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Genetic Analysis of Components of Resistance and Quantitative Trait Loci Mapping of Philippine Downy Mildew Resistance Gene in Maize (*Zea mays* L.)

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ABSTRACT

Downy mildew caused by *Peronosclerospora philippinensis* (Weston) Shaw is one of the most persistent diseases encountered by maize producers in the Philippines. Improved durable resistant host plant could provide an effective way of both increasing and stabilizing corn production in affected areas. An extensive research to study the genetics of downy mildew resistance in maize against the UPLB isolate pathogen using generation mean analysis and to locate QTL conferring resistance against *P. philippinensis* causing Philippine downy mildew were done. The genetics of resistance to *P. philippinensis* was studied in progenies derived from crosses between the resistant inbred P 345 and Nei 9008 and susceptible inbred Pi 17 and Pi 23. Plant generations used in this study were the P_S (susceptible lines); P_R (resistant lines); F_1, F_2, F_S, B_S and B_R. Analysis of generation means indicates that additive-dominance gene effect plays an important role in all components of resistance in all crosses involving Nei 9008. On the other hand, additive-dominance with epistatic gene effect plays an important role for all components of resistance involving P 345. QTL conferring resistance against *P. philippinensis* was observed in a BC_1F_2 population developed from a cross between Pi 23 and P 345. QTL analysis of the different components of resistance showed that a total of twenty-seven regions distributed in nine chromosomes of the maize genome were associated in varying degrees with resistance to *P. philippinensis*. Four regions located in chromosomes 1, 5, 6 and 8 were associated with disease incidence and disease severity. Results suggest that it may be possible to incorporate QTLs of various components of resistance into the elite inbred line Pi 23 using marker aided selection.

Key words: Maize, Philippine downy mildew, resistance, quantitative trait loci

INTRODUCTION

Downy mildew caused by *Peronosclerospora philippinensis* (Weston) Shaw is one of the most persistent diseases encountered by maize producers in the Philippines. Improved more durable resistant host plant could provide an effective way of both increasing and stabilizing corn production in affected areas.

The efficient breeding strategy requires the knowledge on the genetic basis of inheritance of resistance. The inheritance of resistance to *P. philippinensis* is mostly investigated using disease incidence as component of resistance. The studies showed results from simple dominance

(Mochizuki *et al.*, 1974; Kaneko and Aday, 1980) to polygenic inheritance (Gomez *et al.*, 1963; Handoo *et al.*, 1970; Rifin, 1983). Moreover, the mode of polygenic inheritance of resistance gene is further complicated by non-allelic interaction on resistance expression.

Quantitative resistance to Philippine downy mildew pathogen could assist to develop more durable resistance. Components of resistance that reduce the rate of epidemic development of the pathogen have been identified (Ebron and Raymundo, 1987; Ruswandi *et al.*, 2002). These components of resistance are: Disease incidence, disease severity, onset of systemic symptom and the area-under-disease-progress-curve. However, the genetics of these components of resistance is not fully understood.

Development of resistance to *P. philippinensis* entails a great effort by plant breeders and plant pathologists. For one, the resistance is governed by additive genes with dominant and epistatic effects. Selection for this type of resistance will take a longer time to complete. Another constraint is that selection should be done under severe epiphytotic conditions.

Molecular markers linked to Quantitative Trait Loci (QTL) associated with downy mildew resistance traits could allow selection for resistance to different pathogens, i.e., populations in a single location even in the absence of the pathogen. These marker systems are generated in the laboratory so that the confounding effects of environmental variation associated with conventional breeding methods are also eliminated.

The use of molecular markers allows one to proceed with a QTL analysis. QTLs for different diseases in maize have been identified, including QTL for resistance against northern maize leaf blight (Freymark *et al.*, 1993), anthracnose stalk rot (Jung *et al.*, 1994), smut (Lubberstedt *et al.*, 1999), Wheat Streak Mosaic Virus (WSMV) of maize (Marcon *et al.*, 1999) and stewarts wilt, northern corn leaf blight and common rust of sweet corn (Brown *et al.*, 2001).

The objectives of this study were to study the genetics of downy mildew resistance in maize against the UPLB isolate pathogen using generation mean analysis and to locate QTL conferring resistance against *P. philippinensis* causing Philippine downy mildew.

MATERIALS AND METHODS

Genetic analysis of components of resistance against Philippine downy mildew pathogen

Plant materials: Two susceptible line were crossed with two resistant line to generate four F_1 combinations i.e., Pi 17×Nei 9008, Pi 23×Nei 9008, Pi 17×P 345 and Pi 23×P 345. Each F_1 was backcrossed to the susceptible parent (P_S) to generate the B_S population and to the resistant parent (P_R) to generate the B_R populations. Twenty F_1 plants from each cross were self pollinated to produce F_2 populations. Two hundred F_2 plants were used to generate the F_3 populations. Seeds harvested from the different F_2 plants were bulked to represent the F_3 population.

Experimental design: The experiment was laid out in a randomized complete block design with four replications. Forty-eight plants of each of the parental and F_1 populations and a total of ninety-six plants of the backcross (B_S and B_R) populations were evaluated. In addition, a total of 144 individual plants were evaluated from each of the F_2 and F_3 generations.

Screening procedure: Screening for resistance was conducted at the University of the Philippines at Los Banos experimental field. Inoculation was done at the two to three leaf stages. The inoculum

was collected and prepared at the Institute of Plant Breeding (IPB), UPLB following the procedure developed by Barredo and Exconde (1973). One milliliter of conidial suspension was deposited into the whorl of the seedlings after the preparation of the conidial suspension of *P. philippinensis*. To maintain a favorable condition for inoculation, the field experiment was watered using sprinkle irrigation a day before inoculation. Sprinkle irrigation was regularly made until 28 Days after Emergence (DAE) and was afterwards followed by furrow irrigation until 50 DAE.

Resistance evaluation and disease scoring: The response of maize to Philippine downy mildew infection was determined weekly based on five components of resistance-disease incidence, disease severity, onset of systemic symptom, Area under Disease Progress Curve (AUDPC) and rate of downy mildew development (Baria and Raymundo, 1988; Capio, 1990; Ruswandi *et al*., 2002). Disease incidence was assessed based on the number of plants showing systemic symptoms on some leaves times 100 divided by the total number of plants. This parameter was taken at 45 DAE. Disease severity was assessed from the percent of systemic infection of the whole plant on individual plant basis and it was taken at 45 DAE. Onset of systemic symptom was determined based on the number of days from inoculation to the appearance of the symptom. The AUDPC was calculated based on the following equation used by Ebron and Raymundo (1987). Rate of downy mildew development (r) was determined based on the disease severity and was calculated using equation of Van der Plank (1963).

Generation mean analysis: Mean and variance of the seven basic generations were used to estimate genetic effects, genetic variances and environmental components of resistance to Philippine downy mildew by the parametric model proposed by Mather and Jnks (1977).

The joint scaling test as described by Mather and Jnks (1977) was applied. This model was applied when the joint scaling test showed significant deviation from zero. The model included the estimates of non-allelic interactions:

$$[i] = \text{Sum additive} \times \text{additive effects}$$

$$[j] = \text{Sum additive} \times \text{dominance effects}$$

and:

$$[l] = \text{Dominance} \times \text{dominance effects}$$

The genetic effects (m, [d], [h], [i], [j] and [l]) were calculated using weighted linear regression analysis following the procedure of Mather and Jnks (1977). Sequential analysis was applied to find the appropriate model that could explain the genetics of Philippine downy mildew resistance.

The signs of [h] and [j] or [l] were used to determine the type of non-allelic interaction (Gamble, 1962; Mather and Jnks, 1977). If the signs of the two parameters are the same, then the non-allelic interaction is of the complementary type. If the signs are opposite, the interaction is of the duplicate type.

Quantitative trait loci mapping of philippine downy mildew resistance gene in maize (*Zea mays* L.)

Plant materials: Backcross families derived by backcrossing (Pi 23×P 345) F_1 to Pi 23, were used as mapping population in marker analysis and QTL mapping of downy mildew resistance genes. One hundred and forty two BC_1F_1 lines were used for genotyping using the SSR marker system. BC_1F_2 families derived by selfing from the same BC_1F_1 population were then used for phenotyping for downy mildew resistance.

Phenotyping: BC_1F_2 families together with their parental lines Pi 23 and P 345, one resistant check line Nei 9008 and two susceptible check lines Pi 17 and sweet corn, were evaluated for resistance to *P. philippinensis* using artificial inoculation. The experiment was laid out using a 16×16 alpha lattice design with two replications.

Screening for reaction was done at the UPLB-CA experimental station along Pili Drive. Inoculation was done at the two to three leaf stages. The details of inoculation resource and procedure, resistance evaluation and disease scoring were the same as those presented earlier.

SSR analysis: Genomic DNA was isolated from the parental lines Pi 23 and P 345, their F_1 and individual BC_1F_1 plants. DNA isolation was used following the technique described by Hoisington *et al.* (1997). A total of 50 polymorphic primers were used in the study. The primers used were 20 mer oligo-nucleotides from commercially available primers such as nc, dup, bngl and phi (Research Genetics Co.). The polymerase chain reactions were performed in 10 μL total volume containing 1×PCR buffer, 1 U Taq polymerase and 20 ng of genomic DNA. Amplification was carried out in an MJ Research PTC-100 Programmable Thermocycler with the following profile: One cycle of 1 min 93°C initial denaturation, 30 cycles of 1 min 93°C denaturation, 2 min 56/58/60°C annealing and 2 min 72°C extension, followed by 1 cycle of 5 min 72°C for final extension.

Amplification products were resolved on 3% MS4 agarose gels in 1×TBE at 70 V for 3-4 h and were visualized under UV light after staining with ethidium bromide. Amplification products which could not be resolved in agarose, were analyzed using 5% polyacrylamide gel in 1×TBE running at 70-75 W for 45 min to 1 h. The gel was silver stained as described by the manufacturer (Balatero, 2000).

Each genotype was scored for the presence of amplification product as M (homozygous) or H (heterozygous). Segregation of each marker was checked for deviation from expected Mendelian ratio in a BC_1F_1 population (1:1) by standard χ^2 test. The chi-square test was computed using the Q stat software that is integrated with QTL Cartographer software (Wang *et al.*, 2001).

Fifty polymorphic SSR markers were added to a previously constructed framework map consisting of 66 RFLP and RGA markers (Hautea *et al.*, 2001), using the software package MAPMAKER for PC Version 3.0 (Lander *et al.*, 1987). A LOD threshold of 3.50 and a maximum recombination frequency of 0.40 were used to confirm the significance of linkage between 2 markers. Genetic map distance between markers was estimated by recombination frequencies and transformed into cM (centi Morgan) using the Kosambi mapping function.

QTL mapping for downy mildew resistance: QTL analysis was based on genetic map constructed previously by Hautea *et al.* (2001) and Ruswandi *et al.* (2001) spanning 1822 cM. The

average marker density for the QTL analysis was 18.22 with at least 2 marker loci per chromosome. Phenotypic data for QTL analysis obtained from 142 BC_1F_2 lines. Prior to analysis, data on onset of systemic symptom (days after inoculation or DAI), AUDPC, disease severity and disease incidence was transformed to square root, log and arcsine, respectively. QTL analysis was performed with the computer package the Win QTL Cartographer software (Wang *et al*., 2001) based on Composite Interval Mapping (CIM) multitraits analysis applying the additive model. For QTL detection, a LOD threshold of 2.5 was applied corresponding to an alpha level of 0.0032 for the 100 marker interval. The QTL position was determined when the LOD score reached its maximum. The occurrence of pleiotropic effect was determined using joint analysis with a LOD threshold of 4.0.

RESULTS AND DISCUSSION

Genetic analysis of components of resistance against philippine downy mildew pathogen

Nei 9008: The data for onset of systemic symptom, AUDPC and disease incidence fit the simple AD model for both cross combinations Pi 23×Nei 9008 and Pi 17×Nei 9008 (Table 1). The inheritance pattern of resistance gene in Nei 9008 is different from that of the resistance gene in P 345.

Table 1: Estimates of genetic effect resistance to *Peronosclerospora philippinensis* in cross combinations of Pi 17×Nei 9008 and Pi 23×Nei 9008

	Susceptible×resistant crosses	
Genetic effect	Pi 17×Nei 9008	Pi 23×Nei 9008
Disease incidence (%)		
Simple additive dominance model		
M	2.96±0.45**	2.91±0.27**
(d)	1.83±0.45**	1.49±0.26**
(h)	2.70±0.98**	0,76±0,42ns
df	4	4
χ^2	3.62	7.44
Adjusted R^2	92.4	94.2
Disease severity (%)		
Simple additive dominance model		
M	2.90±0.45**	2.86±0.27**
[d]	1.82±0.45**	1.49±0.27**
[h]	2,69±0,99**	0.78±0.41ns
df	4	4
χ^2	3.86	7.24
Adjusted R^2	91.6	95.8
Onset (days after inoculation)		
Simple additive dominance model		
M	3.84±0.37**	3.67±0.42**
[d]	1.15±0.37**	0.85±0.42**
[h]	0.31±0.75 ns	0.22±0.15ns
df	4	4
χ^2	9.20	3.03
Adjusted R^2	77.8	99.3

Table 1: Continue

Genetic effect	Susceptible×resistant crosses	
	Pi 17×Nei 9008	Pi 23×Nei 9008
Area Under Disease Progress Curve (AUDPC)		
Simple additive dominance model		
M	1.77±0.10**	1.55±0.14**
[d]	0.62±0.10**	0.60±0.13**
[h]	0.43±0.23 ns	0.21±0.20ns
df	4	4
χ^2	5.58	6.96
Adjusted R^2	92.2	92.2
Rate of downy mildew development (unit day^{-1})		
Simple additive dominance model		
M	0.0224±0.0165**	-0.0036±0.1104**
[d]	0.0386±0.0520**	0.0492±0.0220**
[h]	0.1581±0.0400**	0.2130±0.0790**
df	4	4
χ^2	0.76	0.39
Adjusted R^2	90.4	93.9

Critical value for Chi-square 4 df = 9.49, 3 df = 7.82, 2 df = 5.99 and 1 df = 3.84; *,**Significant at p = 0.55 and 0.01, respectively

In Nei 9008, simple Additive Dominance (AD) model can explain the inheritance of resistance as indicated by the small χ^2 values (Table 1). Sequential regression analysis also indicated that a simple AD genetic model could likely explain the inheritance of Philippine downy mildew resistance in this resistant source regardless of the susceptible parent used.

P 345C4S2B46-2-2-1-2-B-B-B: For the cross combination between resistant line P 345 and susceptible line Pi 23, the data for all parameters of resistance failed to fit the simple additive-dominance model (m, [d], thus [h]), indicating the presence of epistasis (Table 2). When the model was extended to include digenic epistasis, still the χ^2 values were all significant indicating that higher order epistasis interactions are involved. This may indicate that either trigenic epistasis or linkage or both are important (Hayman, 1958). The sequential regression analysis also indicated that none of the genetic models involving digenic epistasis was sufficient to explain the genetics of Philippine downy mildew resistance in this particular cross.

On the other hand, when the resistant line P 345 was crossed to Pi 17, digenic epistasis models could explain the data (Table 2). This indicates that digenic epistasis is involved in the genetics of resistance against *P. philippinensis*. In addition, sequential regression analysis also indicated that the genetic models involving digenic epistasis was pertinent in explaining the genetics of Philippine downy mildew resistance in this particular cross. For all cross combination involving resistant line P 345, additive genetic effects are more significant than dominance genetic effects for all components of quantitative resistance for all cross combinations. This is an indication that higher order interactions could involve at least two genes interacting in additive manner. Comparison of the signs of dominance effect [h] to additive×dominance gene effect [j] in these crosses suggested the presence of duplicate type of epistasis for all components of resistance except for AUDPC for the cross between Pi 17 and P 345 where complementary type of epistasis may be at work.

The additive×additive [I] gene effect was more important than other non-allelic interactions for all components of resistance, except for disease incidence in Pi 17×P 345 and onset of systemic symptom in Pi 23×P 345. In the first case, Pi 17×P 345, additive×additive gene effect was not

Table 2: Estimates of genetic effect resistance to *Peronosclerospora philippinensis* in cross combinations of Pi 17×P 345 and Pi 23×P 345

	Susceptible×resistant crosses	
Genetic effect	Pi 17×P 345	Pi 23×P 345
Disease incidence (%)		
Additive dominance model with epistasis		
M	3.49±0.065**	13.69±6.00**
[d]	1.30±0.065**	13.92±3.00**
[h]	1.43±0.300**	7.17±8.70
[i]	Not fit in the model	12.30±6.00**
[j]	-0.34±0.290	-14.54±9.50
[l]	-3.36±0.310**	Not fit in the model
df	1	1
χ^2	2.14	46.30**
Adjusted R^2	99.4	91.6
Disease severity (%)		
Additive dominance model with epistasis		
M	3.42±0.124**	24.49±3.45**
[d]	1.30±0.124**	13.39±3.47**
[h]	1.45±0.532**	-21.87±14.27
[j]	-0.27±0.310	-11.97±11.48
[l]	-3.36±0.740**	-18.25±9.35**
df	1	2
χ^2	9.13	177.50**
Adjusted R^2	99.7	88.1
Onset (days after inoculation)		
Additive dominance model with epistasis		
M	1.3 5±0.28**	4.89±0.50**
[d]	-1.2 3±0.17**	-1.22±0.38**
[h]	2.92±0.52**	-1.02±1.33
[i]	2.57±0.26**	-0.97±0.60
[j]	-0.58±0.36	0.12±1.90
df	1	1
χ^2	1.75	57.22**
Adjusted R^2	97.8	82.9
Area Under Disease Progress Curve (AUDPC)		
Additive dominance model with epistasis		
M	2.84±0.31**	1.12±0.17**
[d]	0.57±0.18**	0.38±0.04**
[h]	-1.39±0.59**	0.64±0.51
[i]	-1.02±0.29**	0.64±0.18**
[j]	-0.15±0.42	-0.12±0.87
df	1	1
χ^2	1.16	41.29**
Adjusted R^2	98..9	94.7

Table 2: Continue

Genetic effect	Susceptible×resistant crosses	
	Pi 17×P 345	Pi 23×P 345
Rate of downy mildew development (unit day^{-1})		
Additive dominance model with epistasis		
M	0.0224±0.0165**	-0.0036±0.1104**
[d]	0.0386±0.0520**	0.0492±0.0220**
[h]	0.1581±0.0400**	0.2130±0.0790**
[i]	0.0515±0.0180**	0.0811±0.0060**
[l]	-0.1588±0.0381**	-0.1944±0.0736**
df	1	2
χ^2	37.55	29.55
Adjusted R^2	98.8	94.1

Critical value for Chi-square 4 df = 9.49, 3 df = 7.82, 2 df = 5.99 and 1 df = 3.84; *,**Significant at p = 0.55 and 0.01, respectively

included in the digenic epistasis model since any digenic epistasis which included [I] gene effect would cause inadequacy of the application of the digenic epistasis model. In the second case, onset of systemic symptom in the cross Pi 23×P 345, additive×additive gene effect was not significant.

In general, resistance to *P. philippinensis* which involved digenic or trigenic non-allelic interaction, was exhibited in crosses involving the resistant line P 345. Digenic epistasis is implicated since the 6-parameter epistatic model could explain the expression of resistance to *P. philippinensis* for all parameters of resistance in the cross combination Pi 17×P 345. On the other hand, in the cross Pi 23×P 345, higher order interactions involving at least three genes are implicated since the 6-parameter epistatic model was not adequate to explain all the genetic variation.

The genetics of resistance to Philippine downy mildew pathogen showed that digenic additive-dominance model alone or together with epistasis gave an acceptable fit to the expression of resistance. These may be due to the different genetic background of parental sources, the pathogen populations used that were likely to be highly variable and the experiments that may have employed uneven disease pressures.

In the presence of epistasis, estimates of additive and dominance effects might become unbiased. It is because the distribution of positive and negative gene effects in the parents may result in different degrees of cancellation of gene effects in the expression of the means (Thompson *et al*., 1963). Linkage among genes that confer resistance to downy mildew is possible. Linkage may cause serious bias in the estimates and when epistasis is present, bias due to linkage relations would exist in the estimates of gene effects, especially in the additive×additive [i] and dominance×dominance [l] effects. Trigenic or higher order epistasis could cause the appearance of linkage (Gamble, 1962).

Quantitative trait loci mapping of Philippine downy mildew resistance gene in maize (*Zea mays* L.): Composite Interval Mapping (CIM) indicated several QTLs controlling resistance to Philippine downy mildew pathogen (Table 3, Fig. 1). The result is in agreement with the first study using the classical Generation Mean Analysis (GMA). The results of the GMA showed that the inheritance of resistance gene to *P. philippinensis* in maize is complex involving significant non-allelic interactions that include at least two interacting genes.

Table 3: Chromosomal locations and mode of gene action of QTL for resistance of corn to *Peronosclerospora philippinensis*

Component of resistant	Chromosome	Flanking (nearest marker)	QTL position (cM)	LOD	Additive effect	Direction
Disease incidence	1	umc76	123.0	3.0	-11.95	Pi 23
		npi209	146.0	3.0	-8.96	Pi 23
	5	dupSSR20	108.0	3.0	8.39	P 345
	6	umc65-umc59	112.0	5.8	20.63	P 345
	8	umc150-asg52	67.0	8.9	28.44	P 345
Disease severity	1	umc76	123.0	146.0	-11.21	Pi 23
		npi209	3.3	2.7	-8.72	Pi 23
	4	umc164	89.0	2.7	-8.70	Pi 23
	5	dupSSR20	108.0	3.5	9.89	P 345
	6	umc65-umc59	112.0	6.5	19.96	P 345
	7	(No marker)	71.0	7.0	-23.28	Pi 23
		Rga16	141.0	3.1	10.86	P 345
	8	umc150-asg52	67.0	9.2	27.40	P 345
		rga17-2	191.0	3.3	9.34	P 345
Onset	3	Phi47	243.0	6.3	-8.60	P 345
	4	rga17-1-bng1421	35.0	3.6	-5.51	Pi 23
		umc66	82.0	5.2	8.51	P 345
	5	umc68	212.0	3.4	-8.29	Pi 23
		umc104	228.0	4.2	-8.29	Pi 23
	7	rga16	141.0	4.7	-9.34	Pi 23
	8	asg52	75.0	8.2	-18.01	Pi 23
		asg52-umc89	78.0	7.3	-17.85	Pi 23
	9	phi32	28.0	3.8	-7.14	Pi 23
AUDPC	2	Csu54	121.0	3.0	-9.26	Pi 23
	3	umc17	204.0	3.5	12.32	P 345
	4	umc104	89.0	2.8	7.07	Pi 23
		bngl589	212.0	6.0	-16.90	Pi 23
	8	umc150	61.0	3.6	10.99	P 345
		rga17-2	192.0	3.0	7.00	P 345
Rate(r) of development	1	npi209	146.0	3.0	-0.01	Pi 23
	2	bngl166-Csu-4	104.0	3.9	0.04	P 345
	4	umc104	90.0	2.8	-0.02	Pi 23
	6	umc159	174.0	2.8	-0.02	Pi 23
		bnl5.21	83.0	3.0	-0.02	Pi 23
	8	rgal-l-umc150	51.0	12.7	0.05	P 345
		umc150-asg52	65.0	10.3	0.50	P 345
	9	umc95-bnl8.17	6.0	7.2	0.40	P 345
		umc81	48.0	3.0	-0.01	Pi 23

QTL mapping for resistance to *P. philippinensis* identified four QTLs for reducing disease incidence, seven QTLs for reducing disease severity, nine QTLs for prolonging onset of systemic symptom, six QTLs for reducing AUDPC and seven QTL for reducing rate of downy mildew development. Similar result was reported by Asian Maize Biotechnology Network (AMBIONET) which mapped four QTLs region associated with disease incidence (George *et al*., 2004). QTL mapping for resistance to different pathogens showed that the number of QTLs varied from two to more than ten. It is common to find only three to five loci (Young, 1996). Lubberstedt *et al*. (1998) identified twenty QTLs conferring partial resistance to common rust of corn distributed over all ten

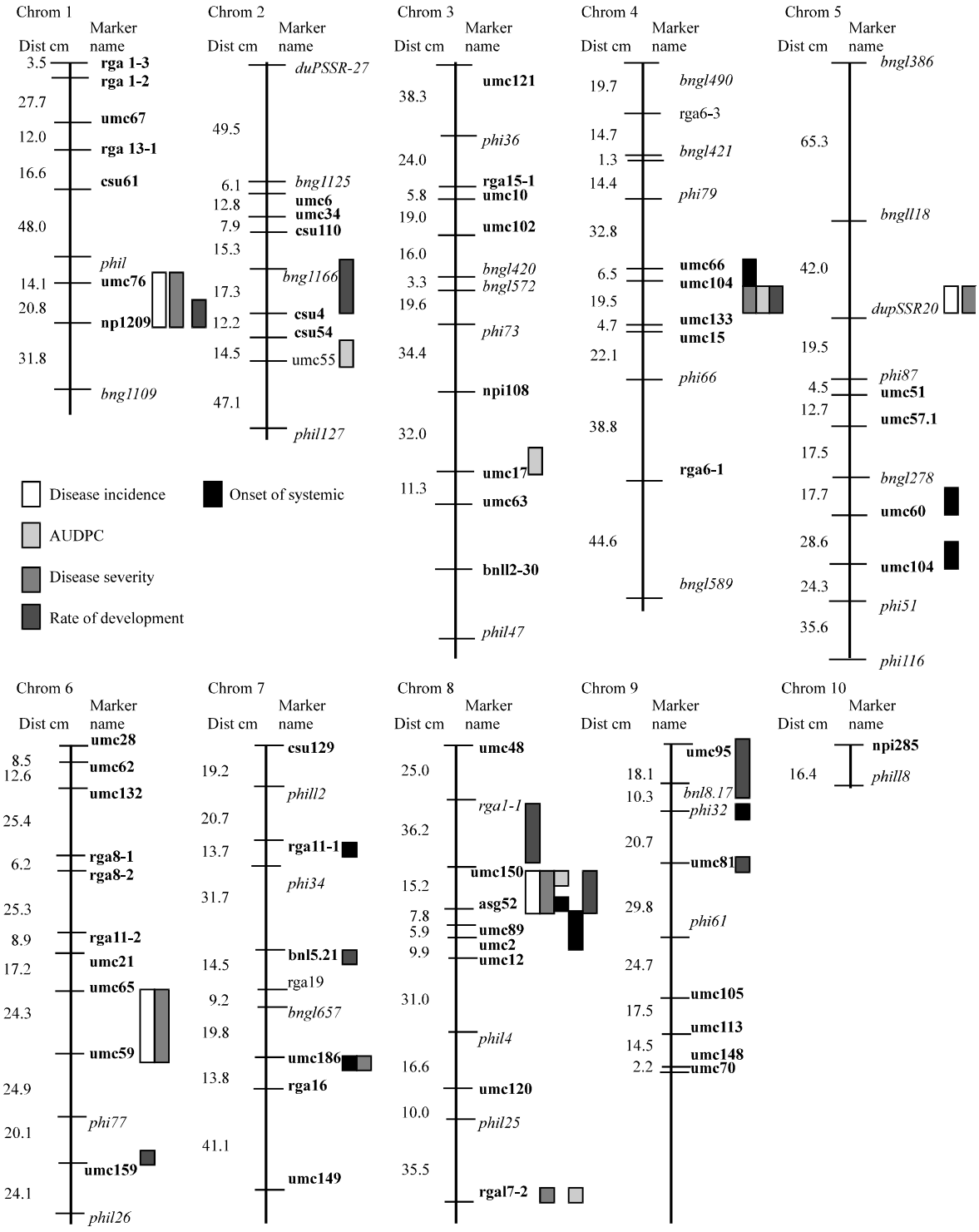

Fig. 1: Map positions of QTLs for downy mildew resistance traits. Segregation analysis was performed on a backcross mapping population derived from Pi 23×P 345. Markers of each linkage group are ordered with a LOD score of 3.3. The molecular linkage map consisting of RFLP and RGA markers (bold font) were previously constructed (Hautea). SSR markers (italic) were integrated into the RFLP/RGA map

Table 4: Joint QTL mapping for % incidence, disease severity, onset of systemic symptom and test of pleiotropy AUDPC and rate of downy mildew development (r) for QTL effects

Chromosome	Flanking (nearest marker)	Component of resistant	QTL position (cm)	LOD for pleiotropy
1	umc76	Incidence-disease severity (%)	123	4.0
5	dupSSR20	Incidence-disease severity (%)	107	4.4
6	umc 65-umc59	Incidence-disease severity (%)	112	7.3
7	rga16	Onset-disease severity	141	6.3
8	umc150	Incidence-disease severity AUDPC-rate of development (%)	61	8.4
8	umc150-asg52	Incidence-disease severity AUDPC-rate of development (%)	66	11.8
8	umc150-asg52	Incidence-disease severity onset (%)	73	9.1
8	umc150-asg52	Onset-rate of development	72	9.8

Test was significant at the respective threshold: Threshold of LR-test used for testing pleiotropy LR = 13.83, corresponding to LOD = 3.0

chromosomes. Young (1996) explained that cases of few QTL could be due to an artefact of small population sizes, inappropriate choice of the cut-off for declaring a QTL or inadequate disease scaling methods. On the other hand, when numerous QTL were detected it could be due to a cut-off that was too lenient (p value too high), particularly when single-marker analysis was applied.

In the present study, pleiotropic effects were observed in chromosomes 1, 5, 6, 7 and 8. The identification of pleiotropy of QTLs for reducing disease incidence, reducing severity, prolonging onset of systemic symptom, reducing AUDPC and reducing rate of downy mildew development are presented in Table 4.

Among pleiotropic effects are detected in five chromosomes, the most importance pleiotropy effects is fond in cchromosome 8. This pleiotropic effect is detected at 66 cM and is flanked to RFLP marker umc150 and asg52 (LOD 11.8). This region controls the expression of disease incidence, disease severity and rate of downy mildew development. P 345 contributes to the reduction of the expression of these traits. The genomic region flanked to RFLP marker umc150 and asg52 (LOD 9.1) at 73 cM influenced disease incidence and disease severity and onset of systemic symptom with P 345 contributing to reduce values for each trait.

For the past fifty years, maize lines resistant against Philippine downy mildew pathogen on corn can be efficiently identified with conventional phenotypic selection indices since artificial inoculation and visual rating of disease incidence are fairly rapid and highly reproducible. However, this method had disadvantages because of (1) The complex, quantitative inheritance of partial resistance to Philippine downy mildew as measured by the components of quantitative resistance, (2) The strong influence of the environment on the expression of quantitative resistance to Philippine downy mildew, (3) The high costs for resistance evaluation, particularly when component of resistance are used as a selection indices for resistance and (4) Other excellent genes like those for high yield may be lost during selection under severe infection. Use of marker-assisted selection can help to resolve all these problems.

QTLs for five components of resistance have been detected in this study. Results suggested that introgression these QTL in the cross Pi 23×P 345 are possible facilitating with Marker Aided Selection (MAS). MAS could be used to incorporate all resistance genes, particularly the QTL with minor effects, into one or both maize inbreds to ascertain resistance of the resulting hybrid. However, a large population size are needed since the presence of desired alleles are low, example the frequency of an individual homozygous for the desired alleles at five unlinked loci in a F_2

populations I $(0.25)^2$. In addition, highly efficient marker system such as multiplexed SSRs would be required in a breeding program particularly if SSR marker associated to partial resistance to Philippine downy mildew, such as phi47, phi32 and dupSSR20 are applied. Since plant death is mainly caused by severe infection at seedling stage, protections against *P. philippinensis* is required especially at early stages of plant development. Consequently, QTLs for delaying onset of systemic symptom in chromosome 3,7, 8 and 9 should be considered to be used for MAS. On the other hand, QTLs in chromosomes 1, 5, 6, 8 and 9 would be useful for suppression of infection caused by *P. philippinensis* at later stages of plant development since it can reduce the disease incidence, disease severity, AUDPC and rate of downy mildew development.

CONCLUSION

Many genes with important additive effect controlled the resistance against *P. philippinensis* in cross combination with P 345. The additive genetic effect was always significantly greater than the dominance genetic effects in P 345-derived resistance. Non-allelic interaction also plays an important role, however, the significance of additive×additive, additive×dominance and dominance×dominance interactions varied depending on the genetic background of the susceptible parent.

Quantitative Trait Loci (QTL) mapping using molecular markers indicated a significant QTLs on chromosomes 1, 5, 6 and 8; chromosomes 1, 4, 5, 6, 7 and 8; chromosomes 3, 4, 5, 7, 8 and 9; chromosomes 2, 3, 4 and 8 and chromosomes 1, 2, 4, 6, 7, 8 and 9 for disease incidence, onset of systemic symptom, disease severity, Area Under Disease Progress Curve (AUDPC) and rate of downy mildew development, respectively.

Composite interval mapping could detect five pleiotropic regions on chromosomes 1, 5 and 6 (for disease incidence and disease severity), 7 (for onset of systemic symptom and disease severity) and 8 (for onset of systemic symptom, disease incidence, disease severity, AUDPC and rate of development). The results on QTL analysis using molecular markers agree with the first study using classical Generation Mean Analysis (GMA) of the cross combination Pi 23×P 345.

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