Partial Resistance to *Erwinia carotovora* subsp. *carotovora* and Plant Vigour among F_1 Hybrids of *Zantedeschia* Cultivars

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Abstract

The potential of breeding *Zantedeschia* cultivars for resistance to soft rot caused by *Erwinia carotovora* subsp. *carotovora* (syn. *Pectobacterium carotovorum* subsp. *carotovorum*) was determined. Hybrids of six cultivars ('Back Magic', Galaxy', Pink Persuasion', Sensation', 'Treasure' and 'Florex Gold') were screened using a non-destructive resistance test (leaf disk test) and scored different levels of susceptibility. Some hybrid families were less susceptible than the very susceptible reference 'Florex Gold', while all hybrid families were more susceptible than the partial resistant reference *Z. rehmannii*. The half-sib family of 'Pink Persuasion' was more resistant, but less vigorous than the half-sib family of 'Florex Gold'. We concluded that resistance and vigour may be negatively correlated in the deployed cultivars and that resistance should be bred from other sources than the used cultivars.

INTRODUCTION

Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* (syn. *Pectobacterium carotovorum* subsp. *carotovorum*) occurs worldwide in many crops. This soil-borne, facultative anaerobic pathogen causes maceration and rotting of parenchymatous tissue of all plant organs, eventually resulting in plant death (Pérombelon and Kelman, 1980). The pathogen is difficult to restrain because of many reasons, such as absence of effective bactericides (Blom and Brown, 1999), its genetic variability (Avrova et al., 2002; Gardan et al., 2003), its wide host range, its broad array of virulence factors and because infection can be latent (Pérombelon, 2002). It is an important disease in *Zantedeschia* spp. and the major disease in cultivars of the section *Aestivae* (Kuehny, 2000; Wright and Burge, 2000).

Plants turn yellow when disease has initiated, produce a foul smell and can become completely macerated resulting in plant death within a few days (Wright, 1998). Bacteria are spread mechanically during cultivation and symptoms can become visible during all stages of plant growth and development. Conditions are favourable for soft rot when plants are under stress during low soil aeration, high temperature or high relative humidity (Funnell and MacKay, 1999; Wright and Burge, 2000). Cultural measures such as drainage, mulching, soil ventilation and time of tuber lifting can reduce disease development (Funnell, 1993; Wright and Burge, 2000; Wright et al., 2002), but better control of the disease could be achieved by the use of resistant cultivars.

Most commercial cultivars have been developed from interspecific hybrids within the section *Aestivae*, mainly of *Z. albomaculata*, *Z. elliotiana*, *Z. rehmannii* and *Z. pentlandii* (Funnell, 1993). The level of genetic control of the resistance should be determined in order to value the potential of this resistant germplasm for breeding.

The aim of the present study was to determine whether resistance is under genetic control and if it is possible to breed for resistance to soft rot using cultivars as parents. To this end, hybrids were developed of six cultivars of the section *Aestivae*. Resistance levels of offspring were determined using a non-destructive leaf disk test.

MATERIALS AND METHODS

Plant Material

Hybrids were retrieved after hybridising six cultivars in all directions ('Black Magic', 'Galaxy', 'Pink Persuasion', 'Sensation', 'Treasure' and 'Florex Gold').

Plants were cultivated as described by (Snijder et al., 2003) with the only modification that tubers of the parents were treated with gibberellic acid (100 ppm) before planting, as is common practice in commercial production. Tubers of the two-year-old seedlings (Table 1) were not treated with gibberellic acid before planting, because gibberellic acid can decrease the number of leaves (Brooking and Cohen, 2002), while leaves were needed for the resistance test.

Growth Characteristics

Growth characteristics (plant length, number of leaves and chlorophyll content) were observed at two months after planting. The tuber weight was determined during planting. Chlorophyll content was determined of the youngest leaf by a SPAD-502 instrument (Minolta Camera Ltd.). This instrument estimates leaf chlorophyll content in situ, by transmitting light through a leaf at wavelengths of 650 and 940 nm. The signal of 650 nm is absorbed by chlorophyll, while the signal of 940 nm is not. The ratio of transmittance of both signals through the leaf is a measure for the amount of chlorophyll, resulting in a unitless SPAD (Soil Plant Analysis Development)-value. Three measurements were made on the abaxial side of the upper half of the oldest leaf, two months after planting and averaged to reduce variability (Adamsen et al., 1999).

Experimental Procedure

Hybrids were analysed for resistance to bacterial isolate *E. carotovora* subsp. *carotovora* PD 1784 (obtained from the Dutch Plant Protection Service) in their second growing season using a leaf disk test, with which individual plants can be tested without being destructed (Snijder et al., 2003). Two to four leaves of each seedling were evaluated in one experiment, which was split into 15 plots. The observations were done at three or four days after inoculation, depending on the progress of the infection on the reference genotypes 'Florex Gold' (susceptible) and *Z. rehmannii* (partial resistant), which were represented in all plots with two leaves.

Resistance Test and Statistical Analyses

The resistance test (Fig. 1) and its statistical analysis were done as described by (Snijder et al., 2003). Results from the different replicates and plots were related to 'Florex Gold', resulting in an index for macerated leaf disk area (M), where 'Florex Gold' was set at 100 as suceptible reference. Growth characteristics were analysed by generalised linear models (GLMs). The chlorophyll content (measured in SPAD units) and plant length were estimated assuming a normal distribution, tuber weight by assuming a lognormal distribution and the number of leaves assuming a Poisson distribution. All analyses were done with the statistical software package Genstat 6, release 6.1 (GenStat, 2002).

RESULTS AND DISCUSSION

After crossing the six cultivars in all directions, marked differences appeared in fruit set and in seed viability. 'Florex Gold' and 'Treasure' were good mothers, because they delivered many viable seeds that produced healthy plants (Table 1.). 'Galaxy' and 'Pink Persuasion', however, were bad mothers, since they did not produce viable offspring and were therefore not incorporated in Table 1.

Some crossings produced chimerical or pale plants that appeared unhealthy and were therefore omitted from the experiment. This hybrid variegation may be caused by combined plastome-genome incompatibility and biparental inheritance of plastids as was observed by Yao and Cohen (2000) in hybrids of *Z. aethiopica* and *Z. odorata* and by

Snijder (2004) in interspecific hybrids of section *Aestivae*.

The selfing of 'Florex Gold' (M=117) was more susceptible than 'Florex Gold' (100) itself. This difference suggests that resistance is negatively correlated to age (Snijder et al., 2003). The seedlings, which were two years old, were more susceptible than their parents, which had been clonally propagated for at least four years. For reliably evaluating the levels of resistance of the progenies, they are best compared to the selfing of 'Florex Gold', instead of with 'Florex Gold' itself.

Many hybrid families were not different from the 'Florex Gold' selfing and these families can therefore be considered as susceptible. The families that were more resistant than the 'Florex Gold' selfing scored an index of 80-90 (Table 2) and are therefore still susceptible (Snijder et al., 2003) and far less resistant than *Z. rehmannii* (44).

The progenies that have 'Pink Persuasion' as father (89) are all less susceptible than the selfing of 'Florex Gold' (117). 'Pink Persuasion' may therefore carry genes for partial resistance and may be a good parent in resistance breeding. Its breeding value however, not only depends on resistance but also on traits that influence plant vigour. It appears that progeny of 'Pink Persuasion' has a lower plant vigour than progeny of 'Florex Gold' (Table 3). So the genes for partial resistance that may be present in 'Pink Persuasion' may be negatively correlated to plant vigour and 'Pink Persuasion' may not be as good a parent as the resistance of its offspring indicates.

Vigour and susceptibility hence appear to be negatively correlated. Slower growing plants may have a higher dry weight, resulting in sturdy tissue that is more difficult to colonise by *E. carotovora* subsp. *carotovora* bacteria, as was observed in potato (Pagel and Heitefuss, 1989) and for *Fusarium* resistance in radish (Hoffland et al., 1996). The less vigorous plants may be in a constant state of stress and therefore have an increased level of induced resistance if classis stress signals are involved (Sticher et al., 1997). Although this type of plants appears resistant, they are not interesting as source of resistance for breeding, because their resistance is associated with decreased plant vigour. Other sources of resistance than the evaluated cultivars should be used as resistance sources in breeding, for example *Z. albomaculata* and Z. rehmannii, in which genetic variation for resistance was described (Snijder et al., 2003).

Literature Cited

Adamsen, F.J., P.J.P. Jr, E.M. Barnes, R.L. LaMorte, G.W. Wall, S.W. Leavitt and B.A. Kimball. 1999. Measuring wheat senescence with a digital camera. Crop Science 39: 719-724.

Avrova, A.O., L.J. Hyman, R.L. Toth and I.K. Toth. 2002. Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. Applied and environmental microbiology 68 (4): 1499–1508.

Blom, T.J. and W. Brown. 1999. Preplant copper-based compounds reduce *Erwinia* soft rot on calla lilies. HortTechnology 9 (1): 56-59.

Brooking, I.R. and D. Cohen. 2002. Gibberellin-induced flowering in small tubers of *Zantedeschia* `Black Magic'. Scientia Horticulturae 95 (1-2): 63-73.

Funnell, K.A. 1993. *Zantedeschia*. In: A.A. De Hertogh and M. LeNard. The physiology of flower bulbs. Elsevier, Amsterdam. pp: 683-703.

Funnell, K.A. and B.R. MacKay. 1999. Directions and challenges of the New Zealand calla industry, and the use of calcium to control soft rot. Proceedings of: International symposium on development of bulbous flower industry, Taichung, Taiwan, R.O.C. pp. 36-44. Taiwan Seed Improvement and Propagation Station.

Gardan, L., C. Gouy, R. Christen and R. Samson. 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. International Journal Of Systematic And Evolutionary Microbiology 2: 381-391.

GenStat. 2002. GenStat for Windows Release 6.1. VSN International Ltd., Oxford. pp.

Hoffland, E., G.J. Niemann, J.A. v. Pelt, J.B.M. Pureveen, G.B. Eijkel, J.J. Boon and H.L.

Lambers. 1996. Relative growth rate correlates negatively with pathogen resistance in radish: the role of plant chemistry. Plant, Cell and Environment 19 (11): 1281-1290.
Kuehny, J.S. 2000. Calla history and culture. HortTechnology 10 (2): 267-274.
Pagel, W. and R. Heitefuss. 1989. Calcium content and cell wall polygalacturonans in potato tubers of cultivars with different susceptibilities to *Erwinia carotovora* subsp. *atroseptica*. Physiological and Molecular Plant Pathology 35 (1): 11-21.
Pérombelon, M.C.M. and A. Kelman. 1980. Ecology of the soft rot Erwinias. Annual Reviews of Phytopathology 18: 361-387.
Pérombelon, M.C.M. 2002. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathology 51 (1): 1-12.
Snijder, R.C., H.-R. Cho, M.M.W.B. Hendriks, P. Lindhout and J.M. van Tuyl. 2003. Genetic variation in *Zantedeschia* spp. (*Araceae*) for resistance to soft rot caused by *Erwinia carotovora* ssp. *carotovora*. Euphytica (in press).
Snijder, R.C. 2004. Genetics of *Erwinia* resistance in *Zantedeschia*: impact of plastome-genome incompatibility. PhD thesis. Wageningen University, Wageningen. pp. 120.
Sticher, L., B. Mauch Mani and J.P. Metraux. 1997. Systemic acquired resistance. Annual Review of Phytopathology 35: 235-270.
Wright, P.J. 1998. A soft rot of calla (*Zantedeschia* spp.) caused by *Erwinia carotovora* subspecies *carotovora*. New Zealand Journal of Crop and Horticultural Science 26 (4): 331-334.
Wright, P.J. and G.K. Burge. 2000. Irrigation, sawdust mulch, and Enhance® biocide affects soft rot incidence, and flower and tuber production of calla. New Zealand Journal of Crop and Horticultural Science 28 (3): 225-231.
Wright, P.J., G.K. Burge and C.M. Triggs. 2002. Effects of cessation of irrigation and time of lifting of tubers on bacterial soft rot of calla (*Zantedeschia* spp.) tubers. New Zealand Journal of Crop and Horticultural Science 30: 265-272.
Yao, J.L. and D. Cohen. 2000. Multiple gene control of plastome-genome incompatibility and plastid DNA inheritance in interspecific hybrids of *Zantedeschia*. Theoretical and applied genetics 101 (3): 400-406.

Tables

Table 1. Number of hybrids that were obtained after crossing six cultivars (‘Black Magic’, ‘Galaxy’, ‘Pink Persuasion’, ‘Sensation’, ‘Treasure’ and ‘Florex Gold’) in all directions.

	Father					
Mother	‘BM’	‘FG’	‘Gal’	‘PP’	‘Sen’	‘Trea’
‘Black Magic’	6	1		4	4	5
‘Florex Gold’	18	11	10	13	22	19
‘Sensation’		4		4	1	6
‘Treasure’		47	31	13	39	41

Table 2. Levels of resistance in index of macerated leaf disk area (M) for the hybrid families.

	Father					
Mother	'BM'	'FG'	'Gal'	'PP'	'Sen'	'Trea'
'Black Magic'	106cd	119 cd		89 bc		104 cd
'Florex Gold'	82 b	117 d	86 b	89 c	107 cd	107 cd
'Sensation'		111 cd				97 cd
'Treasure'		98 c	91 c	89 bc	93 c	102 cd
Florex Gold	100 c					
Z. rehmannii	44 a					

Table 3. Vigour and level of resistance (index of macerated leaf disk area (M)) of half-sib families of fathers 'Florex Gold' and 'Pink Persuasion'.

Father	N	M	Tuber (gr)	Height (cm)	Leaf number	Chlorophyll (SPAD)
'Florex Gold'	63	108 b	8.8 b	43 b	10 b	52 b
'Pink Persuasion'	36	92 a	4.3 a	26 a	6 a	35 a

Figures

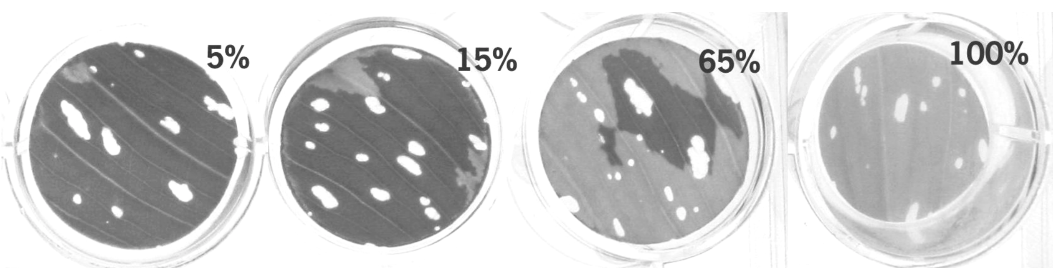

Fig. 1. Leaf disk test as described by Snijder et al. (2003). The area of maceration was visually estimated.