

QTL Mapping linked to downy mildew resistance genes in maize (*Zea mays*)

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Abstract. Ashan MD, Miftahudin, Reflinur, Pabendon MB, Santoso SB, Salim A. 2020. *QTL Mapping linked to downy mildew resistance genes in maize (Zea mays)*. *Biodiversitas* 21: 3735-3743. Downy Mildew (DM) caused by *Peronosclerospora* spp. is one of the most destructive diseases and problems in yield losses of maize production worldwide. Identification of molecular marker-linked to the DM resistance genes is considered as an important step in the improvement of the DM resistance nature in maize breeding program. The objective of the study was to identify molecular markers linked to the DM resistance genes in maize. A total of 198 F₃ maize lines generated from a cross between R10-4430 and Kandora was used in microsatellite genetic map construction and the corresponding F_{3:4} families were used in phenotypic evaluation to identify quantitative trait loci (QTLs) responsible for DM resistance-related trait. The entire genetic linkage map constructed with 28 SSR markers resulted in 460.3 cM with an average distance between markers of 26.85 cM. Seven main-effect QTLs controlling the DM resistance genes were identified in the entire genome map of F₃ population with the phenotypic variation explained (PVE) values ranged from 43.35 to 53.71%. Among detected QTLs, three QTLs, *qDM-Pp1a*, *qDM-Pp1b1*, and *qDM-Pp1b2* were detected on chromosome 1, one QTL, *qDM-Pp5* was on chromosome 5, two QTLs, *qDM-Pp6b1* and *qDM-Pp6b2* were on chromosome 6 and one QTL, *qDM-Pp10* was on chromosome 10. The effect of detected QTLs responsible for DM resistance trait ranged from 43.35 to 53.71% and those will be potential to be further used as molecular aided selection in maize breeding for DM resistance.

Keywords: Disease resistance, downy mildew, maize, molecular markers

INTRODUCTION

Maize is one of the important carbohydrate-producing crops in the world, besides rice and wheat. Central American, South American, African, and Asian countries generally make maize as their staple food, especially Africa and some regions in Indonesia. Globally, maize production continues to rise along with increasing fulfillment of future maize needs. In Indonesian, during 2014 to 2018 maize production increased annually of which at 19,008,426 ton, 19,612,435 ton, 23,578,413 ton, 28,924,015 ton, and 30,253,938 ton, respectively (FAO 2020). However, Indonesian maize productivity is still relatively low. It is reported that in 2019, maize productivity in Indonesia was 5.33 tons/ha (FAO, 2020) and this productivity is lower in comparison with world's maize productivity. Some of the factors causing the low productivity of maize in Indonesia are the limited amount of maize germplasm, changing environmental conditions in each region, and the markers linked to less specific resistance genes, especially the downy mildew disease (DM). Downy Mildew is caused by the fungus *Peronosclerospora* which is one of the most important diseases in maize and can cause serious damage in crops throughout the world, especially in tropical Asia

(Rashid et al. 2018; Kim et al. 2020). Daryono et al. (2018) reported that the level of this pathogen attack reached ± 90% in South of Sulawesi. As one of the maize production centers in Asia, therefore, Indonesia needs to be aware of such a disease. Generally, the spread of DM in Indonesia is caused by two species, such as *Peronosclerospora maydis* (Pm) which is widely distributed in Java and *Peronosclerospora philippinensis* (Pp) found in South Sulawesi (Muis et al. 2015). Some efforts to control this disease, such as regulating the period of land-free plantations, environmental sanitation and improvement of cropping patterns have not shown a significant suppression of maize yield loss. The use of resistant varieties is a very strategic approach in plant in controlling DM disease because it is more effective and relative safety for the environment. Advances in biotechnology, especially through the application of molecular markers as a selection tool for lines carrying the desired character can accelerate the plant breeding cycle with high accuracy (Gedil & Menkir 2019).

The availability of molecular markers linked to DM disease resistance trait in maize is expected to shorten the time required to fix breeding lines which could not be resolved by conventional breeding. In addition, the main

obstacle faced in the conventional selection process is the difficulty in choosing the right genes that are targeted for selection to be expressed in their morphological or agronomic traits (Ashkani et al. 2015). Therefore, the availability of molecular markers associated with desired traits would be benefit to overcome such a problem. The identification of quantitative trait loci (QTLs) responsible for the DM resistance genes in maize is still very limited. Several QTL mapping studies responsible for to the DM resistance in maize have been reported (Agrama et al. 1999; Azrai et al. 2015; Roberdi et al. 2010; Jampatong et al. 2013; Lohithaswa et al. 2015). Initial research conducted by Agrama et al. (1999) using F₂ families and RFLP markers of 109 pairs of markers representing 10 chromosomes of corn was able to explain the construction of the genetic map along 1648 cM. Three QTLs were detected as significant QTL on DM, two of them were mapped very close on chromosome 1, while another one was on chromosome 9. The percentage of phenotypic variations explained (PVE) that could be explained against the degree of resistance to DM was 53.6%. The identification of QTL activity was also carried out by Jampatong et al. (2013) using 760 pairs of SSR markers spread throughout the maize genome. Among these markers, as many as 195 pairs of markers are polymorphic. Based on the linkage analysis of these markers, nine QTLs were linked to downy mildew resistance gene, one QTL each on chromosomes 2, 3, 4 and 6, three QTL on chromosome 5 and two QTL on chromosome 9. The closest markers determine the location of the QTL mentioned above are umc1736 (chromosome 2), bngg1902 (chromosome 5), bng1867 (chromosome 6). Ruswandi et al. (2015) identified the locus of DM disease resistance on chromosome 6 with a 112 cM position using the BC₁F₂ population derived from a cross between P345 (resistant parent) and P23 (susceptible parent) which tested against the DM disease in the Philippines. Meanwhile, Jampatong et al. (2013) mention that a Simple Sequence Repeats (SSR) marker at chromosome 1, 2, 6, 7, and 10 related to the DM resistance using mapping population derived from Ki3 x CML139 crosses. Population differences (origin of parents) used in each genetic mapping activity usually produce molecular markers linked to desired traits with a specific population and those do not necessarily work if the markers are applied in populations with different genetic backgrounds.

In present study, identification of molecular markers associate with the DM resistance genes was carried out on a set of mapping populations generated from the maize germplasm collected in Indonesian Center Cereals Research Institute, Maros, South Sulawesi, Indonesia. The objective of this study was to identify QTL for the DM resistance gene in maize. It is expected that molecular markers associated with DM resistance characters derived from Indonesian maize population can be utilized in future maize breeding programs.

MATERIALS AND METHODS

Genetic materials

Genetic materials used in this study consisted of two sets of maize population (F₃ and F_{3:4}) consist of 198 F₃ maize lines generated from a cross between R10-4430 (susceptible parent) and Kandora (resistant parent). The F₃ and F_{3:4} populations, each along with their parental parents were used in genotypic and phenotypic analysis, respectively. to construct genetic map and the corresponding F_{3:4} families subjected to the phenotypic evaluation. The female parent, R10-4430 was susceptible to DM with good agronomic performance, while the male as donor resistant to the DM. A set of maize SSR markers deposited on maize database (maizegdb.org) was applied on genotyping such population including two parental lines.

The research work was conducted in the Molecular Biology Laboratory of Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRAD), Bogor, West Java, and in the research station of Indonesian Cereals Research Institute (ICERI) in Bonto Bili, Gowa, South Sulawesi, Indonesia.

Segregation analysis of downy mildew in maize population F_{3:4}

A total of 198 individual F_{3:4} maize population including parental lines (R10-4430 and Kandora) were screened phenotypically for the DM resistance in the research station of Indonesian Cereals Research Institute (ICERI) in Bonto Bili, Gowa, South Sulawesi as one of the most endemic areas of the DM disease. The experiment was arranged in a randomized block design with three replications. Each individual F_{3:4} plant of maize was planted in standard 4-m-long rows with 30 plants per row and plant to plant spacing of 70 cm x 20 cm. However, 5 out of 30 plants for each line were then removed at days after planting (DAP) from a row, so that only 25 plants were maintained for further observation. Susceptible maize genotypes (Anoman) were sown as spreader rows three weeks before planting entries or the test materials. Observation of the individual F_{3:4} lines against the downy mildew disease was carried out for 5 times, including vegetative and generative phases of plants (14 DAP to 42 DAP).

Analysis of leaf damage level

Phenotypic analysis was carried out to observe the morphological expression of maize plants resistant to DM disease, especially that in the leaves. Each maize line was observed and the level of plant damage caused by the DM disease was compared with checked plants including both resistant and susceptible parents. Disease incidence was calculated according to the following formula recommended by Rashid et al. (2013):

$$\text{Percentage of the DM incidence} = (\text{Number of plants infected per bed} / \text{Total number of plants per bed}) * 100$$

DNA isolation

DNA isolation of F₃ plant populations including the two parents was carried out based on DNA extraction method as described by Diyasree et al. (2014). Genomic DNA was isolated from healthy and young maize leaves (ages 21 to 28 DAP). Commonly, this method consisted of four main steps, such as destruction of maize leaf cell wall with cetyltrimethylammonium bromide (CTAB) extraction buffer, separation of solvent and dissolved solutions by centrifugation, DNA precipitation using isopropanol, Na acetate solution followed by incubation step at -20 °C, and washing of DNA pellets with ethanol of 70 %. The quality and quantity of DNA were measured by both a nanodrop spectrophotometer (Thermo Scientific 2000) and agarose gel electrophoresis 1 %.

Polymorphic marker survey

A polymorphic marker survey was carried out on the two parents using a set of SSR markers spread across the chromosomes (275 markers) from maize genome database (maizegdb.org). Polymerase Chain Reaction (PCR) reaction was carried out in a total reaction volume of 12.5 µL. Composition of the PCR reaction consisted of 10 ng/µL maize DNA, ddH₂O, 10X buffer PCR, 0.125 mM dNTPs, 0.5 µM primer, and 1 unit of Taq DNA polymerase enzyme. PCR amplification was carried out as many as 35 cycles with annealing temperature of each marker that previously optimized. Amplification was performed in a 96-well GeneAmp with the temperatures and times used for PCR were as follows: In the first cycle, the mixtures were pre-denatured at 95°C for 5 min, and followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at each temperature optimization (°C) for primer annealing, and primer extension at 72°C for 30 seconds. The final extension time for PCR amplification was 7 min at 72°C was programmed to allow for the completion of primer extension on a BioRad T-100 system (CA, USA). PCR products were separated using an 8% non denaturing polyacrylamide gel electrophoresis. A total of 3 µL of PCR product mixed with loading dye was injected into the well of the polyacrylamide gel and then electrophoretically run in TBE 1X buffer at 80 Volts for 120 minutes. Gel was stained in an ethidium bromide solution and visualized using UV light through a Chemidoc (Biorad) machine. SSR markers that provided different sizes in both parents were recorded as polymorphic markers.

Segregation analysis of molecular markers in F₃ population

Banding patterns of F₃ lines amplified by polymorphic SSR markers were scored as “A” if the allele size same with Kandora and “B” if it is same as R10-4430 allele types. While, if the lines showed both parent alleles type were scored as “H” or heterozygous allele. The scoring data was then arranged in an input file (using Microsoft Excel) which subjected as the genotypic data in the analysis of the association between molecular markers and the DM resistance trait.

Linkage map construction and association analysis of molecular markers with DM resistance trait

Construction of a linkage map between SSR markers was conducted using Mapmaker program version 3.0 with the like-hood of odds (LOD) value of 3 (Cui et al. 2016). A maximum distance was set up to 50 centiMorgan (cM) as a threshold to express the linkage among DNA markers and Kosambi function was used to convert recombinant values into genetic distances between these markers (Huehn 2011; Zhang et al. 2019). Linkage analysis between phenotypic and genotypic data was carried out by Composite Interval Mapping (CIM) method which implemented in the Windows QTLs Cartographer program version 2.5 (Wang et al. 2012). CIM analysis was used to perform joint analysis of data across environments to map QTLs and estimate their genetic effects (Wang et al. 2019). A series of two models were used in the analysis. Model 6, which is a standard model for CIM was used as control parameter, walk speed was set up to 2.0 cM, LOD value more than 3.0, and threshold value was set by 1000 permutation (significant level of 0.05) (Cui et al. 2016). The additive effect and phenotypic variation explained (PVE) of QTLs from environment were obtained under the assumptions of Model 3. Multiple regression analysis was performed to estimate the total proportion of phenotypic variation due to the additive effects (R²) (Wang et al. 2012).

RESULTS AND DISCUSSION

Downy mildew disease evaluation

Appearance of DM disease resistance to maize was analyzed through leaf morphology changes at 14 to 42 HST (Figures 1.A-B). The frequency of DM disease was performed and grouped into five criteria: highly resistant (HR), resistant (R), moderate (M), susceptible (S), highly susceptible (HS) (Kim et al. 2016). Distribution of the 198 F₃ populations against the DM disease varied greatly, of which a total 20, 62, 87, 28, and 1 maize lines were highly resistant, resistant, moderately, susceptible, and highly susceptible, respectively against the disease (Figure 1.C). Of them, a total of 62 plants (31.3%) were attacked in the same range with the checked resistance varieties (disease incidence value of > 0 % to 10 %), while 1 line (0.5%) was attacked in the same range with checked susceptible varieties (disease incidence value of > 50%), and the rest were in the same range with the second check varieties (tend to resistant). The average line value of DM disease resistance populations showed significant genotypic variability.

Polymorphic SSR markers survey and F₃ lines segregation patterns

Based on the polymorphic survey of SSR markers genotyped on the two parents, of the total 275 SSR marker, 108 SSR markers were identified as polymorphic markers. Figure 2 presented seven polymorphic primers produced in this study. The different band patterns between the two parents with F₃ lines indicated that the markers used are polymorphic in selecting that lines during genotypic analysis.

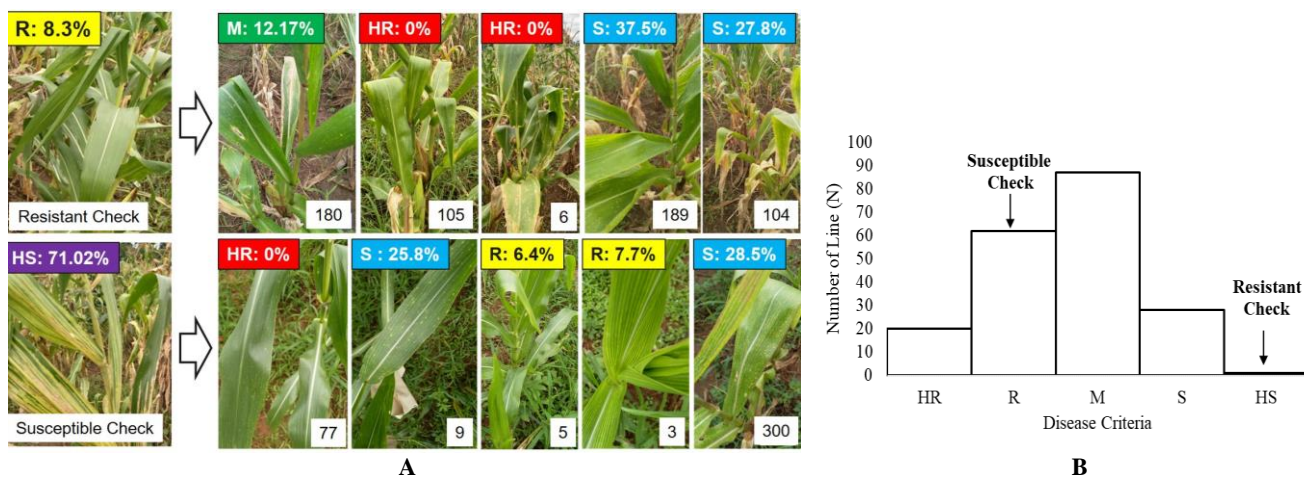


Figure 1. Phenotypic observation in the field. A. Performances of $F_{3:4}$ maize population along with control plants under field trial for evaluation of DM resistance; B. Frequency distribution of DM disease reaction in $F_{3:4}$ populations.

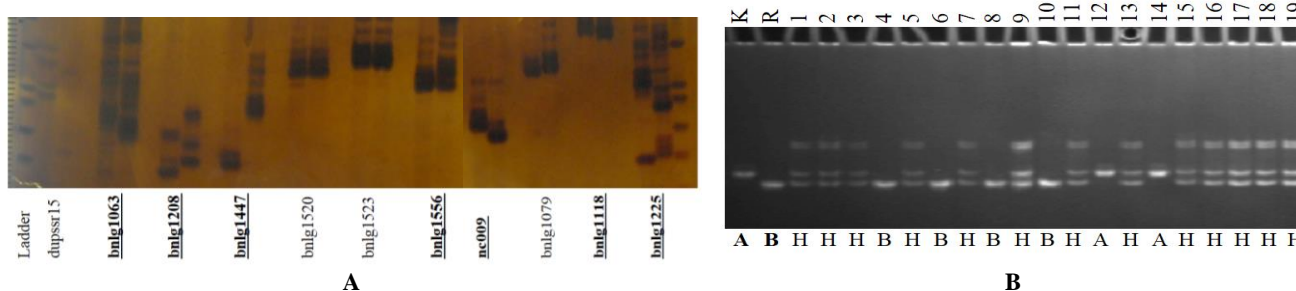


Figure 2. A. Polymorphic markers survey, and B. Segregation test of F_3 maize populations using nc009 polymorphic marker. K: Kandora male parent, R10: R10-440 female parent, 1-19: F_3 maize populations (partially), A: male homozygous allele score, B: female homozygous score, and H: heterozygous allele score, Ld: Ladder 1 Kb base⁻¹ size marker.

Segregation distortion was also examined in this study by looking at the deviation of the segregation ratio of a locus from the expected Mendelian ratio. Parental allele frequencies for all framework markers in both populations were calculated (Table 1), and used to assess the presence of distorted markers.

It can be inferred that genetic factors are responsible for segregation distortion if the markers are distorted in F_3 population. A total of 20 markers were distorted in F_3 populations (Table 1). Of them, 6 markers were on chromosome 1 (umc2225, umc1685, bnlg109, bnlg1564, bnlg1884 and bnlg1556), two markers on chromosome 2 (bnlg1258 and bnlg1138), two markers on chromosome 4 (umc2279 and bnlg1241), two markers on chromosome 5 (phi048 and bnlg1118), three markers on chromosome 6 (nc010, bnlg1371 and bnlg1443), one marker on chromosome 8 (umc2042), two markers on chromosome 9 (bnlg1583 and bnlg128) and two markers on chromosome 10 (bnlg1655 and bnlg2190). Results showed severe segregation distortion in this population, seven markers were favoring the Kandora, one marker favoring the heterozygote, and 12 markers favoring the R10-4430 allele. The result indicated that genetic from R10-4430 had

stronger effect than that from Kandora on transmission ratio distortion.

Linkage map construction and QTL analysis

The linkage map was constructed using 198 F_3 populations with 108 pairs of polymorphic SSR markers. Of these, only 44 out of 108 polymorphic markers were informative according to their segregation pattern in the F_3 maize population, one of which was nc009 marker shown in Figure 2. Genetic intervals between the markers obtained in the same chromosome on the basis of the recombination value with the Kosambi function were presented in Figure 3. Genetic linkage maps constructed from the F_3 maize population were successfully mapped 28 out of 44 polymorphic SSR markers at the significance of LOD value of 3.0 as a threshold. Overall, genetic linkage map resulted in present study was 460.3 cM with an average distance between markers was 26.85 cM. A large genetic difference between the polymorphic markers was observed in present study and this phenomenon has commonly occurred in the construction of a genetic linkage map with low polymorphic markers available. In other words, molecular markers that showed unlinked to each other revealed a

large distance between marker space (> 50 cM). In order to obtain a high-density linkage map, therefore, it is suggested that in future work more polymorphic markers between the two adjacent markers are needed to explore. The magnitude of the linkage map formed in this study was about 39.75% of the total maize genome in comparison to the maize genetic map reported by Kim et al. (2020).

QTL analysis

Based on the linkage analysis between genotype and phenotype data using the Windows QTL Cartographer ver. 2.5 program with the CIM analysis method, a total of seven QTLs were significantly linked to the DM resistance character. The seven QTLs were detected on four maize chromosomes, including chromosomes 1, 5, 6, and 10. Three QTLs were located on chromosome 1 (*qDM-Pp1a*, *qDM-Pp1b1*, and *qDM-Pp1b2*), one QTL was on chromosome 5 (*qDM-Pp5*), two QTL were on chromosome 6 (*qDM-Pp6b1* and *qDM-Pp6b2*) and one QTL was on chromosome 10 (*qDM-Pp10*), and those with the peak position above the LOD value (3.0) (Figure 4). The information of flanking QTLs of the SSR markers, QTL positions, markers interval, LOD value, additive effects, and PVE were presented in Table 2.

Table 1. Chi-square test for segregation distortion of markers and genetic factors influencing distorted markers in F₃ generated from Kandora and R10-4430

Chr	Marker	(Kandora x R10-4430)				Chi-square	Direction of skew
		Genotype		K/R	R/R		
1	umc2225	153	0	45	29.75**	Kandora	
1	umc1685	161	0	36	40.26**	Kandora	
1	bnlg109	159	0	39	36.73**	Kandora	
1	bnlg1564	73	0	124	6.70**	R10-4430	
1	bnlg1614	113	0	84	2.17 ^{ns}	-	
1	bnlg1884	30	0	168	48.58**	R10-4430	
1	bnlg1556	27	102	69	4.50*	Heterozygot	
2	bnlg1225	43	88	66	1.36 ^{ns}	-	
2	bnlg1258	73	0	124	6.70**	R10-4430	

2	bnlg1138	144	7	47	24.00**	Kandora
3	bnlg1246	59	50	86	1.92 ^{ns}	-
3	bnlg1035	87	10	101	0.50 ^{ns}	-
3	bnlg1447	39	98	61	1.23 ^{ns}	-
3	dupssr17	41	93	56	0.62 ^{ns}	-
4	umc2281	21	163	14	0.12 ^{ns}	-
4	umc1008	48	97	53	0.06 ^{ns}	-
4	umc2039	26	111	59	2.83 ^{ns}	-
4	umc2410	31	130	35	0.04 ^{ns}	-
4	umc2279	86	64	47	3.92*	Kandora
4	bnlg1241	13	0	162	72.49**	R10-4430
5	phi048	31	4	162	44.22**	R10-4430
5	bnlg118	58	103	32	1.81 ^{ns}	-
5	bnlg1118	77	0	121	4.94*	R10-4430
6	bnlg1759	59	90	46	0.44 ^{ns}	-
6	bnlg1740	47	110	40	0.13 ^{ns}	-
6	nc010	28	0	170	51.43**	R10-4430
6	nc009	36	120	41	0.06 ^{ns}	-
6	bnlg1371	48	36	113	10.89**	R10-4430
6	bnlg1443	40	5	153	32.57**	R10-4430
7	umc1401	46	108	43	0.02 ^{ns}	-
7	bnlg1200	56	85	48	0.18 ^{ns}	-
8	umc2042	5	4	188	86.29**	R10-4430
8	bnlg1446	22	123	44	1.35 ^{ns}	-
9	bnlg1583	21	39	138	34.92**	R10-4430
9	umc2337	45	123	28	0.75 ^{ns}	-
9	umc1037	25	134	37	0.37 ^{ns}	-
9	phi028	32	120	41	0.22 ^{ns}	-
9	bnlg128	68	76	30	4.77*	Kandora
9	bnlg1209	49	80	59	0.28 ^{ns}	-
9	bnlg1525	49	101	48	0.00 ^{ns}	-
10	bnlg1655	194	0	4	92.08**	Kandora
10	bnlg2190	73	2	123	6.38*	R10-4430
10	bnlg1028	38	101	58	1.03 ^{ns}	-
10	phi118	36	99	46	0.31 ^{ns}	-

Note: Chr.: Chromosome, Marker: Significant SSR marker (Mapmaker), Genotype K/K: The number of alleles that follow the Kandora (Resistant allele for DM), K/R: The number of alleles that follow heterozygous allele, R/R: The number of alleles that follow the R104430 (Susceptible allele for DM), Chi-square: A chi-square: a test that measures how expectations compare to actual observed data, Direction of Skewed: a measure of the asymmetry of the probability distribution of 198 maize lines (F_{3:4}).

Table 2. QTLs for the Downy Mildew (DM) resistance identified in the F₃ maize population generated from Kandora and R10-4430 cross

Chr.	QTL	Flanking marker (bin)	QTL Position	Interval Marker (cM)	LOD	Additive effect	a	d	Gene action	PVE (%)	Favorable allele
1a	<i>qDM-Pp1a</i>	umc2225 (1.01) - umc1685 (1.01)	6.01	0.0-23.4	3.13	1.33	1.33	22.64	OD	47.74	Kandora
1b	<i>qDM-Pp1b1</i>	bnlg1564 (1.07) - bnlg1884 (1.05)	4.01	0.0-38.7	6.85	-0.89	-0.89	23.67	OD	45.7	R10-4430
1b	<i>qDM-Pp1b2</i>	bnlg1564 (1.07) - bnlg1884 (1.05)	36.01	0.0-38.7	6.7	0.81	0.81	24.43	OD	43.35	Kandora
5	<i>qDM-Pp5</i>	phi048 (5.07) - bnlg1118 (5.07)	6.01	0.0-38.0	4.69	0.2	0.20	20.61	OD	51.12	Kandora
6b	<i>qDM-Pp6b1</i>	nc010 (6.04) - bnlg1371 (6.02)	4.01	0.0-31.2	7.49	0.87	0.87	22.70	OD	47.73	Kandora
6b	<i>qDM-Pp6b2</i>	bnlg1371 (6.02) - bnlg1443 (6.05)	77.21	31.2-84.6	7.12	-0.55	-0.55	20.87	OD	53.71	R10-4430
10	<i>qDM-Pp10</i>	bnlg2190 (10.06) - bnlg1028 (10.06)	6.01	0.0-44.2	5.22	-0.78	-0.78	20.84	OD	50.68	R10-4430

Note: Chr.: Chromosome, *qDM-Pp*: QTL Downy Mildew *Peronosclerospora philippinensis*, marker that was provided underlines is the closest marker to the QTL position, Marker intervals: the distance between markers in centimorgan units (cM), a: additive effect (a), d: Dominance effect (d), PVE (Phenotypic Variation Explained) is phenotypic variations presentation (%) of the total value of additive effects and dominant effect for putative QTL (model 3), Additive effect: positive value indicates the contribution of the donor alleles (Kandora) to the F₃ population to increase DM disease resistance

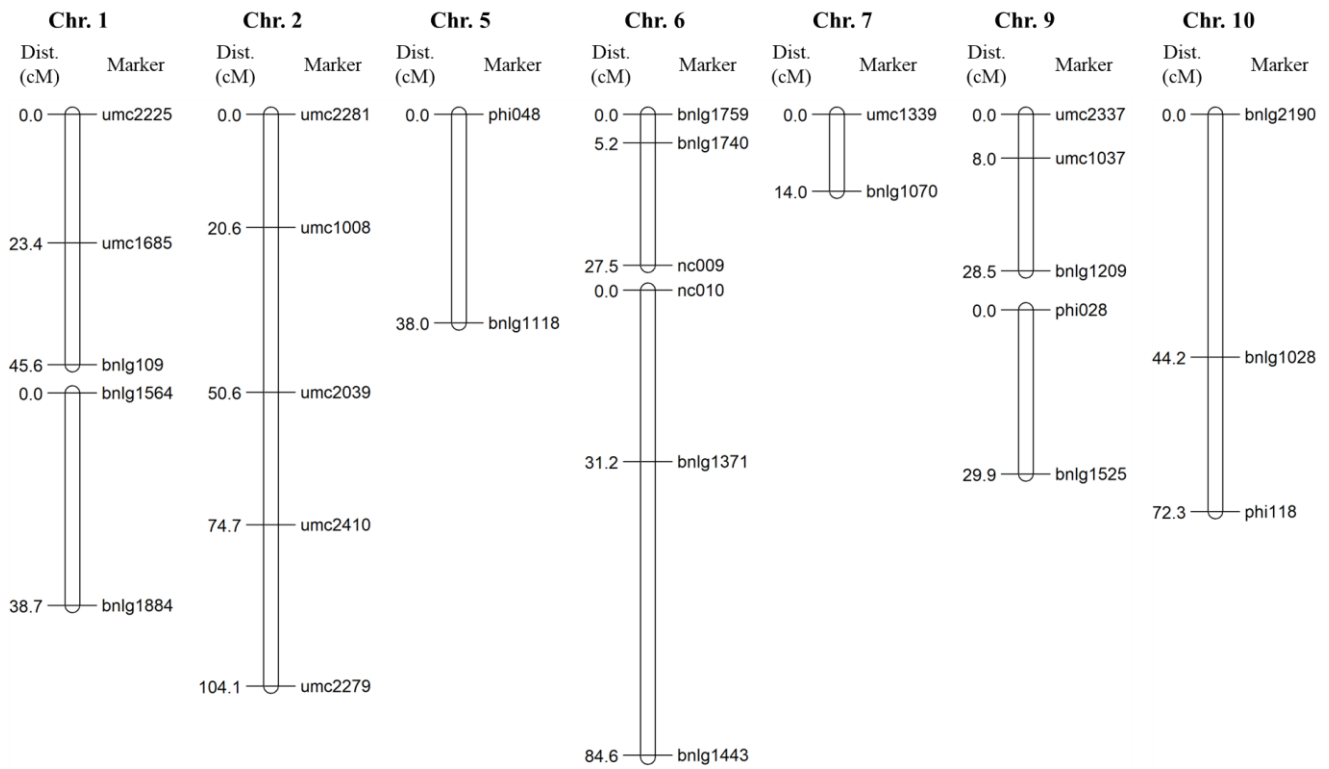


Figure 3. Linkage map of SSR markers constructed in present study. Intervals between the marker (cM) are linked to each chromosome (Chr.-n)

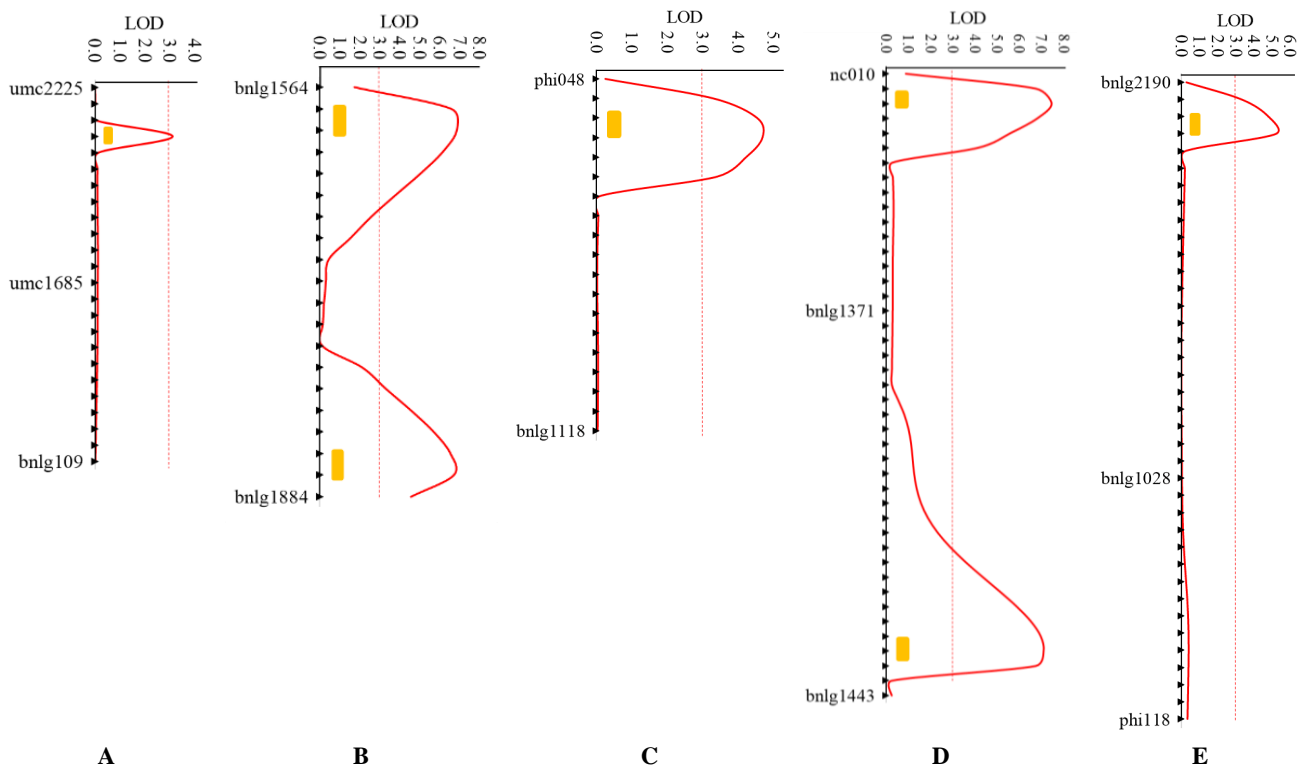


Figure 4. QTL position to DM resistance character of F₃ populations. A. Chromosome 1a; B. Chromosome 1b; C. Chromosome 5; D. Chromosome 6b; E. Chromosome 10. *qDM-Pp*: QTL Downy Mildew *Peronosclerospora philippinensis*, LOD: Likelihood of odds, cM: centi Morgan

With regards to the QTL positions to the DM trait ranged from 4.01 cM (*qDM-Pp1b₁* and *qDM-Pp6b₁*) to 77.21 (*qDM-Pp6b₂*). Based on the closest QTL position to the DM resistance character, *qDM-Pp1b₁* was flanked by bnlg1564 and bnlg1884 markers along chromosome 1 and *qDM-Pp6b₁*, was flanked by nc010 and bnlg1371 markers along chromosome 6. Of these, the bnlg1564 and nc010 markers were the closest marker to the QTL *qDM-Pp1b₁* and *qDM-Pp6b₁* peaks, respectively. Based on gene action observed in present study, each parental lines provided an additive effect on the QTL responsible to the DM disease. In *qDM-Pp1b₁*, *qDM-Pp6b₂*, and *qDM-Pp10*, the additive effect (it) came from R10-4430 (susceptible) parent while the other QTL came from Kandora (resistant) parent. Over-dominance effect was observed for increasing the DM at all QTL loci was derived from Kandora parent. The effect of QTL found in present study showed a large effect on phenotype reflected by a high value of phenotypic variation explained (PVE), value ranged from 43.35% to 53.71% (Table 2). Kandora allele contributed to an increase in DM resistance at four putative QTLs designated as *qDM-Pp1a*, *qDM-Pp1b₂*, *qDM-Pp5*, and *qDM-Pp6b₁* loci on chromosome 1, 2, 5 and 6.

Discussion

To reduce DM infection in maize crops have been performed various attempts such as lines screening, chemical application, and management pattern cultivation. It, however, still provided negative impact on production losses and genetic variability on the pathogen's adaptability. Roberdi et al. (2010) explained that the distribution factor of resistance character was very dependent on the genetic variability level, phenotypic variability, and interaction between genetic and environment. The formation of resistant varieties was important to be developed, considering that in nature the maize parents have not been found to encode the downy resistance character. Based on phenotypic characterization of the 198 F_{3:4} maize population resulted in 20 maize lines were highly resistance (HR), 62 lines were resistant (R), 87 lines were moderately resistance (MR), 28 lines were susceptible (S) and 1 line was highly susceptible (HS) against the DM disease. Of these, 62 plants (31.3%) were attacked in the same range as resistant varieties (> 0 to 10 %). The distribution of HR plants showed that DM resistance response from donor parents has been introgressed into their progenies, so that it needed for relatively more homogeneous population studies (Roberdi et al. 2010; Boddy 2015)

Molecular marker, one of new technology in biotechnology, was a selection tool for lines possessing the desired character, so that, it can accelerate the plant breeding cycle with high accuracy (Kordrostami and Rahimi 2015). The availability of molecular markers linked to DM disease resistance trait in maize plants will potentially benefit to cut the conventional breeding cycle which requires a relatively long time (Jiang 2013). Based on the polymorphic survey of 275 SSR markers pairs on the two parents, 108 SSR primers showed polymorphically.

Of them, a total of 44 pairs of markers were informatively segregated in the 198 F₃ maize lines, while the rest of the primers did not provide banding patterns suggesting that more markers are needed to apply in order to identify precise loci associated with target genes. Therefore, the availability of a set of polymorphic markers dispersed evenly along all the maize chromosomes, population type and total population would give high opportunity in identifying molecular markers associated with DM resistance trait (Ferreira et al. 2006). Regardless of population size, most published experiments with replicated trials have used between 100 and 200 progenies in QTLs study (Semagn et al. 2010). Recent QTLs study related to the downy mildew resistance genes conducted by Lohithaswa et al. (2015) used a total of 185 lines of sorghum F₂ population, Ruswandi et al. (2015) have also succeeded to identify QTLs responsible to DM resistance using 142 maize BC1F1 lines. Therefore, in present study, a population size contained 198 maize lines is comparable with that in previous study to detection of QTLs with large phenotypic effect.

In present study, of the ten chromosomes in maize genome, the SSR markers could only be mapped in seven chromosomes based on genotyping analysis on the F₃ maize population. The SSR markers were not successfully constructed on chromosomes 2, 3, and 8 in present study due to they were not linked to each other. It can be caused by the large distance between polymorphic markers might be the reason for such unlinked markers observed in current study. Among the linked SSR markers constructed in present study, those on chromosome 6 showed a high number of SSR markers that could be mapped (six pairs of markers). Linkage map constructed on chromosomes 1, 6, and 9 each was divided into two maps, namely chromosomes 1a and 1b (chromosome 1), chromosomes 6a and 6b (chromosome 6), and chromosome 9a and 9b (chromosome 9), respectively. The separation of the linkage maps was caused by the recombination value of each marker in the different chromosomes from each other with the position of the nearest marker was sorted based on the maximum distance (maximum-likelihood) between the markers in one chromosome and breakpoints segment more than 50 cM (Portwood et al. 2019). These results showed similar events with those reported by Sabry et al. (2006), in which found 89 polymorphic SSR markers constructed in their study showed higher map distances (exceeding 60 cM) with adjacent markers in the linkage group, significantly expanding genetic map distance. This phenomenon occurred due to the unavailability of the polymorphic SSR locus at bin location 6.01-6.04, making chromosome 6 was divided into two sub-groups, called as 6A and 6B. The final map produced using 80 SSR markers has 11 linking groups. Through segregation analysis, DNA marker sequence and the relative distance between these markers can be determined. The value of recombination frequency between two markers that get smaller shows the closeness of the location of the two markers in a chromosome. Conversely, the higher the frequency of recombination between two markers, the farther the

distance between them on a chromosome. A marker or gene is said to be linked if it has a recombination frequency value of less than 50% (Hartl 1988).

The linkage between phenotypic and genotypic data obtained seven QTL that was spread on four maize chromosomes (1, 5, 6, and 10). *qDM-Pp1b₁* was flanked by bnlg1564 and bnlg1884 markers on chromosome 1 and *qDM-Pp6b₁*, was flanked by nc010 and bnlg1371 markers on chromosome 6. Of these, the bnlg1564 and nc010 markers were the closest marker to the QTL *qDM-Pp1b₁* and *qDM-Pp6b₁* peaks, respectively. Based on gene action observed in present study, each parental line provided an additive effect on the QTL responsible to the DM disease. In *qDM-Pp1b₁*, *qDM-Pp6b₂*, and *qDM-Pp10*, the additive effect (it) came from R10-4430 (susceptible) parent while the other QTL came from Kandora (resistant) parent. The contribution of donor parent alleles inherited to their progenies can be known from the additive effect value in which three QTL was influenced by the allele R10-4430 (susceptible) while the other QTL was dominated by Kandora (resistant) parents allele. De Almeida et al. (2016) reported that additive effect was closely related to the contribution of donor alleles that were polygenically inherited to certain resistance characters. PVE values for all QTLs were expressed as major gene effects, whereas minor gene effects are stated when PVE values more than 10% (Nagabhusan et al. 2014). Mechanism of the effects of the major or minor genes can be seen from the number of plants successfully infected by pathogens that cause DM (Li et al. 2011; Sukhwinder-Singh et al. 2012; Shah et al. 2017). The PVE diversity in the linkage area was caused by infection pathogenic factor to the response of the resistance maize lines. Based on previous study, Kim et al. (2017) and Ruswandi et al. (2015) have identified QTL for downy resistance on chromosome 1 using F₂ and BC₁F₂ populations, respectively. Furthermore, it was also reported by Jampatong et al. (2013) that QTL related to Pp in the RILs, Lohithaswa et al. (2015) found QTL related to Ps (*Peronosclerospora sorghi*) in F₂ population, and QTL linked Ph (*Peronosclerospora heteropogoni*) character was obtained BC₁F₂ population (Kim et al. 2020). Based on this study, we conclude that response of 198 F_{3:4} lines on the DM disease in the field varied from highly resistance to highly susceptible and the DM resistance related QTLs found in present study would give new insight on the future molecular maize breeding program for resistant against downy mildew disease.

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