA,Calif.	1968
Soquel	Cruz × Aïko	USA	1983
Souvenir de Charles Machiroux	Tardive de Leopold × Ville de Paris	FR	1942
Spaete Leopold	Comet × Sämling	GE	1920
Saint Louis d'Orléans	-[b]	FR	-[b]
Saint Pierre	Chandler × Jewel	CND	1989
Surprise des Halles	-[b]	FR	1925
Talisman	New Jersey 1051 × Auchincruive Climax	GBR	1955
Valeta	Sivetta × Holiday	NL	1983
Versallaise	-[b]	GBR	1850
Ville de Caen	Empereur du Maroc × Madame Moutot	FR	1922
Ville de Paris	Capron royal × Princess Dagmar	FR	1929

[a]main data source: Genberry Database, http://www.bordeaux.inra.fr/eustrawberrydb; [b]no data; [c]uncertain released year.

2.3. Inoculation tests

All plants were placed at 4°C three days before inoculation for thawing. Cold stored plants were washed under cold running water on the day before inoculation and their roots were cut 6–8 cm below the crown.

Plants were inoculated as described before by Pitrat and Risser [10]. After gently wounding crowns using a scalpel to lay a few vascular vessels open, a mycelium of PDA-disk of 5 mm diameter was placed on the wound and held in place by sealing the crown and the mycelium disk with a plastic film wrapped around the crown. Control plants were likely treated without mycelium disk. Plants were then transplanted into 10-cm plastic pots containing a mixture of pasteurized sand and soil (1:3, v/v). Pots were placed in a greenhouse with 22 ± 4°C day temperature and 17 ± 4°C night temperature. High relative humidity, 75–85% rh, was maintained during the week after inoculation using a fog system. Inoculation tests were performed from end of February to end of April with day length varying from 10h30 to 14h00 respectively. No extra light was added.

For each experiment, 10 plants were inoculated and five control plants were included to confirm that commercial samples of genotypes were free of diseases. The 10 inoculated plants were randomized in bulks of five in two blocks while the five control plants were placed into a different compartment in the greenhouse to avoid cross contamination

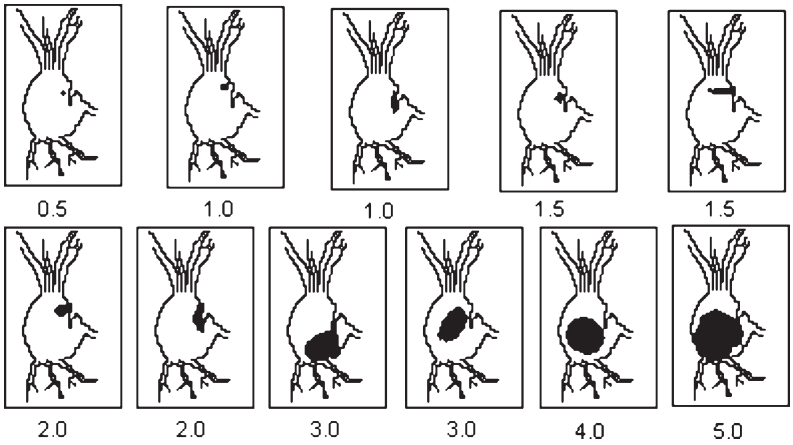

Fig. 1. Examples of the disease response scored 28 days after inoculation on a scale from 0 to 5 based on the degree of necrosis present in the crown. 0 = no symptoms, 1 = necrosis inferior to 1 mm, 2 = necrosis of 2-3 mm; 3 = necrosis of a quarter to one third of the crown; 4 = necrosis of half the crown; 5 = necrosis spread into the entire crown.

by splash dispersal. Experiments were performed twice a year and during three successive years. During the first year, the effect of the type of plants - plug plants and cold stored plants-was evaluated on a limited number of genotypes, while, during the second and the third year, the variability of susceptibility of a large range of genotypes was evaluated using cold stored plants.

2.4. Disease response

Eight days after inoculation, the plants were treated against *Botrytis cinnerea* (Rovral 2g/l). On day 15, the growth of plants was visually assessed on control cold stored plants. When plants failed to grow for some genotypes, we considered that the quality of these cold stored plants was poor and these genotypes were discarded for further analysis for the experiment in which they failed.

Crown disease responses were recorded 28 days after inoculation on a scale of 0 to 5 according to the size of the necrosis. The crown was cut longitudinally in slices of 0.5–1 mm thickness to have a three-dimensional representation of the necrosis and hence an accurate reading of it. A definition of the main scores assigned to this scale is as follows: 0 = no symptoms, 1 = necrosis inferior to 1 mm, 2 = necrosis of 1–2 mm; 3 = necrosis of 2–3 mm; 4 = necrosis of half the crown; 5 = necrosis spread into the entire crown (Fig. 1).

2.5. Statistical analyses

Analysis of variance (ANOVA) was performed for disease response followed by a Bonferroni test using SAS software (SAS Institute, Inc., Cary, NC). Since the scale of disease responses comprises 11 steps, with intermediate scores, they were considered as quantitative and subjected to a variance analysis performed with the general linear model procedure (PROC GLM in SAS). A Bonferroni test was used to separate means.

3. Results

All inoculation tests were successful and wilt symptoms started to appear on the most susceptible genotypes about two weeks after inoculation. In each of experiment, 10% of genotypes were removed because at least one of their control plants did not grow 15 days after inoculation.

Table 2
Results of ANOVA for disease response in strawberry genotypes (G) inoculated with *Phytophthora cactorum* using two types of plants (Tplt), plug and cold stored plants

Source	df	Sum of squares	Mean squares	F value	Pr>F
Genotype (G)	14	104.72	7.48	3.80	<0.0001
Type of plant (Tplt)	1	47.64	47.64	24.22	<0.0001
Interaction (G × Tplt)	13	86.97	6.69	3.40	<0.0001

3.1. Effect of type of plants

When plug plants were inoculated, very few symptoms were observed on crowns of the 15 genotypes compared to inoculation on cold stored plants. For most of the genotypes, results of the Student test performed for each genotype lead to classify cold stored plants as more susceptible than plug plants except for Charlotte (data not shown). ANOVA was carried out on crown disease responses scored 28 days after inoculation from the 15 genotypes tested for both types of plants (Table 2). All tested effects, genotype, type of plants and [genotype × type of plants] interaction, were highly significant. Since the [genotype × type of plants] interaction was significant, we carried out an ANOVA for each type of plants, followed by a Bonferroni test in order to identify which type of plants was the most consistent for evaluating the susceptibility to *P. cactorum* (Table 3). Only results of inoculation on cold stored plants clearly separated the genotypes according to their susceptibility whereas results of inoculation on plug plants gave statistically similar crown disease responses whatever the genotype (Table 3). Therefore, in order to screen for cultivar resistance to *P. cactorum*, we chose hereafter inoculation on cold stored plants rather than on plug plants.

3.2. Screening test on varieties and advanced lines using cold stored plants

Among the 107 genotypes, four were evaluated in only one experiment, 40 in two experiments, 25 in three experiments and 38 in all four experiments. ANOVA was carried out using the four experiments performed on two successive years. The effects of genotype and experiment were significant while the effect of [genotype × experiment] interaction was not significant (Table 4). Therefore, we applied the Bonferroni test on data using all experiments to classify the experiments and the genotypes according to their susceptibility to *P. cactorum*. The two experiments performed at the end of February in 2009 and 2010 were more severe (disease response means of 1.55 and 1.42 respectively) than the two experiments performed at the end of March the same years (disease response means of 1.22 and 1.18, respectively).

Genotypes displayed continuous values of crown disease responses from very resistant (e.g. crown disease response of 3.06 for Cambridge Favourite) to very susceptible (e.g. crown disease response of 0.36 for Senga Sengana) with all intermediate degree of susceptibility as shown in Fig. 2 (Table 5). Results of Bonferroni test allowed the classification of the 107 genotypes into overlapping groups. Sixty five cultivars, with crown disease responses ranged from 3.72 (Segaline) to 1.1 (Hative de Caen) (Table 5), displayed a susceptibility statistically similar to the one of the cultivar Cambridge Favourite ranged as very susceptible [10, 23]. Nine cultivars displayed a level of resistance similar to the one of Senga Sengana, used as resistant control in numerous works [review in 15]. Among these nine genotypes, Docteur Morere is a very old variety released in 1871. Some genotypes, such as Cigaline, showed large variation between experiments (crown disease responses varied from 1.24 to 2.60) while low variation was observed for other genotypes such as CF3008 (crown disease responses varied from 1.11 to 1.25).

4. Discussion

In Europe, the development of cultivation techniques was accompanied by the development of different types of plants. As a consequence, nurseries adapted their cultures to obtain different types of plants such as plug, tray, fresh and cold stored plants. Here, we reported that inoculation of *P. cactorum* on cold stored plants gave more

Table 3
Susceptibility of 15 varieties and advanced lines according to the type of plant

Name of genotype/cultivar	Number of plants	Disease response[a]	Standard deviation[a]	Bonferroni test[b]
For cold stored plants				
CF1116	27	2.94	2.16	a
Gariguette	10	2.45	1.38	ab
Valeta	5	2.30	1.72	ab
Ciflorette	10	2.05	1.36	abc
Addie	20	1.78	2.20	a-d
Elsanta	20	1.73	1.57	a-d
Darselect	20	1.55	1.50	a-d
Capitola	29	1.52	1.33	a-d
Pajaro	20	1.43	1.87	a-d
CF2559	19	1.18	1.68	a-d
CF1778	10	1.00	0.71	a-d
Mara des Bois	20	0.93	1.76	bcd
Dover	10	0.20	0.35	cd
Charlotte	20	0.05	0.15	d
Cirafine	10	0.00	0.00	d
For plug plants				
CF1116	20	0.65	1.27	a
Gariguette	25	1.30	1.53	a
Valeta	15	1.23	1.74	a
Ciflorette	20	1.20	1.33	a
Addie	20	0.58	1.14	a
Elsanta	20	0.76	0.85	a
Darselect	20	0.35	0.52	a
Capitola	10	0.70	0.72	a
Pajaro	19	0.45	0.83	a
CF2559	19	0.21	0.35	a
CF1778	9	1.39	1.85	a
Mara des bois	20	1.33	1.72	a
Dover	10	0.26	0.35	a
Charlotte	15	1.17	1.21	a

[a]Disease response was scored 28 days after inoculation on a scale from 0 to 5 based on the degree of necrosis present in the crown. Mean and standard deviation of disease response were reported. Cold stored and plug plants were inoculated by depositing a mycelium disk on the wounded crown. [b]Values in a column followed by different letters are different at $P = 0.001$.

Table 4
Results of ANOVA of disease response in strawberry genotypes (G) inoculated with *Phytophthora cactorum* using cold stored plants. Plants were inoculated in a total of four experiments (exp)

Effect	df	Sum of squares	Mean squares	F value	Pr>F
Genotype (G)	109	1576.43	14.46	6.05	<0.0001
Experiement (E)	3	82.39	27.46	11.48	<0.0001
Interaction (G × E)	204	567.76	2.78	1.16	0.062

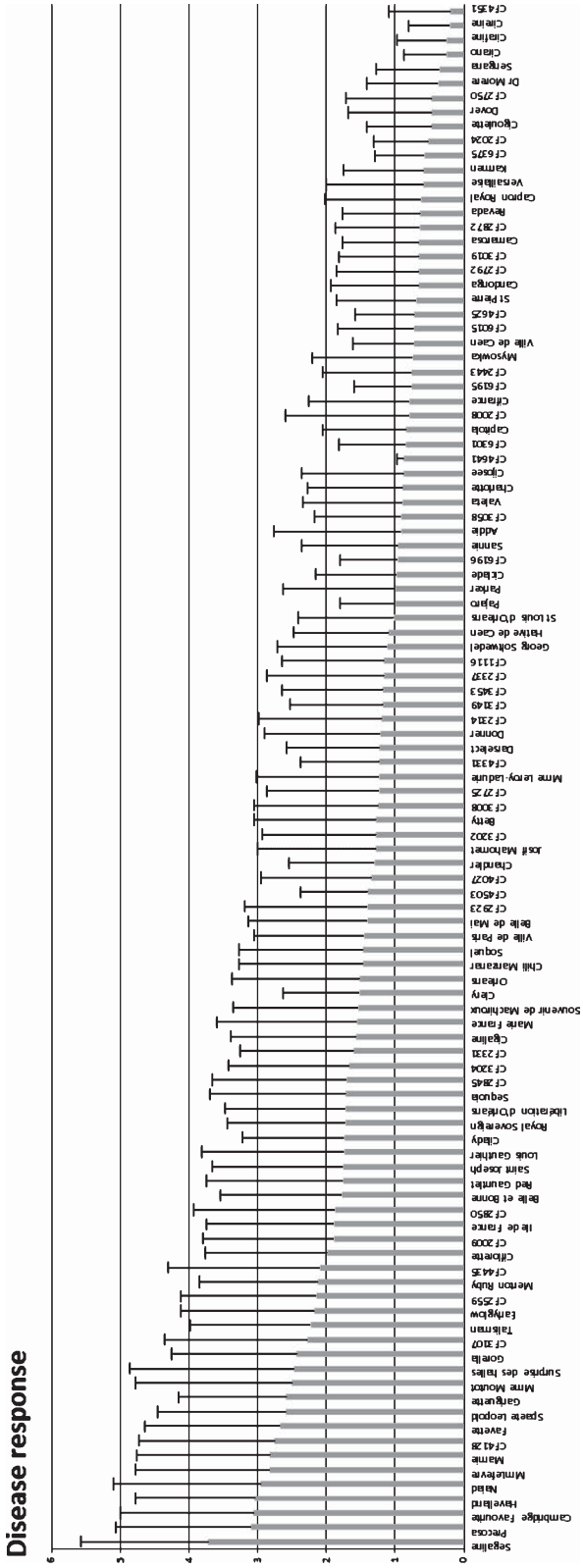

Fig. 2. Disease response of 70 varieties and 37 advanced lines from the breeding program of CIREF (CF followed by a number). Each bar represents the mean of disease response of one genotype with its standard deviation. Disease response was scored 28 days after inoculation on a scale from 0 to 5 based on the degree of necrosis present in the crown. Cold stored plants were inoculated by depositing a mycelium disk on the wounded crown.

Table 5
Disease response of 70 varieties and 37 advanced lines inoculated with *Phytophthora cactorum*. Cold stored plants were inoculated with one isolate of *P. cactorum*, S22, by wounding the crown

Genotype	N° exp[a]	N° Year[a]	Freq.[b]	DR-mean[c]	DR-std[c]	Bonf-class[d]
Segaline	3	2	30	3.72	1.86	a
Senga Precosa	4	2	36	3.1	1.97	ab
Cambridge Favourite	2	1	17	3.06	1.94	ab
Havelland	3	2	28	3.04	1.74	abc
Naiad	2	2	13	2.96	2.14	a-d
MmLefevre	3	2	26	2.83	1.94	a-e
Mamie	2	1	17	2.82	1.94	a-e
CF4128	2	1	18	2.75	1.98	a-f
Favette	3	2	28	2.68	1.96	a-g
Spaete Leopold	4	2	33	2.59	1.87	a-h
Gariguette	3	2	25	2.58	1.57	a-h
Mme Moutot	3	2	30	2.5	2.28	a-i
Surprise des halles	2	1	19	2.47	2.39	a-j
Gorella	2	1	20	2.43	1.82	a-j
CF3107	2	1	20	2.28	2.08	a-k
Talisman	4	2	37	2.24	1.75	a-k
Earlyglow	3	2	30	2.18	1.94	a-l
CF2559	2	1	20	2.15	1.96	a-l
Merton Ruby	3	2	28	2.13	1.72	a-l
CF4435	1	1	10	2.1	2.2	a-l
Ciflorette	3	2	40	1.99	1.78	a-l
CF2009	2	1	20	1.9	1.89	a-l
Ile de France	3	2	30	1.9	1.84	a-l
CF2850	2	1	20	1.88	2.05	a-l
Belle et Bonne	4	2	40	1.78	1.76	a-l
Red Gauntlet	2	1	19	1.76	1.99	a-l
Saint Joseph	4	2	40	1.76	1.9	a-l
Louis Gauthier	2	1	20	1.75	2.06	a-l
Cilady	2	1	20	1.75	1.47	a-l
Royal Sovereign	3	2	26	1.73	1.72	a-l
Libération d'Orléans	2	1	20	1.73	1.74	a-l
Sequoia	4	2	37	1.72	1.98	a-l
CF2845	2	1	19	1.71	1.95	a-l
CF3204	3	2	30	1.67	1.75	b-l
CF2331	2	1	20	1.6	1.65	b-l
Cigaline	4	2	47	1.57	1.82	b-l
Marie France	3	2	27	1.56	2.04	b-l
Souvenir de Machiroux	4	2	40	1.54	1.81	b-l
Clery	4	2	38	1.53	1.1	b-l
Orleans	3	2	27	1.52	1.86	b-l
Chili Manzanar	4	2	38	1.47	1.81	b-l
Soquel	3	2	30	1.47	1.81	b-l
Ville de Paris	3	2	26	1.46	1.59	b-l
Belle de Mai	4	2	35	1.41	1.73	b-l
CF2923	4	2	40	1.41	1.78	b-l

Table 5
(*Continued*)

Genotype	N° exp[a]	N° Year[a]	Freq.[b]	DR-mean[c]	DR-std[c]	Bonf-class[d]
CF4503	2	1	19	1.4	0.97	b-l
CF4027	2	1	20	1.35	1.6	b-l
Chandler	3	2	29	1.31	1.24	b-l
Josif Mahomet	4	2	39	1.28	1.72	b-l
CF3202	4	2	38	1.28	1.65	b-l
Betty	2	1	20	1.28	1.78	b-l
CF3008	4	2	34	1.25	1.81	b-l
CF2725	4	2	35	1.24	1.62	b-l
Mme Leroy-Ladurie	4	2	40	1.24	1.78	b-l
CF4331	2	1	20	1.23	1.14	b-l
Darselect	2	2	20	1.23	1.35	b-l
Donner	4	2	34	1.22	1.68	b-l
CF2314	2	1	20	1.2	1.78	b-l
CF3149	4	2	38	1.18	1.35	b-l
CF3453	2	1	20	1.18	1.46	b-l
CF2337	2	1	19	1.16	1.7	b-l
CF1116	2	1	19	1.16	1.48	b-l
Georg Soltwedel	3	2	29	1.12	1.59	b-l
Hative de Caen	2	1	20	1.1	1.38	b-l
St Louis d'Orleans	3	2	30	1.02	1.39	d-l
Pajaro	2	1	20	1	0.81	d-l
Parker	3	2	29	1	1.63	d-l
Ciclade	4	2	40	0.99	1.17	d-l
CF6196	2	1	15	0.97	0.83	d-l
Sannie	4	2	37	0.96	1.4	d-l
Addie	2	1	19	0.92	1.85	e-l
CF3058	3	2	25	0.92	1.26	e-l
Valeta	4	2	40	0.9	1.45	e-l
Charlotte	4	2	63	0.89	1.38	e-l
Cijosee	4	2	37	0.88	1.48	e-l
CF4641	2	1	20	0.88	0.1	e-l
CF6301	1	1	10	0.85	0.97	e-l
Capitola	2	2	19	0.84	1.21	e-l
CF2008	1	1	5	0.8	1.79	f-l
Cifrance	2	1	20	0.8	1.45	f-l
CF6195	2	1	24	0.77	0.83	f-l
CF2443	3	2	28	0.77	1.29	f-l
Mysowka	3	2	28	0.75	1.45	f-l
Ville de Caen	4	2	39	0.73	0.88	g-l
CF6015	2	1	20	0.73	1.11	g-l
CF4625	1	1	9	0.72	0.87	g-l
St Pierre	4	2	37	0.7	1.15	g-l
Candonga	4	2	34	0.66	1.27	h-l
CF2792	4	2	44	0.66	1.2	h-l
CF3019	4	2	38	0.66	1.16	h-l
Camarosa	4	2	38	0.66	1.1	h-l

Table 5
(*Continued*)

Genotype	N° exp[a]	N° Year[a]	Freq.[b]	DR-mean[c]	DR-std[c]	Bonf-class[d]
CF2872	2	1	20	0.65	1.22	h-l
Revada	3	2	28	0.64	1.13	h-l
Capron Royal	2	2	20	0.63	1.4	h-l
Versaillaise	4	2	38	0.59	1.41	h-l
Karmen	4	2	40	0.59	1.16	h-l
CF6375	2	1	20	0.58	0.71	h-l
CF2024	3	2	30	0.52	0.8	i-l
Cigoulette	4	2	38	0.47	0.95	jkl
Dover	2	1	19	0.47	1.21	jkl
CF2750	2	1	15	0.47	1.25	jkl
Dr Morere	4	2	38	0.38	1.04	kl
Senga Sengana	4	2	37	0.36	0.91	kl
Cirano	4	2	40	0.26	0.62	kl
Cirafine	4	2	40	0.26	0.72	kl
Cireine	4	2	39	0.21	0.59	l
CF4351	2	1	20	0.2	0.89	l

[a]Number of experiment and Number of years. [b]Number of inoculated cold stored plants. [c]Disease response was scored 28 days after inoculation on a scale from 0 to 5 based on the degree of necrosis present in the crown. Mean and standard deviation of disease response were reported. Cold stored plants were inoculated by depositing a mycelium disk on the wounded crown. [d]Bonferroni test was carried out for all experiments. Values in a column followed by different letters are different at $P = 0.001$.

consistent results than on plug plants, which are plants obtained in July from runners and raised under tunnel until they were placed at -1.5°C for chilling requirement. The last were very low affected by the wounding inoculation with *P. cactorum* whatever the genotype, whereas results obtained using cold stored plants consistently reflected the genotype susceptibility of the crown to *P. cactorum*. In addition by using cold stored plants, we reported the largest study on genetic screening for susceptibility to *P. cactorum* in the cultivated strawberry.

In Eikemo et al. [12], authors reported that inoculation of cold stored plug plants gave more uniform results than inoculation on non-cold stored plug plants. Hypothesis of cold storage to shift plants into their most susceptible state was suggested. In our experiment, both types of plants received cold storage treatment, naturally in the nursery field for cold stored plants and controllably in the climatic chamber for plug plants. Low temperature observed in natural conditions would injure crowns more severely and as a result increase their susceptibility to crown rot [18]. Crown rot in cold stored plants could also result of infections prior to cold storage [11]. To avoid this problem, absence of symptoms all along the inoculation test on non-inoculated control plants is critical.

Other factors than the type of plants may influence screening test. One of them is the preparations of inoculum of *P. cactorum*, which can be prepared as zoospores poured onto the crown [9, 11, 15], as sporangia added in container [14], as agar disks of mycelium placed on the wounded crown [10], as homogenates of mycelium on strawberry *in vitro* shoots [13], or V8 juice-vermiculite-oat medium permeated with *P. cactorum* incorporated in soil [16]. In this study, use of mycelium disks was preferred, because it has given consistent results over several years for disease responses of genotypes and for inoculum preparation. Another factor is the strain used in the screening test since specific interaction can exist between genotypes and isolates as previously described for anthracnose in strawberry [19, 20]. However, pathogenicity of *P. cactorum*, isolated from crown rot did not show large variability [21, 22] whereas crown rot isolates were different from leather rot isolates [21].

To be an efficient screening test for evaluating resistance to *P. cactorum*, inoculation test was repeated to confirm the level of genotype resistance in addition to the presence of replicates for each experiment. In this study, two years of inoculation test with two tests per year were enough to give consistent results. Variability observed between inoculation tests could be due to the influence of environmental conditions (Pitrat and Risser xxx). In order to standardize the

protocol and overcome problem of repeatability, control genotypes have to be included in each experiment. In our work, the cultivars Senga Sengana and Cambridge Favourite were useful as resistant and susceptible cultivars [10, 12, 23].

Strawberry breeding began in England in the late 1700 s, followed by France and Germany. The first selected European cultivars were used as genitors in early American breeding programs, together with American native cultivars [24]. The origin of strawberry and these early breeding practices reduced initial genetic variability. As an example, pedigrees of 134 North American cultivars were traced and shown to originate from only 17 cytoplasmic sources [25]. Despite introgressions of wild strawberry germplasm increased initial diversity of the cultivated strawberry, loss of diversity was observed in modern strawberry cultivars [26, 27]. However, our results showed that advanced lines can display large variability from high to low level of resistance (Table 5). Therefore, breeding for resistance is a powerful strategy for controlling diseases in the cultivated strawberry.

Efficiency of breeding programs could be improved by developing marker assisted selections as developed for anthracnose resistance [19]. Since the mode of inheritance for resistance to *P. cactorum* is likely polygenic [16, 17], which is suggested by the continuum of level of disease resistance in a range of cultivar, a strategy for the identification of QTLs will be developed. Then, markers flanking the most significant QTLs will be used for validation on germplasm. For this strategy, the use of the diploid *Fragaria*, which genome sequence is available [28] will be very useful, since the octoploid and the diploid genomes are almost collinear [29, 30].

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