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Inheritance of resistance to downy mildew (*Peronosclerospora maydis*) in crossing of Madura Maize Plant (*Zea mays* L.)

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Hybridization of Back cross is one method to get varieties that are resistant to downy mildew. The purpose of this study was to obtain information on inheritance characteristics of downy mildew resistance. This research was conducted at the experiment center of Agro-Technology Study Program of Agriculture Faculty, University of Trunojoyo Madura. Research of Assessment of resistance to Downy Mildew used a randomized block design with 18 treatments (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 in three sets of crosses, namely LGL x Mdr-3, T12 x Mdr-1 and E02 x Mdr-2) and three replications so there were 54 experimental units. Identification of polymorphic RAPD markers for endurance to downy mildew through Bulk Segregant Analysis (BSA) was done by amplifying the DNA in the resistant pool and susceptible pool. The random primers used were 120 primers from 6 operon groups, namely OPA, OPB, OPC, OPD, OPF and OPG. The results showed that the inheritance pattern of maize genetic resistance to downy mildew followed a segregation pattern of 3:1 with a degree of dominance between -1 and 0, and was controlled by incomplete partially negative dominant gene. OPC-07 was a marker that was linkage close to the resistance to downy mildew with a genetic distance of 1.9 cM.

Keywords: inheritance, downy midew, madura maize, back cross, bulk segragant analysis

INTRODUCTION

Madura Island is an island located in the East Java region of Indonesia which has a crop area for maize of approximately 360,000 hectares (30% of the area of maize in East Java), but productivity at the farm level is still low at an average of 2.7 tons per hectare (BPS, 2015). These results are very low compared to other maize-producing regions. The low productivity of maize on the island of Madura is caused by low soil fertility and not yet assembled superior maize for the island of Madura (Amzeri et al., 2011).

Increased productivity can be done by assembling superior varieties that have high production and resistant to the main diseases of maize plants. Downy mildew is a major disease in maize plants caused by the fungus Peronesclerospora. Downy mildew caused a decrease in yield of 10% to 90% (Talanca, 2010) even reaching 100% (Soebandi et al., 1996; Yasin et al. 2008).

Assembling varieties that have high production and resistance to downy mildew can be done by crossing maize plants that have high production with plants that are resistant to downy mildew (Amzeri, 2015). The method of crossing that can be used to produce varieties resistant to disease is back cross (Poespodarsono, 1998; Mangendidjojo, 2003; Amzeri, 2015). The back cross method has produced many varieties that are resistant to disease. In addition, molecular markers can be used as a selection aids (MAS = Marker Assisted Selection), where selection is based on plant genetic traits without

environmental influences, so that plant breeding is faster, right, save money and time (Pabendon et al., 2007; Kumar and Gurusubramanian, 2011).

The purpose of this study was to obtain information on inheritance characteristics of downy mildew resistance. Information about inheritance of a character has an important meaning in determining plant breeding strategies so that breeding programs to improve the desired character are more effective.

MATERIALS AND METHODS

Genetic Material

This research was conducted at the experiment center and biotechnology laboratory of Agro-Technology Study Program of Agriculture Faculty, University of Trunojoyo Madura, Indonesia, in July 2017-June 2018. The parents of maize used were parents resistant and not resistant to downy mildew. The genetic material evaluated were P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 in three crossing sets namely LGL x Mdr-3, T12 x Mdr-1 and E02 x Mdr-2.

Field Experiments and Assessment of Resistance to Downy Mildew

This research used a randomized block design with 18 treatments (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 in three crossing sets namely LGL x Mdr-3, T12 x Mdr-1 and E02 x Mdr-2) and three replications so there were 54 experimental units. Each experimental unit consists of 400 plants planted with a spacing of 20 cm x 70 cm. waxy corn is a plant that is susceptible to downy mildew planted as a spreader plant (spreader row) as many as three rows on the edge and block of plant population tested. Inoculation was done by spraying suspension spores on plants aged 7, 9 and 11 days after planting, both on the spreader plant lines and on the plant population tested. Observation of the occurrence of downy mildew is done when plants are 21, 28 and 35 days after planting. The number of sample plants observed in P_1, P_2 and F_1 (40 sample plants), BC_1P_1 and BC_1P_2 (80 sample plants) and F_2 (200 sample plants). Resistance to downy mildew is tested based on the percentage of attacks : score 0 = very resistant (0-10%), score 1 = resistant (>10-20%), score 2 = rather resistant (>20-40%), score 3 = susceptible (>40-60%), score 4 = very susceptipble (60-100%) (Talanca, 2009).

The degree of dominance is measured based on the parents middle value (P_1 and P_2) and F_1 using the formula for estimating the potential ratio (hp) (Griffing, 1950).

$$hp = \frac{(F1-MP)}{1/2(HP-LP)}$$

where : hp = value of potential ratio; HP = value of highest parent average; LP = value of lowest parent average; MP = the average value of the two parents.

Based on the value of potential ratios can be predicted the degree of dominance as follows:

hp = 0 = additive gene action
-1 = hp = +1 = perfect dominant
-1 < hp < 0 = incomplete partially negative dominant gene
0 < hp < 1 = incomplete partially positive dominant gene
-1 < hp < 1 = over dominant

The segregation pattern on F2 was tested using the chi-square test (Suryo, 2008).

$$\chi 2 = \frac{(Oi-Ei)}{Ei}$$

where : Oi = number of phenotypes observation to-i; Ei = number of phenotype to-i based on hypothesis

DNA preparation

The DNA of F_2 plant groups from the three crosses that resistant (score 0) and very susceptible (score 4) to downy mildew each was isolated following the procedure of Daryono and Natsuaki (2002). The DNA of each group was combined proportionally based on the concentration level to form 1 DNA pool.

DNA amplification

DNA amplification followed the method of Te-Chato et al., (2005). The amplification reaction was done using 20µl of the solution that consist of master mix 11 µl, primer 2,2 µl, DNA 3 µl and dH2O/1XTE buffer 3,8 µl. The tube containing a mixture of PCR was rotated in a centrifuge with 8000 rpm for 30 seconds to 1 minute. Furthermore, amplification was done in the PCR machine for approximately 4 hours 30 minutes with the program as follows:one pre PCR cycle (92^0C, 2 minute), 40 cycles {denaturation (92^0C, 1 minute), annealing (37^0C,1 minute), elongation (72^0C, 2 minute) }, post elongation (72^0C, 10 minute). The amplification results were visualized by agarose gel electrophoresis.

Linkage Analysis of RAPD Marker with resistance to downy mildew

Identification of polymorphic RAPD markers for resistance to downy mildew was done by

amplifying resistant DNA pools and susceptible DNA pools using random primers. The random primers that were used 120 primers from 6 operon groups, namely OPA, OPB, OPC, OPD, OPF and OPG. Each group consists of 20 primers with 10 base lenghts. Primers that capable to amplifying DNA in the resistant pool were applied to resistant and susceptible parents. From the specific polymorphic markers of the resistant parent obtained, then it was applied to the population F_2 for analysis of linkage resistance to downy mildew. The linkage test used 120 plant populations in three crosses. Linkage analysis between RAPD markers and resistance control was done with the MAPMAKER computer application program.

RESULTS AND DISCUSSION

In three crosses, the rate of downy mildew attack in Family F_1 was between the values of the mid-parents and the resistant parents (Table 1). Schematically the relative position of F_1 for both parents is shown in Figure 1. Takdir et al., (2003) conducted a cross between resistant and susceptible maize produced the rates of downy mildew attack in the F_1 Family whose value was between the middle values of parents and and resistant parents. The value of the potential ratio in the three crosses has a value between -0.384 to -0.652. The value of the potential ratio (hp) which is in the range of -1 and 0 indicates that the character is controlled by incomplete partially negative dominant gene (Petr and Frey, 1966).

The segregation pattern in F_2 population given an indication that the resistant character shows a simple genetic inheritance pattern and follows the pattern of segregation of Mendel's law (Table 2). The chi-square test ($\chi 2$) for the degree of suitability of the F_2 population segregation ratio for various hypothetical ratios based on two endurance classes shows that the segregation pattern of the three F_2 populations matches the 3: 1 ratio. At the LGL x Mdr-3 crossing had a value of $\chi 2 = 0.0061$ with an opportunity value (P) of 0.95-0.90. T12 x Mdr-1 crossing had a value of $\chi 2 = 0.2960$ with an opportunity value (P) of 0.70-0.50. E02 x Mdr-2 crossing had a value of $\chi 2 = 2.0575$ with an opportunity value (P) of 0.20-0.10.

Population analysis of back cross (BC_1P_1 and BC_1P_2) show that crossing of LGLxMdr-3, T12xMdr-1 dan E02xMdr-2, back cross to male parent (resistant/BC_1P_1) and back cross to female parent (susceptible/BC_1P_2) had a value that matches the 1: 1 ratio at the 0.05 level. The population of BC_1P_1 which derived from a crossing between F_1 and P_1 (resistant parent) approaches the resistant parent. In contrast, the population of BC_1P_2 which derived of crossing between F_1 with P_2 (susceptible parent) approaches the susceptible parent. This event was suitability with Mendel Laws of 1 that in the formation of gametes the two genes that are pairs will be segregated into two daughter cells (Stansfield, 1991).

The results of amplification of the resistant DNA pool and susceptible DNA pool using 120 random primers were obtained that 46 primers that could amplify the resistant and susceptible DNA pool. These primers were used to identify 4 F_2 plants in each pool (4 F_2 plants of resistant and 4 F_2 plants of susceptible). The results of this study indicate that OPC-7 ('GTCCCGACGA') resulted in the application of bands present to resistant parents, bulk resistant and 4 resistant F2 plants. This band was not found in susceptible parents, susceptible parent bulk and 4 susceptible F_2 plants. This marker had around 475 base pairs (bp) (Figures 5 and 6). OPC-07 was a marker that was linkage close to the resistance to downy mildew with a genetic distance of 1.9 cM.

Table 1. The rate downy mildew attack on P_1, P_2, F_1 dan potensi rasio (hp)

Family	Attack rate		
	LGL x Mdr-3	T12 x Mdr-1	E02 x Mdr-2
P_1	20,000	10,000	15,000
P_2	52,500	87,500	72,500
F_1	30,000	35,000	25,000
hp	-0,384	-0,355	-0,652

Table 2. Segregation of resistance to downy mildew in crossing of LGL x Mdr-3, T12 x Mdr-1 and E02 x Mdr-2.

Crossing	Genera-tion	Number of plants				Expected ratio	χ^2	(P)
		Observation		Expected				
		Resistant	Susceptible	Resistant	Susceptible			
LGL x Mdr-3	P_1	32	8	-	-			
	P_2	19	21	-	-			
	F_1	28	12	-	-			
	F_2	143	57	143,50	56,50	3:1	0,0061	0,95-0,90
	BCP_1	55	25	56,00	24,00	1:1	0,0596	0,80-0,70
	BCP_2	51	29	51,20	28,80	1:1	0,0729	0,80-0,70
T12 x Mdr-1	P_1	36	4	-	-			
	P_2	5	35	-	-			
	F_1	26	14	-	-			
	F_2	138	62	141,50	58,50	3:1	0,2960	0,70-0,50
	BCP_1	51	29	52,00	28,00	1:1	0,0549	0,90-0,80
	BCP_2	43	37	41,20	38,80	1:1	0,1621	0,70-0,50
E02 x Mdr-2	P_1	34	6	-	-			
	P_2	11	29	-	-			
	F_1	30	10	-	-			
	F_2	132	68	141,24	58,76	3:1	2,0575	0,20-0,10
	BCP_1	59	21	60,00	20,00	1:1	0,0667	0,80-0,70
	BCP_2	44	36	44,5	35,50	1:1	0,0629	0,80-0,70

$\chi^2.05 = 3.841$ and $\chi^2.01 = 6,635$

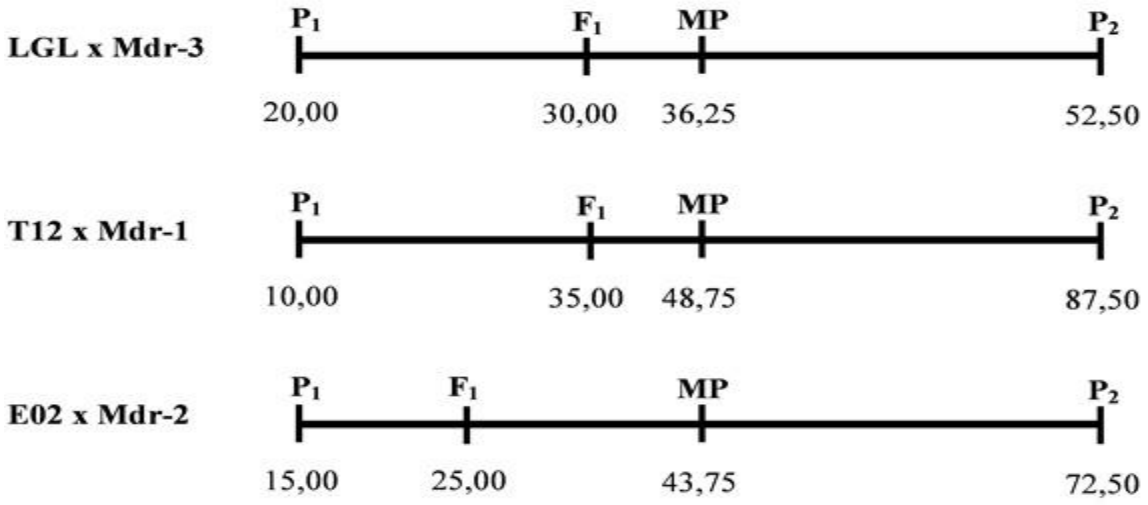

Figure 1. Schematic of the relative position of the F_1 middle value of both parents

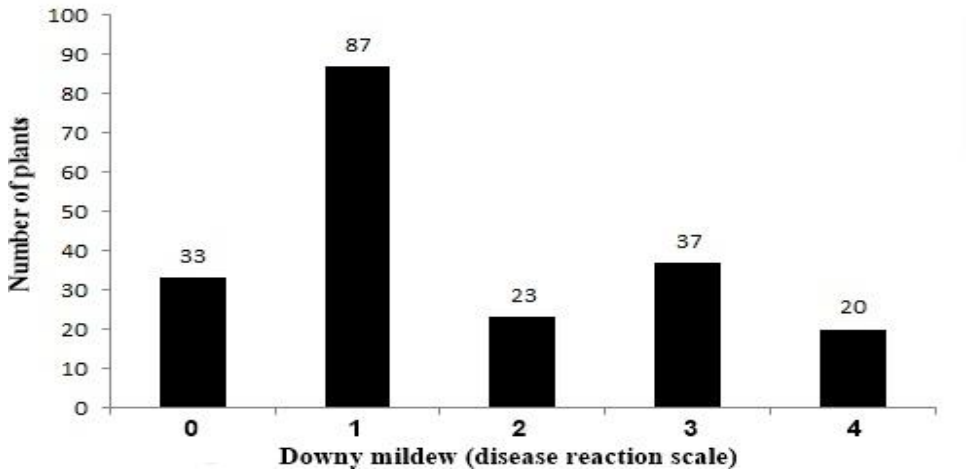

Figure 2. Distribution of F_2 plants as a crossing result of resistant maize plants (LGL) with susceptible maize plants (Mdr-3) based on the level of resistance to mildew.

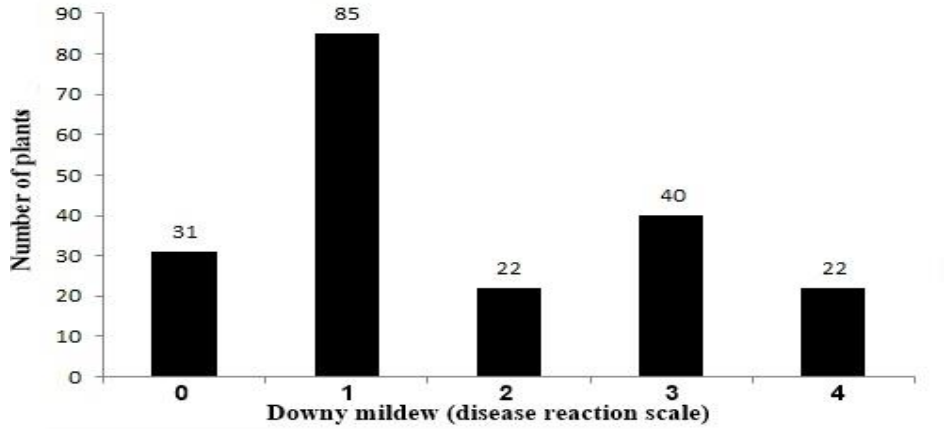

Figure 3. Distribution of F_2 plants as a crossing result of resistant maize plants (E02) with susceptible maize plants (Mdr-2) based on the level of resistance to mildew.

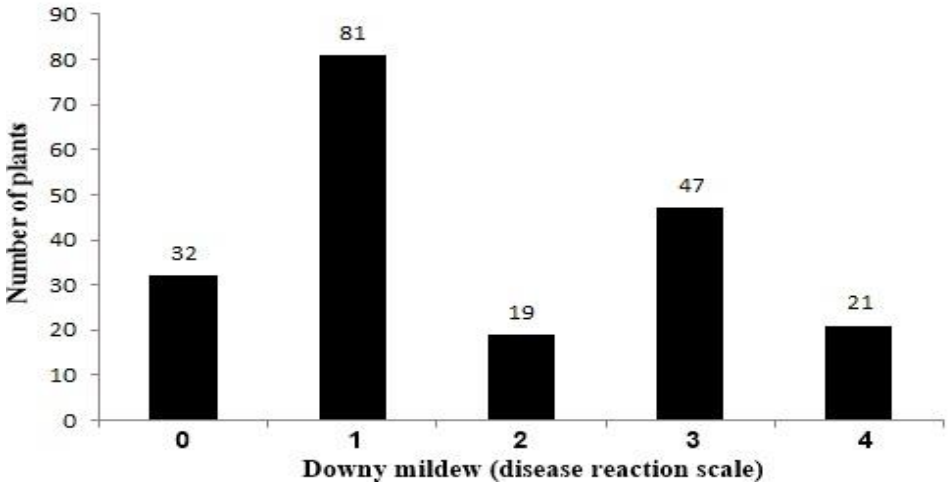

Figure 4. Distribution of F_2 plants as a crossing result of resistant maize plants (T12) with susceptible maize plants (Mdr-1) based on the level of resistance to mildew.

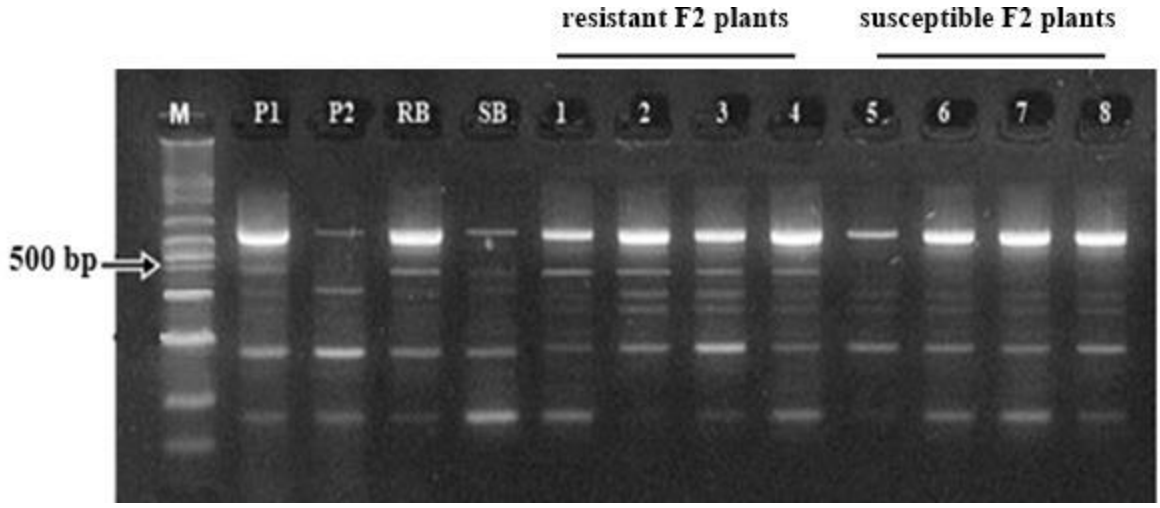

Figure 5. The profile of the DNA fragment amplified with OPC-7 primer in the F_2 population derive from the crossing of LGL x Mdr-3

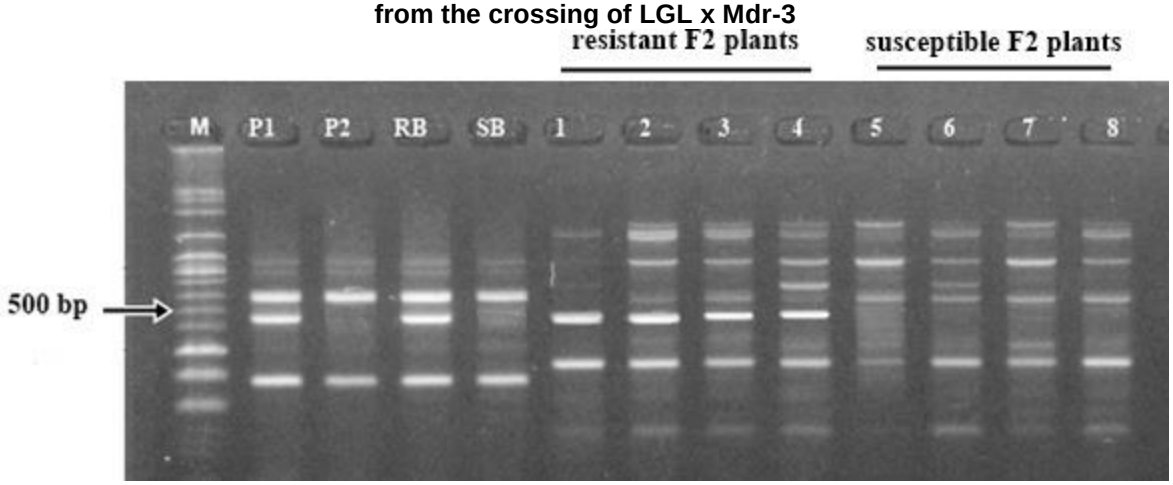

Figure 6. The profile of the DNA fragment amplified with OPC-7 primer in the F_2 population derive from the crossing of T12 x Mdr-1

CONCLUSION

The results showed that the inheritance pattern of maize genetic resistance to downy mildew followed a segregation pattern of 3:1 with a degree of dominance between -1 and 0, and was controlled by incomplete partially negative dominant gene. OPC-07 was a marker that was linkage close to the resistance to downy mildew with a genetic distance of 1.9 cM.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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