Genetic Basis of Resistance to Systemic Infection by *Xanthomonas axonopodis* pv. *dieffenbachiae* in Anthurium

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ABSTRACT

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The genetic basis of systemic resistance to bacterial blight disease (blight) of anthurium (*Anthurium andraeanum*) caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* was investigated in progenies of 53 crosses involving 31 parent cultivars using segregation analysis. Inoculation of parents and progenies was achieved by injecting the petiole base of the most recent fully expanded leaf with 100 μ l of 10^9 colony forming units per ml of the blight pathogen (strain X4gfp) transformed with the green fluorescent protein (GFP) gene. The time to death and the presence or absence of GFP fluorescence on newly emerging leaves was monitored over a period of 30 weeks after inoculation (WAI), on an individual plant basis. The expected resistance to susceptible ratios based on a digenic model involving two dominant genes, designated A and B, interacting

according to a duplicate recessive epistasis model fitted the observed segregation ratios in the crosses. Based on the segregation ratios obtained, the parental cultivars were assigned plausible genotypes. There were significant differences ($P < 0.001$) in time to death following inoculation between the various genotypic designations. Cultivars with genotypes AABB, AABb, AaBB, and AaBb died within 10 WAI and designated as susceptible; AAbb and aaBB died from 18.8 to 25.6 WAI and were designated as moderately resistant; and Aabb, aaBb, and aabb produced resistant phenotypes. There was also some evidence for dosage effect especially in the highly resistant category. Hence, (AABb = AaBB = AaBb) < (aaBB = AAbb) < Aabb = aaBb = aabb. An approach to fixing resistance to blight in anthurium is discussed.

Additional keywords: interallelic interaction, nonadditive effects, oligogenic inheritance.

The bacterial blight disease (blight) of *Anthurium andraeanum* (Hort.) (18) caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (31), previously known as *X. campestris* pv. *dieffenbachiae* (4,24) was primarily responsible for the rapid decline in anthurium production in Hawaii during the 1980s (13,17, 21,25) and the Caribbean during the late 1980s (10,34) and 1990s (8).

The disease has two phases, a foliar and a systemic phase (11). In the foliar phase the pathogen invades the foliage of the plant via hydathodes (29). In susceptible cultivars, the foliar infections can rapidly progress into a systemic phase resulting in plant death. Anthurium cultivars show differential susceptibility to the foliar and systemic phases of blight (11). The symptoms are characterized by water-soaked leaf spots with a dark center surrounded by a yellow halo (26); leaf chlorosis (25); yellowing of older leaves and petioles causing the infected leaves and flowers to break off easily revealing dark brown streaks at their bases (26); and the eventual collapse of the plant. Infections may also advance without visual symptoms (27) until the infected plant collapses and dies (11).

Long-term management of blight requires an integrated approach involving genetic resistance (19,20) along with other supplemental disease management tactics including pathogen exclusion, cultural practices and sanitation (6). The development of anthurium cultivars resistant to blight can reduce the level of capital and management costs associated with the cultural control of the disease, and improve profitability of anthurium cultivation

in the Caribbean (2) and in other anthurium producing countries where the disease has been reported (1).

A recent review of the status of the disease in Hawaii (1) reported that natural genetic resistance to blight is not present in *A. andraeanum* (Linden) ex André. A number of screening approaches were developed in the 1990s in Hawaii (11,12,13) and resistance to blight was successfully transferred from *A. antioquiense* to *A. andraeanum*. The efficiency of such conventional breeding efforts could however, be further improved through a clear understanding of the mechanism of resistance to blight and its genetic basis. One attempt at nonconventional breeding using bioengineering (22) increased tolerance to blight but did not confer resistance.

Recently, a rapid green fluorescent protein (GFP) based screening method for the identification of systemic resistance to blight in anthurium was developed (8). This method has led to the identification of numerous sources of systemic resistance to blight in *A. andraeanum* (Hort.). This method also lends itself for the screening of large segregating populations on an individual plant basis and is hence amenable to genetic studies.

In this study, we describe the genetic basis of systemic resistance to blight in *A. andraeanum* (Hort.).

MATERIALS AND METHODS

Anthurium cultivars. Thirty-one *A. andraeanum* (Hort.) cultivars, 12 belonging to the resistant, four belonging to the moderately resistant and 15 belonging to the susceptible categories (8), at the systemic level, were used in the study. The cultivars originated from a variety of sources including the Netherlands (Anthura, Avo, Florist, Fla, Theo, and Voguls farms), Hawaii, and the Caribbean. These cultivars were planted and maintained in a

shade house (75% shade saran; overlaid with clear plastic) on beds of coconut husk at a commercial anthurium farm, Kairi Blooms Ltd., located in Arima, Trinidad.

Hybridization. Fifty-three biparental crosses were generated involving the various categories of resistance/susceptibility according to established methods (14), during the period January to April 2000. All pollinations were conducted in shade houses between 0600 to 1000 h. Each cross was made at least seven times to generate adequate seeds per cross.

Germination and aftercare. After 6 to 7 months, the ripe (yellow) berries each containing one to two green seeds, were collected and squeezed gently to remove the seeds. The seeds were planted in labeled (name, sowing date) seedling trays containing peat moss (Premier Horticulture Ltd., Quebec, G5R6C1, Canada) in a nursery house covered with plastic and saran (75% shade) and equipped with a mist irrigation and fertigation system. The nursery was misted daily for 10 s at 15-min intervals between 0900 and 1600 h. Six months after germination, the anthurium seedlings were transplanted into labeled 8 cm² (8 cm deep) pots containing peat moss. After one year, seedlings were transplanted into labeled 10.5 cm² (12.5-cm deep) pots containing peat moss and allowed to grow for another year before screening. The plants were fertilized (1.5 g) on a monthly basis with either triple super phosphate (Keisurit, Germany) or 12-11-13 NPK (El Cosechero, Venezuela). The pH of the growing medium was maintained at 5.5 by continuous monitoring and applying calcium carbonate (Limestone, TRINCARB, Port-of-Spain, Trinidad), as necessary.

Fig. 1. Frequency distribution of time to death of progenies of **A**, susceptible × susceptible *Anthurium andraeanum* (Hort.) crosses, and **B**, susceptible × resistant crosses screened for resistance to bacterial blight disease caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*.

The nematicide, Temik (Aldicarb, Shandong, China), was applied once to prevent nematode infestation.

Inoculation. Disease screening was carried out in a screen house, which was located well away from the breeding and nursery houses and drained separately. The segregating progenies (2.5 years of age) were individually labeled and transferred to the screen house, and were screened for blight resistance in two batches. Each batch was arranged randomly on concrete benches, regardless of the cross, along with the controls (10 plants each of the parental anthurium cvs. Lydia, Rosa, Cuba, Success, and Midori).

After allowing 6 weeks to acclimatize to the new environment, the plants were inoculated, individually using the optimized method (8). The method involved injecting 100 µl of 10⁹ colony forming units (CFU) per ml of inoculum (strain X4gfp) into the 2.5-cm-long cut petiole stub of the second youngest leaf, and covering the cut surface of the stub overnight, using a 1,000-µl pipette tip sealed at the end. The first batch was screened during the period April to November 2003, and the second batch during the period June 2003 to February 2004.

The inoculated plants were watered three times a day with a hose to minimize splashing. The environmental conditions that prevailed during the screening period were monitored using a data logger (Hobo, England).

Data collection. Time taken for each individual plant to collapse/die was measured once a week over a period of 30 weeks after inoculation (WAI). Representative samples of petioles and leaves of each plant were harvested and evaluated for the presence of green fluorescence. The dead plants were removed once a week from the screen house in large garbage bags and burnt. All plants that were symptomless at 30 WAI were tested for the presence of green fluorescence by sampling the petiole bases of leaves. The progeny data were divided into two categories; (a) susceptible, those that died between 0 to 12 WAI, and (b) resistant, those that show delayed symptoms/GFP fluorescence or remained free of symptoms/GFP fluorescence (Leica fluorescence microscope, BM IRB model, Germany) at the end of the study period (8).

Data analysis. Segregation analysis was carried out by fitting genetic ratios to the observed ratios of resistance/susceptible progenies for the various crosses. The goodness of fit of the genetic ratios to observed ratios were tested using the chi-square test with Yates' correction. Parental cultivars were assigned genotypes based on the genetic hypothesis and confirmed using other crosses involving the same parents.

Crosses with the same assigned genotype were pooled and a pooled chi-square test ($P \leq 0.05$) was performed using Yates'

Fig. 2. Mean weighted time to death (weeks) in response to inoculation by *Xanthomonas axonopodis* pv. *dieffenbachiae*, as influenced by the genotypic designation for the duplicate recessive bacterial blight resistant genes, A and B for 31 *Anthurium andraeanum* (Hort.) cultivars.

correction to determine the significance of fit between the observed ratios and expected ratios. The genotypic designations for the 31 parents with respect to the duplicate recessive blight resistant genes, A and B, determined in this study, were compared to their resistance status (weighted time to death) reported in a previous study (8). A general linear model analysis of variance (ANOVA) (NCSS, 2001, Kaysville, UT) was performed to determine whether there were significant differences between cultivar genotype designations based on weighted time to death. For this purpose, anthurium plants that did not die were given a time to death (dummy value) of 30 weeks.

RESULTS

Environmental conditions of the screen house. The environmental conditions that prevailed during the screening of progenies over the two batches were very similar. The average relative humidity was 83%; the average temperature was 27.7°C, and the mean daily light intensity was 57 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the study period. The comparable results obtained with the control clones over the two inoculation batches suggested that the inoculum potential over the two inoculation trials was similar (data not shown).

Progeny frequency distributions. The progeny frequency distributions for time to symptom development/death were bimodal for all crosses, with some variation around the two modes. Progeny frequency distributions for susceptible $\times$ susceptible crosses and susceptible $\times$ resistant crosses are shown in Figures 1 and 2. Both categories of crosses produced susceptible and resistant cohorts, but at different proportions. All the plants that remained alive at the end of the experimental period were consistently free of GFP fluorescence. Those that remained free of symptoms at the end of the study period had not shown symptoms or died even 2 years after the end of this study.

Segregation analysis. Monogenic models were too simplistic and were not able to explain the segregation ratios in resistant $\times$ susceptible data. Further, the appearance of blight resistant genotypes in susceptible $\times$ susceptible crosses (Fig. 1A) and susceptible genotypes in resistant $\times$ resistant crosses (Fig. 1B) suggested the possible influence of modifying factors such as dominance and epistasis in the inheritance.

In some susceptible $\times$ susceptible crosses, the dihybrid ratio, 9:7 susceptible:resistant (S:R) was observed, suggesting duplicate recessive epistasis. The two genes were denoted, 'A' and 'B'. In the model, 'A' is dominant to 'a', 'B' is dominant to 'b', 'aa' is epistatic to 'B-' and 'bb' is epistatic to 'A-'. The presence of

genotype 'aa' or 'bb', either singly or in combination, produced moderately resistant to resistant phenotypes.

Based on the model, susceptible cultivars were assigned genotypes AABB, AaBB, AABb, and AaBb, which is expected to yield S:R ratios of 9:7, 3:1, or 1:0 in crosses between susceptible cultivars. Fifteen of the sixteen susceptible $\times$ susceptible crosses fitted one of the above three ratios based on χ^2 tests (Table 1). Putative genotypes were assigned to the susceptible cultivars and were tested in all crosses of these cultivars with others. Based on designation of genotypes, the moderately resistant cultivars (showing delayed symptoms or death) were assigned AABb or aaBB, except Honduras which was assigned Aabb. The resistant ones were assigned Aabb, aaBb, or aabb, except Kalapana which was assigned AABb.

Genotypes assigned to the 31 parent anthurium cultivars from the 53 crosses based on segregation ratios (S:R) supported by chi-square tests with Yate's correction are shown in Tables 1, 2, 3, and 4. Among the resistant $\times$ susceptible crosses, 18 out of the 21 crosses fitted (χ^2 test; $P > 0.05$) the expected S:R ratios based on the digenic duplicate recessive epistatic model (Table 2). All 11 of the moderately resistant $\times$ susceptible crosses fitted the expected S:R ratio (χ^2 test; $P > 0.05$) based on the same genetic model (Table 3), therefore confirming the genotype designations. All of the five resistant $\times$ resistant crosses closely fitted the expected ratios based on the assigned genotypes and were supported by χ^2 tests (Table 4). Overall, even in the cases where the χ^2 values did not quite support the S:R ratio, the ratios presented were the most plausible based on a digenic model; all the other possible ratios produced much higher χ^2 values.

When crosses with the same genotypes were grouped and the chi-square test performed (Table 5), the fit was as good as or better than for single crosses. These together confirmed the digenic model with duplicate recessive epistasis.

Magnitude of dosage effect of recessive alleles. An ANOVA showed that there were significant differences ($P < 0.001$) between the genotypic designations (blight resistant genes A and B) of the 31 parents for weighted time to death. Tukey-Kramer multiple comparison test placed the genotypes in one of three categories. The genotypes designated as AABb, AaBB, and AaBb (susceptible cultivars) were not significantly different from each other; those designated as aaBB and AABb (moderately resistant cultivars) were not significantly different from each other; and those designated as Aabb, aaBb, and aabb (resistant cultivars) were not significantly different from each other. None of the susceptible genotypes were designated AABB.

TABLE 1. Goodness of fit tests (chi-squared test) for expected ratios (and plausible genotypes) based on the digenic duplicate recessive epistatic model versus observed ratios of resistance:susceptible cohorts of progenies of 16 susceptible $\times$ susceptible crosses of *Anthurium andraeanum* (Hort.) screened for resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae*

Female	Genotype	Male	Genotype	No. of plants	Ratios		χ^2	Probability
					Observed S:R ^a	Fitted S:R		
Alexis	AABb	Cumbia	AaBb	107	74:33	3:1	1.648	>0.10
Champagne	AaBb	Hawaii	AABb	68	49:19	3:1	0.176	>0.50
Champagne	AaBb	Lydia	AaBB	29	20:9	3:1	0.288	>0.50
Champagne	AaBb	Pierrot	AaBb	122	76:46	9:7	1.575	>0.20
Champagne	AaBb	Gloria	AABb	30	25:5	3:1	0.711	>0.30
Fantasia	AABb	Hawaii	AABb	49	31:18	3:1	3.000	>0.05
Gloria	AABb	Pierrot	AaBb	126	88:38	3:1	1.524	>0.20
Laguna	AaBb	LMR ^b	AaBb	36	13:23 (?)	9:7	5.143	<0.05
Linda	AaBB	Midori	AaBb	64	53:11	3:1	1.688	>0.10
Pierrot	AaBb	Cuba	AaBB	36	25:11	3:1	0.333	>0.50
Pierrot	AaBb	Midori	AaBb	35	16:19	9:7	1.182	>0.25
Rosa	AaBb	LMR	AaBb	24	13:11	9:7	0.000	1.00
Rosa	AaBb	Lydia	AaBB	20	15:5	3:1	0.000	1.00
Champagne	AaBb	Victoria	AaBb	60	32:28	9:7	0.106	>0.70
Laguna	AaBb	Victoria	AaBb	45	29:16	9:7	0.919	>0.30
Pierrot	AaBb	Victoria	AaBb	26	13:13	9:7	0.199	>0.50

^a Susceptible:resistant to bacterial blight disease.

^b Local Mina Red.

DISCUSSION

It can be noticed from Figure 2 that cultivars with the genotype designations, AaBB (three cultivars), AABb (four cultivars), and AaBb (eight cultivars) all died within 10 WAI (susceptible category). The weighted time to death for the single cultivar designated as 'aaBB' was 18.8 weeks, whereas those for cultivars designated as AaBb (three cultivars) ranged from 18.8 weeks to 25.6 weeks with the exception of Kalapana, which was 28.4 weeks. Cultivars with at least three recessive alleles fell into the highly resistant category. Cultivars designated as aaBb (five cultivars) or aabb (two cultivars) did not become infected at the end of the experiment or two years thereafter. In the highly resistant category of genotypes that had a genotypic designation 'Aabb' (five cultivars), only a few plants became infected systemically, and even those showed a much delayed decline. These results show that a digenic model with duplicate recessive epistasis (with dosage effects) can explain resistance to systemic infection by *X. axonopodis* pv. *dieffenbachiae* in anthurium.

Anthurium cultivars have been shown to display differential susceptibility to foliar and systemic phases of bacterial blight infection (11). Resistance to both phases of infection will be required to reduce the damaging effects of the pathogen. In this study, we focused mainly on host resistance at the systemic phase of the infection.

Understanding the genetic basis of resistance to blight in anthurium is important both for selecting parents for breeding and for designing an efficient breeding programme. This study, using 3,444 progenies from 53 crosses involving 31 parental cultivars, showed that resistance to blight in anthurium is determined by a digenic model with dominance (in the direction of susceptibility) and duplicate recessive epistasis. If the two genes are labeled 'A' and 'B', then either gene in the recessive form will impart moderate resistance/resistance to blight. By fitting ratios for the progenies

TABLE 2. Goodness of fit tests (chi-squared test) for expected ratios (and plausible genotypes) based on the digenic duplicate recessive epistatic model versus observed ratios of resistance:susceptible cohorts of progenies of 21 susceptible × resistant crosses of *Anthurium andraeanum* (Hort.) screened for resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae*

Female	Genotype	Male	Genotype	No. of plants	Ratios		χ^2	Probability
					Observed S:R ^a	Fitted S:R		
Acropolis (r) ^b	Aabb	Gloria	AABb	59	23:36	1:1	2.440	>0.10
Acropolis (r)	Aabb	Hawaii	AABb	113	47:66	1:1	2.868	>0.05
Acropolis (r)	Aabb	Victoria	AaBb	104	40:64	3:5	0.010	>0.90
Bianco (r)	aaBb	Pierrot	AaBb	40	14:26	3:5	0.027	>0.80
Kalapana (r)	AABb	Hawaii	AABb	31	19:12	1:1	1.162	>0.25
Kalapana (r)	AABb	Loc M Red ^c	AaBb	30	15:15	1:1	0.000	1.00
LMW ^d (r)	aaBb	Cuba	AaBB	96	58:38	1:1	3.760	>0.05
Alexis	AABb	Loc Or ^e (r)	aaBb	94	70:24	3:1	0.000	1.00
Pierrot	AaBb	Loc Or (r)	aaBb	73	24:49	3:5	0.485	>0.30
Cuba	AaBB	Local Pink (r)	aaBb	52	22:30	1:1	0.942	>0.30
Laguna	AaBb	Local Pink (r)	aaBb	124	52:72	3:5	0.861	>0.30
Lydia	AaBB	Local Pink (r)	aaBb	52	20:32	1:1	2.326	>0.10
Rosa	AaBb	Local Pink (r)	aaBb	28	14:14 (?)	3:5	1.371	>0.20
Mirjam (r)	Aabb	Gloria	AABb	65	25:40	1:1	3.015	>0.05
Champagne	AaBb	Venus (r)	Aabb	95	22:73	3:5	7.743	<0.05
Fantasia	AABb	Venus (r)	Aabb	51	25:26	1:1	0.000	1.00
Gloria	AABb	Venus (r)	Aabb	71	51:20	1:1	12.676	<0.05
Midori	AaBb	Venus (r)	Aabb	32	9:23	3:5	0.834	>0.30
Pierrot	AaBb	Venus (r)	Aabb	60	27:33	3:5	1.138	>0.25
Midori	AaBb	Sweetie (r)	aabb	53	8:45	1:3	2.271	>0.10
Senator (r)	Aabb	Hawaii	AABb	135	54:81	1:1	5.007	<0.05

^a Susceptible:resistant to bacterial blight disease.

^b Resistant to bacterial blight disease.

^c Local Mina Red.

^d Local Mina White.

^e Local Orange.

TABLE 3. Goodness of fit tests (chi-squared test) for expected ratios (and plausible genotypes) based on the digenic duplicate recessive epistatic model versus observed ratios of resistance:susceptible cohorts of progenies of 11 susceptible × moderately resistant crosses of *Anthurium andraeanum* (Hort.) screened for resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae*

Female	Genotype	Male	Genotype	No. of plants	Ratios		Probability	χ^2
					Observed S:R ^a	Fitted S:R		
Alexis	AABb	Sonate (mr)	AABb	28	13:15	1:1	0.036	>0.80
Champagne	AaBb	Honduras (mr)	Aabb	176	63:113	3:5	0.152	>0.50
Champagne	AaBb	Sonate (mr)	AABb	37	16:21	1:1	0.432	>0.50
Laguna	AaBb	Sonate (mr)	AABb	62	25:37	1:1	1.952	>0.10
Champagne	AaBb	Success (mr)	aaBB	23	11:12	1:1	0.000	1.00
Cuba	AaBB	Success (mr)	aaBB	50	30:20	1:1	1.620	>0.20
Gloria	AABb	Honduras (mr)	Aabb	66	39:27	1:1	1.834	>0.10
Honduras (mr) ^b	Aabb	Loc M Red ^c	AaBb	92	36:56	3:5	0.046	>0.80
Honduras (mr)	Aabb	Cuba	AaBB	39	27:12	3:1	0.419	>0.50
Honduras (mr)	Aabb	Victoria	AaBb	45	18:27	3:5	0.037	>0.80
Loc Wh Pink ^d (mr)	AABb	Pierrot	AaBb	135	76:59	1:1	1.896	>0.10

^a Susceptible:resistant to bacterial blight disease.

^b Moderately resistant to bacterial blight disease.

^c Local Mina Red.

^d Local whitish Pink.

of the crosses, it was possible to assign genotypes for all the parents. All possible genotypes were represented within the parental population used in crosses, except 'AABB'. It is possible that cultivars with the genotype 'AABB' were extremely susceptible to blight and may not have survived in the farm.

Similarly, oligogenic inheritance has been reported for resistance to a number of diseases caused by pathovars of *X. campestris*, which are related to *X. axonopodis* pathovars. For instance, resistance to *X. campestris* pv. *campestris* in cabbage was reported to be controlled by one dominant gene (15,16,32), one or more dominant genes (3), or a recessive gene, influenced by one recessive and one dominant modifying gene (7,33). Similarly, resistance to *X. campestris* pv. *oryzae* in rice has been shown to be determined by two dominant genes (5,30), or by one dominant gene as observed in some cultivars (9,28). Resistance to bacterial leaf pustule in soybean caused by the closely related *X. axonopodis* pv. *glycines* was found to be determined by two genes interacting in a duplicate recessive epistasis manner (23).

When the genotypic designation of the parents (blight resistant genes, A and B) were compared with their level of resistance and susceptibility previously reported (8), there was clear evidence suggesting a dosage-effect of recessive resistant genes. For instance, the level of resistance (indicated by the weighted length of time taken to death) in genotypes with at least three recessive alleles (mean 29.3 WAI) was higher and significantly different from those with two recessive alleles at the same locus (mean 22.9 WAI), and those with four recessive alleles did not die at the end of the experiment or thereafter, nor did they show evidence of GFP fluorescence at the end of the experiment. Even in those genotypes with three recessive alleles GFP fluorescence and plant death was seen in only a few of the plants inoculated (8). It is possible that the systemic symptoms may have been masked in genotypes with three recessive alleles and one dominant allele. Although not significant, even within the susceptible category, genotypes with two recessive alleles across two loci (AaBb) had a mean time to death of 9 WAI whereas those with one recessive

allele died on average in 7.2 WAI. The dosage effect therefore, could at least partially account for the continuous distributions around the two modes observed in the progeny frequency distributions (Figs. 1 and 2). It can be postulated that (AABb = AaBB = AaBb) < (aaBB = AAbb) < (Aabb = aaBb = aabb).

There was some evidence suggesting a role for minor modifiers in the inheritance of systemic resistance to blight. For instance, cvs. Kalapana and Honduras showed levels of resistance to blight (weighted time to death) (8) that were higher and lower, respectively, than what was expected based on their genotype designations. These suggest that there may be other minor modifier genes that may augment the level of systemic resistance. Modifier genes have been reported to affect resistance to black rot, caused by *X. campestris* pv. *campestris*, in cabbages (7,33).

A recent review of bacterial blight disease in anthurium contended that natural resistance does not exist in *A. andraeanum* (Linden) ex André (1). This study supports a previous study that resistance to bacterial blight disease exists in *A. andraeanum* (Hort.) (8) and further describes the genetics of resistance for the first time. In fact, several of the parental cultivars were resistant to blight, and 'Sweety' and 'Ibara' were highly resistant with a possible genotype of 'aabb'. The progenies identified as resistant in this study have remained disease-free for 2 years following the end of the study in a commercial anthurium farm which further shows the validity of this approach.

Based on the results, screening should be continued up to at least 30 weeks through basal petiole sampling, so that genotypes with high levels of resistance can be discerned. Using this approach, one is able to eliminate genotypes with four, three, or two susceptible (dominant) alleles. Genotypes with three recessive alleles could not be separated clearly from those with four alleles, since escapes are possible. Those with one susceptible dominant allele can however be deciphered by performing progeny tests with cultivars with the genotypic designation 'AABb' (Fantasia, Hawaii, and Gloria) or 'AaBB' (Cuba and Linda). The study therefore provides a means of fixing resistance to blight in anthurium.

TABLE 4. Goodness of fit tests (chi-squared test) for expected ratios (and plausible genotypes) based on the digenic duplicate recessive epistatic model versus observed ratios of resistance:susceptible cohorts of progenies of five resistant × resistant crosses of *Anthurium andraeanum* (Hort.) screened for resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae*

Female	Genotype	Male	Genotype	No. of plants	Ratios		χ^2	Probability
					Observed S:R ^a	Fitted S:R		
Mirjam	Aabb	Ibara	Aabb; aabb	50	2:48	0:1		
Mirjam	Aabb	Kalapana	AABb	60	2:58	0:1		
Mirjam	Aabb	Local						
Orange	aaBb			42	5:37	1:3	3.175	>0.05
Mirjam	Aabb	Tropical	aaBb	26	7:19	1:3	0.000	1.00
Local								
Orange	aaBb	Venus	Aabb	71	14:57	1:3	0.793	>0.30

^a Susceptible:resistant to bacterial blight disease.

TABLE 5. Goodness of fit tests (chi-squared test) for expected ratios (over pooled genotypes) based on the digenic duplicate recessive epistatic model versus observed ratios of resistance:susceptible cohorts of *Anthurium andraeanum* (Hort.) crosses with the same genotypes when screened for resistance to *Xanthomonas axonopodis* pv. *dieffenbachiae*

Genotype 1	Genotype 2	No. of crosses	Total number of plants	Ratios		χ^2	Probability
				Observed S:R ^a	Fitted S:R		
AABb (s)	AaBb (s)	4	331	236:95	3:1	2.224	>0.10
AABb (s)	Aabb (r)	7	560	264:296	1:1	1.716	>0.10
AABb (s)	AABb (t)	2	59	32:27	1:1	0.271	>0.50
AaBb (s)	AaBb (s)	7	348	192:156	9:7	0.123	>0.70
AaBb (s)	AaBB (s)	4	149	113:36	3:1	0.020	>0.80
AaBb (s)	Aabb (r)	7	604	212:392	3:5	0.855	>0.30
AaBb (s)	aaBb (r)	4	265	104:161	3:5	0.274	>0.50
AaBb (s)	AABb (t)	4	264	132:132	1:1	0.000	1.00
AaBB (s)	aaBb (r)	3	200	100:100	1:1	0.000	1.00
Aabb (r)	aaBb (r)	3	139	26:113	1:3	2.612	>0.10

^a Susceptible:resistant to bacterial blight disease.

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