AGRIVITA VOLUME 35 No. 2 JUNE - 2013 ISSN : 0126-0537

PATH ANALYSIS OF SOME LEAF CHARACTERS RELATED TO DOWNY MILDEW RESISTANCE IN MAIZE

Eko Hary Pudjiwati[1*)], Kuswanto[2)], Nur Basuki[2)] and Arifin Noor Sugiharto[2)]

[1)] Agrotechnology Department, Faculty of Agriculture Borneo University of Tarakan
Jl. Amal Lama No. 1 Tarakan East Kalimantan Indonesia
[2)] Faculty of Agriculture University of Brawijaya
Jl. Veteran Malang 65145 East Java Indonesia
[*)] Correspondending author Phone:+62- 85236642168 E-mail: eko.pudjiwati@borneo.ac.id

Received: November 7, 2012/ Accepted: April 12, 2013

ABSTRACT

This research was aim to investigate both direct and indirect effects of some characters related to downy mildew resistance in maize. This information is required to determine the selection criteria on maize breeding program for downy mildew resistance. The research was conducted at Research Station of Agriculture Faculty, University of Brawijaya, from January to April 2012. Five varieties of hybrid maize and five inbreeding lines were employed, and Randomized Complete Block Design with two replications was applied. The result showed revealed that trichome and stomatal density on the upper and lower surface of leaves had positif direct effect and genotipic correlation with downy mildew disease incidence. These characters are qualified as effective selection criteria for resistance to downy mildew. In relation to maize breeding program, low trichome density and stomatal density characters enhance downy mildew resistance.

Keywords: path analysis, maize, downy mildew

INTRODUCTION

Maize plays a strategic role as food commodity and raw material of industry. Increasing production of maize has faced some obstacles, and one of them is downy mildew infection. The cause of such disease in maize is the fungal infection caused by *Peronosclerospora* spp. The disease could cause 100% of damage, particularly for the susceptible varieties in Indonesia (Sudjono, 1988). Disease control through inherent plant resistance is desirable since it will reduce dependence on costly fungicides that can damage the environment. Therefore, from both economic and environmental points of view, the development of resistant varieties is very important.

The mechanism of plant defence pathogen attack is complicated. The internal anatomy and surface features of the leaves often determine plant resistance to biotrophic pathogen infection (Smith *et al*., 1996). Among such characters, aspects of stomata and trichome can influence disease resistance (Niks and Rubiales, 2002). Determination of direct and indirect relation between some characters and plant resistance is important in order to determine the aspects of plant selection criteria.

Correlation coefficient determines simple relations among the traits, but it can not determine more specific character that will influence the main character. Path coefficient analysis as to correlation coefficient gives more detailed information on the relations and had been used for complex characters in several crop species to provide information on interrelations of complex characters and to develop selection criteria (Diz *et al.*, 1994).

Many researches about correlation on some characters resistant to diseases have been reported, but a few reviews using path analysis are very rare. Baswarsiati (1994) found significant correlation between number of trichome and stomata with downy mildew resistance in grapes. According to the research finding by Triharso and Kusdiarti, 1976 varieties in maize with the least number and the narrowest opening of stomata were resistant, and, on the contrary, such a correlation was not found in different tested varieties. Makkulawu (2007) stated there was no correlation between stomata frequency of maze and the resistance level to downy mildew in maize. Based on the previous researches, obvious information about the influential character concerning with

Accredited SK No.: 81/DIKTI/Kep/2011

http://dx.doi.org/10.17503/Agrivita-2013-35-2-p167-173

resistance to downy mildew in maize has not been obtained yet.

The objective of this research was to find out both direct and indirect effect of some leaf characters on resistance to downy mildew, which serves as a foundation in determining effective selection criteria with path analysis.

MATERIALS AND METHODS

The research was conducted at Research Station of Agricultural Faculty, University of Brawijaya, Jatikerto from January to April 2012. The materials involved were commercial hybrid maize varieties in Indonesia, namely Pioneer 21, Pertiwi 3, Bisi 12, NK 33, DK 979 and inbreed lines such as G2, G4, G6, G10 and G21 as in the collection of Agriculture Faculty, University of Brawijaya.

Randomized Complete Block Design with two replications was applied in this research. Each variety and inbreed line were planted with 30 plants per plot. Pathogen inoculation was introduced into 10 day after planting plants by spraying inoculum liquid at 4.00 a.m. Approximately 1.8 x 10^5 conidia per ml.

The variables in this research include disease incidence, stomatal density on upper and lower surface of the leaves, density of trichomes, EC (electric conductivity) of leaves, pH of the leaves, length and width stomata on the upper and lower surface of the leaves. The disease incidence was observed on plants at 14, 21, 28, 35 and 42 day after planting, and the data were accumulated and calculated by the following formula:

$$I = \frac{a}{b} x\ 100\%$$

Remarks:

I	=	Disease incidence
a	=	number of plants infected by downy mildew
b	=	number of plants observed

Criterion of resistance was based on Lal and Singh (1984), where (1) it is resistant when the disease incidence ranges from 0 – 10%; (2) moderately-resistant when disease incidence is >10 – 30%; (3) moderately-susceptible when disease incidence is >30 – 50%; (4) susceptible when disease incidence is >50%.

Phenotypic and genotypic correlation coefficients were calculated using formula suggested by Miller *et al*. (1958):

$$r_{gxy} = \frac{\mathrm{Kov}_{gxy}}{\sqrt{\sigma^2_{gx} . \sigma^2_{gy}}}$$

$$r_{pxy} = \frac{\mathrm{Kov}_{pxy}}{\sqrt{\sigma^2_{px} . \sigma^2_{py}}}$$

Remarks:

r_{gxy}	=	genotypic correlation of two variables x and y
r_{pxy}	=	phenotypic correlation of two variables x and y
Kov_{gxy}	=	genotypic covariance of two variables x and y
Kov_{pxy}	=	phenotypic covariance of two variables x and y
σ^2_{px}	=	phenotypic variance of variable x
σ^2_{py}	=	phenotypic variance of variable y
σ^2_{gx}	=	genotypic variance of variable x
σ^2_{gy}	=	genotypic variance of variable y

The genotypic correlation coefficients were partitioned into direct and indirect effects using the path analysis according to Dewey and Lu (1959):

$$r_{ij} = p_{ij} + \sum r_{ik}\ p_{kj}$$

Remarks:

r_{ij}	=	mutual association between the independent character (i) and dependent chracter (j) as measured by the correlation coefficients
p_{ij}	=	components of direct effects of the independent character (i) on the dependent variables (j) as measured by the path coefficients
$\sum r_{ik}\, p_{kj}$	=	summation of components of indirect effects of a given independent character (i) on a given dependent character (j) via all other independent characters (k)

Broad sense heritability was determined based on the following formula:

$$H = \sigma^2_g / \sigma^2_p$$

Remarks:

H	=	heritability
σ^2_g	=	genotypic variance
σ^2_p	=	phenotypic variance

Heritability value is considered high when the value is > 50%, the moderat value ranges from 20% - 50%, and low value is < 20% (Baihaki, 1999).

RESULTS AND DISCUSSION

Some observed hybrid varieties and inbreed lines indicated different level of resistance to downy mildew, ranging from resistant to susceptible (Table 1), while correlation coefficient of genotypic and phenotypic observed are listed in Table 2. Generally, the analysis result showed that genotypic correlation coefficient was higher than that of phenotypic correlation. Also, the pattern of correlation was similar in both genotypic and phenotypic level, indicating a strong inherent relationship among the characters studied. The genotypic correlation coefficient of the width of stomata on the upper surface of the leaves and other characters were presumed zero since the genotypic variance had a negative value. Negative estimates should be considered equal to zero. Some researchers (Hee and Wynne, 1983; Basuki, 1986) have also found negative genotypic variances in some characters of plants. The result of the correlation analysis indicated that some characters had coefficient value more than one point for their genotypic correlation coefficient. Masnenah *et al.* (2004) also found the genotypic correlation coefficient between two characters, which were more than one. It is presumed that close relationship between these two characters were unclear.

Table 2 informs that disease incidence had significant and positive genotypic correlation with stomatal density on the lower surface of leaves (r = 0.468*) and highly significant with the width of stomata on the upper surface of leaves (r = 0.681**). Highly significant and negative genotypic correlation was observed between disease incidence with the length of stomata on the upper surface of leaves (r = -0.647**).

Disease incidence had insignificant genotypic correlation compared with other observed characters, and it also had insignificant pheno-typic correlation among all observed characters.

Path-analysis based on genotypic correlation of the observed characters towards the downy mildew disease incidence are presented in Table 3, and diagramatic representation of direct effects and correlation coefficients of variable on dependent variable are given in Figure 1.

Table 1. Disease incidence and criteria of resistance

Varieties /line	Disease incidence (%)	Criteria of resistance
Pioneer 21	20	moderately-resistant
Pertiwi 3	0	resistant
Bisi 12	0	resistant
NK 33	0	resistant
DK 979	1.67	resistant
G-2	21.67	moderately-resistant
G-4	35	moderately-resistant
G-6	70	susceptible
G-10	18.33	moderately-susceptible
G-21	38.33	moderately-susceptible

The determination of characters used for selection criteria in resistance to downy mildew was based on path-analysis guidelines by Singh and Chaudary (1979). The first guideline states that the correlation represents the true correlation and the direct selection by the variable is considered effective when correlation between variables is similar to their direct effect. Such indication was found in the observation of the trichome density, stomatal density on the upper and lower surface of leaves, also length of stomata on the lower surface of leaves. These caharacters could be considered as selection criteria for resistance to downy mildew.

According to Agrios (1996), one of mecha-nisms on resistant plant toward any disease is due to the structural feature, which makes the plants able to prevent, inhibit or restrain the growth and development of the pathogen. Singh (1986) stated that the avoidance of these leaves from disease-infection might be due to the morphological resistance, the existence of leaf trichome, less number of stomata and inconformity of stomatal opening and stomatal closing with the pathogen-infection process. The presence of trichomes on the leaf surface should therefore determine the attachment of the spores and further development of the hyphae. Trichomes apparently retain water on the plant surface and provide nutrients for microbial growth (Lindow and Brandl, 2003).

The second guideline of path-analysis explains that when a character has a positive correlation but its direct effect is negative and can be ignored, the indirect effect has created the correlation, so that all characters should be considered simultaneously in the selection. This indication occurs in width of stomata on the lower surface of leaves. The use of stomata width on the lower surface of leaf as selection criteria for downy mildew resistance should be done simultaneously with other characters which play indirectly.

The third guideline of path-analysis states if the correlation coefficient is negative but the direct effect is positive and high, restriction is to be imposed to nullify the undesirable indirect effects in order to make use of the direct effect. This indication occurs in character of stomata length on the upper surface of leaves.

Table 2. Genotypic correlation coefficient (thick) and phenotypic correlation coefficient (thin) of some characters in maize

Variable	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
X_1		**0.073**	**0.165**	**0.468***	**0.222**	**-0.042**	**-0.647****	_	**-0.432**	**0.681****
X_2	0.092		**0.465***	**-0.263**	**-0.031**	**-0.392**	**-0.592****	_	**1.137**	**-0.046**
X_3	0.107	0.449*		**-0.485***	**0.138**	**0.179**	**-0.305**	_	**-0.457***	**-0.437**
X_4	0.395	-0.243	-0.264		**0.585****	**-0.093**	**-0.238**	_	**-0.897****	**-0.157**
X_5	0.205	-0.136	-0.002	0.486*		**0.303**	**0.043**	_	**-0.392**	**-0.752****
X_6	-0.017	-0.262	0.044	-0.148	0.281		**0.545***	_	**-0.334**	**-0.66****
X_7	-0.428	-0.379	-0.479	-0.162	-0.09	0.345		_	**1.3**	**1.03**
X_8	0.021	0.004	-0.083	-0.034	0.098	-0.108	0.202		_	_
X_9	-0.164	0.464*	0.086	-0.406	-0.179	-0.048	-0.066	-0.155		**-0.195**
X_{10}	0.268	0.204	0.156	-0.143	-0.343	-0.143	-0.098	0.149	0.525*	

Remarks: X_1 = disease incidence (%); X_2 = density of trichomes per mm^2; X_3 = stomatal density on the upper surface of leaves per mm^2; X_4 = stomatal density on the lower surface of leaves per mm^2; X_5 = EC of leaves; X_6 = pH of leaves; X_7 = length of stomata on the upper surface of leaves (µm); X_8 = width of stomata on the upper surface of leaves (µm); X_9 = length of stomata on the lower surface of leaves (µm); X_{10} = width of stomata on the lower surface of leaves (µm)*, ** = Significant at 5% and 1%, respectively

Table 3. Direct and indirect effects of some leaf characters on downy mildew disease incidence

Character	**Direct effect**	**Indirect effect**								**Correlation coefficient**
		X_1	**X_2**	**X_3**	**X_4**	**X_5**	**X_6**	**X_7**	**X_8**	
X_1	0.516		0.551	-0.519	0.038	-0.019	-0.41	-0.088	0.003	0.073
X_2	1.185	0.24		-0.958	-0.167	0.009	-0.211	0.035	0.032	0.165
X_3	1.975	-0.136	-0.575		-0.708	-0.004	-0.165	0.069	0.011	**0.468**
X_4	-1.21	-0.016	0.164	1.155		0.015	0.03	0.03	0.055	0.222
X_5	0.048	-0.202	0.212	-0.184	-0.367		0.377	0.026	0.048	-0.042
X_6	0.692	-0.305	-0.361	-0.47	-0.052	0.026		-0.1	-0.075	**-0.647**
X_7	-0.077	0.587	-0.542	-1.772	0.474	-0.016	0.9		0.014	-0.432
X_8	-0.073	-0.024	-0.518	-0.31	0.91	-0.032	0.713	0.015		**0.681**

Renarks: X_1 = Density of trichomes; X_2 = Density of stomata on the upper surface of leaves; X_3 = Density of stomata on the lower surface of leaves; X_4 = EC of leaves; X_5 = pH of leaves; X_6 = Length of stomata on the upper surface of leaves; X_7 = Length of stomata on the lower surface of leaves; X_8 = Width of stomata on the lower surface of leaves

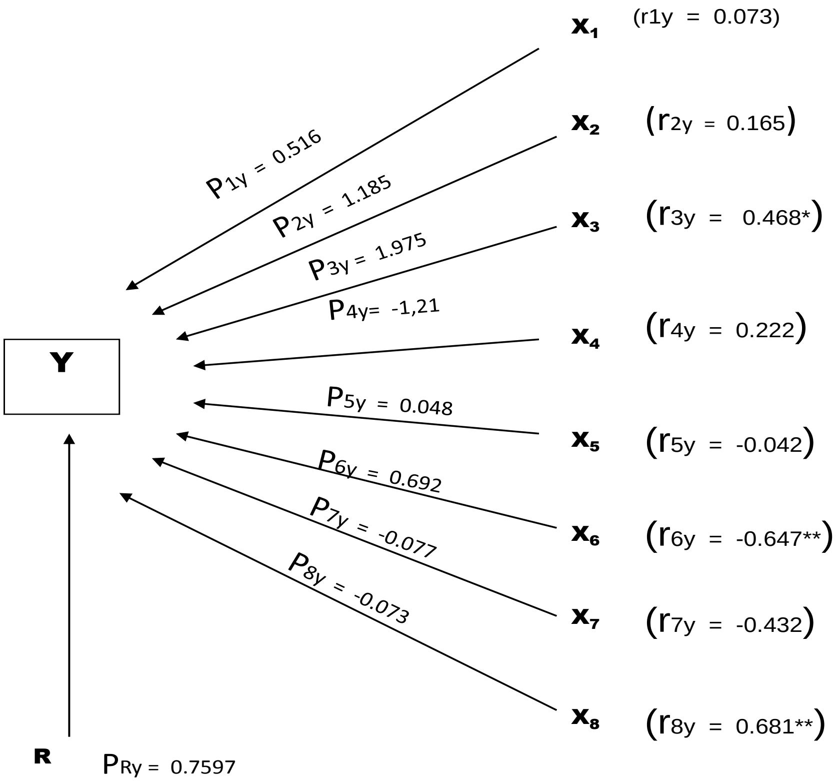

Figure 1. Path-coefficient diagram (P_{xy}), correlation coefficient (r_{xy}), character in density of trichomes (X_1), density of stomata on the upper surface of leaves (X_2), density of stomata on the lower surface of leaves (X_3), EC of leaves (X_4), pH of leaves (X_5), length of stomata on the upper surface of leaves (X_6), length of stomata on the lower surface of leaves (X_7), width of stomata on the lower surface of leaves (X_8), and contributing factors not observed (R) on disease incidence (Y).

Table 4. Heritability estimate of some characters in maize

No.	Characters in Corn	Heritability (%)	Criteria
1.	Disease incidence	94.12	High
2.	Density of stomata on the upper surface of leaves	37.95	Moderat
3.	Density of stomata on the lower surface of leaves	91.36	High
4.	Density of trichomes	50.00	Moderat
5.	Length of stomata on the upper surface of leaves	46.74	Moderat
6.	Length of stomata on the lower surface of leaves	18.79	Low
7.	Width of stomata on the lower surface of leaves	13.04	Low
8.	pH of leaves	93.98	High
9.	EC of leaves	76.54	High

Figure 1 describes that based on the observed-leaf characters; only 24% of them are able to describe diversity of downy mildew-disease incidence, while other factors outside the observed characters still have greater influence (76%) such as microenvironment, chemical interactive process and other factors.

The estimation heritability of each character is presented in Tabel 4. Disease incidence, stomatal density on the lower surface of leaves, EC and pH of leaves had high heritability. The moderate heritability value for stomatal density was found on the upper surface of leaves, the trichomes density and lenght of stomata on the upper surface of leaves, while the lenght and width of stomata on the lower surface of leaves had lower heritability. Character used as the selection criteria should have high heritability in order to be inherited to the next generation.

CONCLUSION AND SUGGESTION

CONCLUSION

Based on the result of the correlation analysis, path analysis and heritability, the qualified characters as effective selection criteria for resistance to downy mildew are trichome density, stomatal density on the upper and lower surface of leaves. In relation to maize breeding program, low trichome density and stomatal density characters enhance downy mildew resistance.

SUGGESTION

High value of the residue effects indicates the need of further research in order to find out other factors, which influence the maize resistance to downy mildew.

ACKNOWLEDGMENT

This manuscript is a part of the first authors dissertation for Doctoral Program at University of Brawijaya. Gratitude is expressed to Directorate General of Higher Education for funding this research through BPPDN.

REFERENCES

Agrios, G.N. 1996. *Phytophatology* (Translated by M. Busnia and T. Martoredjo), Gadjah Mada University Press. Yogyakarta.

Baihaki, A. 1999. The designing technique and breeding research analysis. Written Summary of Lectures. Agriculture Faculty, UNPAD.

Basuki, N. 1986. Estimation of genetic parameter and relationship between yield amd some agronomical features, as well as diallel cross analysis in sweet potato (*Ipomoea batatas* (L) Lamb.) *Dissertation.* Postgraduate Program Bogor Agriculture Institute.

Baswarsiati. 1994. Assessment on stomata and leaf trichome as resistance character in some clones of grapes toward *Plasmopara viticola.* J. Zuriat 5(1): 29-35.

Dewey, D.R. and K.H. Lu. 1959. A correlation and path-coefficient analysis of com-ponents of crescent wheat grass seed production. J. Agron. 51: 515-518.

Diz, D.A., D.S. Wofford and S.C. Schank. 1994. Correlation and path-coefficient analysis of seed-yield components in pearl millet x elephant grass hybrids. Theor. Appl. Genet. 89: 112-115.

Hee, Y.C. and J.C. Wynne. 1983. Heritabilities and genetic correlations for yield and quality traits of advanced generations in a cross of peanut. *Peanut Sci*. 10: 13-17.

Lal, S. and I.S. Singh, 1984. Breeding for resistance to downy mildews and stalk rots in maize. Theor.Appl.Genet. 69: 111–119.

Lindow, S.E. and M.T. Brandl. 2003. Microbiology of the phyllosphere. Applied and Environmental Microbiolgy 69: 1875-1883.

Makkulawu, A.T. 2007. Inheritance of resistance on downy mildew *Peronosclerospora maydis* (Rac.) Shaw in maize (*Zea mays* L.) and correlation between number of stomata and degree of resistance. Indonesian Agricultural Research Abstracts. 24 (1): 27.

Masnenah, E.,H.K. Murdaningsih, R. Setiamihardja, W. Astika and A. Baihaki. 2004. Correlation between some morphology characters and resistance of the soybean to rust infection. J. Zuriat 15 (1): 40-46.

Miller, P.A., J.C. Williams, H.F. Robinson and R.F. Comstock. 1958. Estimation of genotypic and environmental variances and covariance's in upland cotton and their implication in selection. J.Agron. 50: 126-131.

Niks, R.E. and D. Rubiales. 2002. Potentially durable resistance mechanisms in plants to specialized fungal pathogens. Euphytica 124: 201-216.

Singh, R.K. and B.D. Chaudhary. 1979. Biometrical Method in Quantitative Gene-tic Analysis. Kalyani Pub. Ludhiana, New Delhi.

Singh, D.P. 1986. Breeding for Diseases Resistance and Insect Pest. Springer-Verlag. Berlin, Heidelberg, New York, London, Paris, Tokyo.

Smith, P.H., E.M. Foster, L.A. Boyd and J.K.M. Brown. 1996. The early development of *Erysiphe pisi* on *Pisum sativum* L. Plant Pathology 45: 302-309.

Sudjono, M.S. 1988. The maize disease and its control. *In* Subandi, M. Syam, and A. Widjono. Maize. Puslitbangtan Bogor. p. 205-241.

Triharso, T. M. and L. Kusdiarti. 1976. Recent problem and studies on downy mildew of maize in Indonesia. The Kasetsart Journal. 10(2): 101-105.