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Kustanto H

Testing of resistance to downy mildew on several sweet corn inbred lines of 7th generation

Abstract. Sweet corn (*Zea mays saccharata* Sturt) is one of the most well-known horticultural crop commodities and can adapt to various climates and environments. Efforts to control downy mildew disease continue to be carried out in collaboration with various parties in different ways, including genetic improvement of the varieties planted. The purpose of this study was to test the resistance of the 7th generation of sweet corn inbred lines to downy mildew and to find out the phenotypic characters that affect downy mildew. The new inbred lines obtained from the results of a long breeding process will be better tested for resistance to downy mildew before crosses are carried out among these inbred lines to guarantee better the development of new varieties of sweet corn that are resistant to downy mildew. The experiment was conducted from August to November 2019 in Getasrejo, Grobogan, and Central Java. The material consisted of 10 kinds of maize inbred line seeds and two comparison varieties. The experiment was carried out using a randomized block design with three replications. One of the obstacles that is difficult to overcome in sweet corn cultivation is downy mildew disease (*Peronosclerospora spp.).* The conclusions of this study were: (1) Inbred lines HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04, and HSJM 01 were categorized as highly resistant genotypes. HSJM 09 can be categorized as a resistant inbred line. Inbred lines HSJM 10 and HSJM 03 were classified as moderately resistant genotypes. (2) The characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining selection criteria against downy mildew disease on sweet corn.

Keywords: Attack · Downy mildew · Resistance · Sweet corn

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Kustanto H[1]*
[1] Faculty of Agriculture, Universitas Wahid Hasyim, Jl. Menoreh Tengah X/22, Sampangan, Gajahmungkur, Semarang 50232, Indonesia
*Correspondence: heri.kustanto@unwahas.ac.id

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Introduction

Sweet corn (*Zea mays saccharata*) is one of the best-known horticultural commodities. Sweet corn could adapt to various climates and environments so that it could be bred easily by farmers. The sweet corn seeds contain solid components (27.3%) and water (72.7%). The solid parts of the sweet corn seed contain hydrocarbons (81%), protein (13%), fat (3.5%), and other materials (2.5%) (Alfandri et al., 2014; Swapna et al., 2020). Also, sweet corn seeds contain vitamins that benefit the human body (Szymanek, 2012).

As is the case with forage corn, breeding sweet corn faces many obstacles, including the onset of downy mildew (*Peronosclerospora spp*). Downy mildew is the main disease that reduces sweet corn productivity. Today, it is reported that downy mildew has infected new superior varieties of various corn types at the start of the growth phase (Daryono et al., 2018). In addition to the severe infestation, the disease always exists, is latent, and threatens the production of sweet corn (Daryono et al., 2018; Prasetyo et al., 2021). The causes of latent downy mildew infestation are the presence of early inoculum sources, asynchronous corn planting patterns, and the application of susceptible varieties (Hendrayana et al., 2020; Prasetyo et al., 2021).

Efforts to control downy mildew continue to be carried out in collaboration with various parties. Downy mildew can be controlled through crop rotation, eradication, seed treatment, organic and inorganic pesticides, and the use of resistant superior varieties (Alfandri et al., 2014; Mariani et al., 2019; Pajrin et al., 2013; Sonhaji et al., 2013). Efforts to control the disease with superior varieties are made through the breeding process. The conventional breeding process was started by preparing basic population material, the inbred line, and combining ability selection and resistance selection to biotic and abiotic factors. A method of breeding resistance to biotic disturbance is testing the inbred lines against resistance to downy mildew infestation (Ali et al., 2019; Daryono et al., 2018; Kustanto et al., 2012).

It is expected that the selection of lines that are resistant to downy mildew will be able to support the hybrid sweet corn assembly, which is more resistant to downy mildew, so that it can be produced easily and economically (Daryono et al., 2018; Janruang & Unartngam, 2018). The new inbred lines, which were derived from a long breeding process, are better tested for resistance to downy mildew before being crossed between inbred lines. The objectives of the study were to test the resistance of the sweet corn inbred lines of the 7th generation to downy mildew and to find out the phenotypic characteristics of the corn that relate to downy mildew disease to facilitate the determination of selection criteria. By knowing the lines that are resistant to downy mildew, it is expected that new varieties of sweet corn that are more resistant to downy mildew will be produced.

Materials and Methods

The planting materials comprised 10 sweet corn inbred lines of the 7th generation that had been developed by CV. Hakako Seed. The inbred lines were: HSJM 01, HSJM 02, HSJM 03, HSJM 04, HSJM 05, HSJM 06, HSJM 07, HSJM 08, HSJM 09, and HSJM 10, along with 2 comparison varieties, namely VP 01 and VP 02. VP 01 is a commercial sweet corn variety that has high production and better resistance to downy mildew, while VP 02 is a forage corn variety that has high production but is highly susceptible to downy mildew. Other materials were NPK at a dose of 300 kg/ha and Urea at a dose of 50 kg/ha. The devices used were dibbles, meters, Crown brand manual scales, and hand counters. The experiment was conducted from August to November 2019 in Getasrejo, Grobogan, and Central Java. The experiment was carried out using a randomized block design with three replications. The spacing was 70 x 20 cm, and the population per plot was 100 plants.

Planting was done using two seeds per planting hold. Thinning was carried out at 10 days after planting (DAP) to obtain an appropriate population of 100 plants per plot. The fertilization doses were Phonska 300 kg/ha and Urea 250 kg/ha. The first fertilization was applied at 10 DAP using phonska at 300 kg/ha. The second fertilization was applied at 21 DAP using urea at 200 kg/ha, and the third was applied at 35 DAP using urea at 50 kg/ha. The Inoculum was prepared three weeks before the experiment was carried out. The susceptible varieties (*spreader row*) were planted in 4 rows around the replication plot and 4 rows between each replication. After the *spreader row infection* was found to be 20-30%, it was followed by experimental planting. To strengthen the infection, conidia, which were dissolved in sugar solutions, were sprayed on leaves that were infected by downy mildew. And then, the solutions were sprayed on the experiment field when the plants were 10 and 12-days old at 3-4 a.m. in the morning (Hendrayana et al., 2020; Kim et al., 2016).

Some characters that being observed were: (a) leaf width (cm), measured from outside of the right side and the left side (cm), (b) leaf length (cm), measured from the base to the tip of the leaf (cm), (c) leaf wet weight (g), by weighing the wet weight of freshly picked leaves, (c) leaf dry weight, by weighing the leaf dry weight after sun-dried for 3 days until the leaves crumble when crushed, (d) moisture content (%) of the leaves is calculated by the formula: MC = (DW-WW) x 100%, in which MC = moisture content, DW = leaf dry weight, WW = leaf wet weight, (d) number of leaves (pcs), calculate number of leaves from the base to the tip of the flag leaf, (e) plant height (cm), calculate the plant height from the ground level to the base of the flag leaf. Observation on the intensity of the downy mildew infestation was conducted at 14, 21, 28, 35, and 42 DAP. The percentage of the downy mildew infestation was calculated by the formula: I = (A/B) x 100%. In which: I= percentage of the downy mildew infestation, A= number of plants infected by downy mildew, and B= number of plants observed in each tested genotype. Criteria for plant resistance are as follows: (a) Highly Resistant (HR) is the disease onset 0 - 10%, (b) Resistant (R) is the disease onset 10 - 20%, (c) Moderately Resistant (MR) is the disease onset 20 – 40%, (d) Susceptible (S) is the disease onset 40 - 60% and Highly Susceptible (HS) is the disease onset 60 – 100% (Daryono et al., 2018; Khoiri et al., 2021).

Data processing used Anova at level 5%. Post-Hoc Test was carried out to determine the effect of significant difference treatment using Duncan Multiple Range Test (DMRT) at level 5%. Relationship among the observed variables was analyzed with correlation by the formula: rp= $(Cov_{xy})/(\sqrt{\sigma^2 x . \sigma^2 y})$. Where: rp= coefficient of correlation, Cov_{xy} = trait covariance x and y, $\sigma^2 x$ = trait variance x, dan $\sigma^2 y$= trait variance y (Singh & Chaudary, 1998; Kustanto, 2022). Calculation for the correlation values are: (a) not strong (0.1 – 0.2), (b) rather strong (0.2 – 0.4), (c) strong enough (0.4 – 0.6), (d) strong (0.6 – 0.8) and (e)very strong (0.8 – 1.0).

Results and Discussions

Performance of the Vegetative Characters. Results for the combined analysis of variance showed significant influence on characters of leaf width, leaf wet weight, leaf dry weight, leaf moisture content, plant height, and infestation level of downy mildew (*Peronosclerospora* spp). Results for the combined analysis of variance on characters of leaf length and number of leaves per plant did not show any significant influence. Results for analysis of variance, coefficient of variance, and influence of the significantly different treatments with Duncan's Multiple Range Test are presented in Table 1.

Results for the combined analysis of variance toward leaf length showed significant influence among the tested genotypes. Leaf length in all treatments ranged 78.73 – 91.33 cm. HSJM 08 had the lowest leaf length, 78.73 cm, while HSJM 04, HSJM 03, and VP 02 had the highest leaf lengths 91.33, 90.40, and 90.00 cm, respectively, and were not significantly different with HJSM 01, HSJM 02, HSJM05, HSJM 09 and VP 01. Leaf width did not show significant difference among the tested genotypes. In comparison with the character of leaf width, leaf length showed variations on the tested genotypes. Inbred lines of corn showed different appearances in leaf sizes, which are different from the comparative varieties, the hybrids. Sari and Hafsah (2022) reported that crop varieties have a specific characteristic that can be set as superiority value of a given variety. Factors which affect different character in each line can be affected by genetic factors from heredity.

Results for the combined analysis of variance on leaf wet weight showed significant influence among the tested genotypes. Leaf wet weight ranged 12.40 – 16.04 g. The lowest leaf wet weight was 12.40 g for HSJM 10 and it was not significantly different from HSJM 06, HSJM 07, HSJM 08, VP 01 and VP 02. The highest wet weight was 16.04 for HSJM 02 and it was not significantly different from HSJM 01, HSJM 03, HSJM 05, HSJM 08 and HSJM 09. Results for the combined analysis of variance toward leaf dry weight showed significant influence among the tested genotypes. The leaf dry weight ranged from 3.07 – 4.34 g. The lowest leaf dry weight was 3.07 g for VP 02 and it was not significantly different from HSJM 10 and VP 01. The highest leaf dry weight was 4.34 g for HSJM 02 and it was

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not significantly different from HSJM 01, HSJM 03, HSJM 05, HSJM 08 and HSJM 09. Results for the combined analysis of variance toward moisture content in leaf showed significant influence among the tested genotypes. The leaf dry weight ranged from 24.52 – 29.60 g. The lowest moisture content was found on HSJM 07 and HSJM 08, 24.52 and 24.80 g, respectively, and they were not significantly different from HSJM 04, HSJM 05, HSJM 06, HSJM 09 and VP 02. The highest moisture content in leaf was 29.60 on HSJM 02 and was not significantly different from HSJM 01, HSJM 03, HSJM 04, HSJM 06, HSJM 10, VP 01 and VP 02. Characters of leaf wet weight, leaf dry weight and moisture content in leaf showed many variations among the tested genotypes. Not only differences among lines, characters of leaf wet weight, leaf dry weight, and moisture content of leaf also have obvious and significant differences compared to the hybrid variety as the comparison. The growth process is affected by internal and environmental factors. The internal factor is the used genotypes, and the environmental factors are temperature, light, water, and etc. (Mariani et.al. 2019).

Number of leaves per plant did not show any significant difference and ranged 12.20 – 13.30. Results for the combined analysis of variance toward plant height showed significant influence among the tested genotypes. The plant heights ranged 160.40 – 185.40 cm. The lowest plant heights were found on HSJM 02, HSJM 04, HSJM 10 for about 160.63, 161.97, and 160.40 cm, respectively, and were not significantly different from HSJM 03, HSJM 06, HSJM 09, VP 01 and VP 02. The highest plant heights were found on HSJM 01, HSJM 05, HSJM 07 and HSJM 08 for about 195.40, 180.07, 180.4, 180.30 cm, respectively, and were not significantly different from HSJM 03, HSJM 06, HSJM 09, VP 01 and VP 03. Plant heights showed a difference among the tested genotypes. The tested inbred lines of the sweet corns showed high variations among the tested genotypes on character of the plant height. The inbred lines have a homozygous genetic composition and tend to have weak adaptation to the environment (Ali et al., 2019). The hybrid varieties have heterosis effects and high vigor and can adapt well to less supportive environments than their parents (Agustiani et al., 2019; Xiao et al., 2021).

Table 1. Characters of vegetative components of the tested genotypes

Genotype	LL		LW	LWW		LDW		LM		NLP	PH	
HSJM 01	88.17	bcd	8.23	14.71	bcde	3.95	cde	28.59	bc	12.63	185.40	b
HSJM 02	89.30	cd	7.87	16.04	e	4.34	e	29.60	c	13.30	160.63	a
HSJM 03	91.33	d	8.67	15.21	cde	4.11	de	28.80	bc	12.83	170.40	ab
HSJM 04	90.40	d	7.80	12.91	abc	3.68	bcd	27.06	abc	13.27	161.97	a
HSJM 05	83.70	abcd	8.17	14.67	bcde	3.65	bcd	25.67	ab	13.23	180.07	b
HSJM 06	81.03	abc	8.13	12.92	abc	3.63	bcd	26.33	abc	12.23	175.50	ab
HSJM 07	80.57	ab	8.13	13.17	abcd	3.70	bcd	24.52	a	12.37	180.40	b
HSJM 08	78.73	a	8.13	14.00	abcde	4.07	de	24.80	a	12.40	180.30	b
HSJM 09	84.93	abcd	8.00	15.61	de	4.10	de	25.62	ab	12.97	174.47	ab
HSJM 10	85.60	abcd	8.73	12.40	a	3.43	abc	28.88	bc	12.60	160.40	a
VP 01	85.27	abcd	8.17	13.37	abcd	3.37	ab	28.37	bc	12.20	170.50	ab
VP 02	90.00	d	8.77	12.73	ab	3.07	a	26.73	abc	12.87	173.90	ab
Mean	85.75		8.23	13.98		3.76		27.08		12.74	172.83	
CV (%)	5.34		8.19	9.34		8.16		6.61		6.79	5.33	
Sign.	*		ns	**		**		*		ns	*	

Notes: LW: Leaf Width, LL: Leaf Length, LWW: Leaf Wet Weight, LDW: Leaf Dry Weight, LM: Leaf Moisture, NLP: Number of Leaves per Plant, PH: Plant Height.

Resistance to Downy Mildew. Results for the combined analysis of variance toward percentage of the downy mildew infestation showed significant influence among the tested genotypes. Percentage of the downy mildew onset ranged 2.67 – 50.00%, as presented in Table 2. The lowest percentage of the downy mildew infestation was 2.67% on HSJM 08 and it was not significantly different from HSJM 06, HSJM 07 and HSJM 02 that were 3.33%, 3.33% and 6.00%, respectively. The highest percentage of downy mildew infestation was 50% on the comparison variety 02. Percentage of the downy mildew infestation was highly varied from 7, 14, 21, 27 and 28 DAP and showed high coefficient of variances that ranged 36.60 – 46.10%. The incubation periods were varied due to some factors, for instance, pathogen virulence, host resistance, and environmental conditions, such as temperature and humidity that support the pathogen development (Ulhaq and Masnilah, 2019). Percentage of the downy mildew infestation at the age of 34 and 42-day old tended to be stable with lower coefficient of variances, 17.13 – 18.43%. According to the criteria, the comparison genotype of VP 02 is the inbred line which is susceptible, while the inbred lines of HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04 and HSJM 01 are characterized as highly resistant genotypes. HSJM 09 is characterized as resistant inbred line. While the inbred lines of HSJM 10, HSJM 03 and VP 01 are categorized as moderately resistant.

This fact showed that the tested sweet corn inbred lines had good resistant to downy mildew and superior. Inbred lines that have good resistant to downy mildew can be used as the parent in the hybrid varieties assembly (Daryono et al., 2018; Khoiri et al., 2021). Besides the genetic factor, the environmental factors also affect severity of the downy mildew infestation. The genetic resistance is brought about by heredity factor and also it is presumed to be affected by the morphological factors (Ulhaq and Masnilah, 2019). The environmental factors are temperature, humidity, and wind direction. Wind velocity and location of the inoculum determine the occurrence of downy mildew epidemics, while the wind direction affects the infection rate on sweet corn (Purwanto et al., 2016).

Values for coefficient of variance at 7 DAP, 14 DAP, 21 DAP, 27 DAP, and 28 DAP were very high, while at 34 DAP and 42 DAP were medium. The fact might be caused by different incubation periods among genotypes. The incubation period is the time between the onset of infection and the onset of symptoms. The crop which resistant to a disease will indicate longer incubation period (Ulhaq and Masnilah, 2019)

Table 2. Characters of the infestation percentage and categories of the downy mildew infestation on the tested genotypes.

Genotype	% infestation of downy mildew							Category
	7 DAP	14 DAP	21 DAP	27 DAP	28 DAP	34 DAP	42 DAP	
HSJM 01	1.00 ab	1.00 ab	4.00 ab	1.67 a	7.33 abcd	8.00 c	9.00 cd	HR
HSJM 02	1.00 ab	1.00 ab	1.33 a	11.67 abc	4.67 abc	6.00 bc	6.00 abc	HR
HSJM 03	5.33 ab	5.33 ab	7.00 abc	2.33 a	19.00 ef	33.00 f	34.33 f	MR
HSJM 04	1.33 ab	1.33 ab	1.33 a	3.67 ab	4.00 ab	6.33 bc	7.33 bc	HR
HSJM 05	1.67 ab	1.67 ab	3.00 ab	1.00 a	4.00 ab	6.00 bc	8.00 c	HR
HSJM 06	0.00 a	0.00 a	0.33 a	1.33 a	1.33 a	2.67 ab	3.33 ab	HR
HSJM 07	1.00 ab	1.00 ab	1.00 a	1.00 a	1.67 a	2.33 ab	3.33 ab	HR
HSJM 08	0.67 ab	0.67 ab	0.67 a	12.00 abc	1.67 a	1.67 a	2.67 a	HR
HSJM 09	6.33 ab	6.33 ab	10.67 bc	11.00 abc	14.33 cde	15.33 d	13.67 d	R
HSJM 10	7.33 b	7.33 ab	8.67 abc	8.67 abc	13.00 cde	20.33 e	22.00 e	MR
VP 01	3.67 ab	3.67 ab	4.67 abc	17.67 c	12.33 bced	19.00 de	22.67 e	MR
VP 02	8.67 b	8.67 b	13.33 c	6.41	24.33 f	42.67 g	50.00 g	S
Mean	3.17	3.17	4.67	6.42	8.97	13.61	15.19	
CV (%)	46.10	46.07	36.60	40.96	28.34	18.43	17.13	

Notes: %: percentage, DAP: day after planting, HR: Highly Resistant, MR: Moderately Resistant, R: Resistant and S: Susceptible

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Correlation among the observed traits. Correlation between the observed characters and percentage of the downy mildew infestation showed varying outcomes. The character of leaf width showed strong correlation with percentage of downy mildew infestation and has positive value (0.57). Positive correlation refers to an increase in one trait followed by an increase in another trait. On the contrary, negative correlation refers to an increase in one trait will be followed by a decrease in other trait (Pudjiwati et al., 2013; Novrika et al., 2016; Sudjana, 1992).

The character of leaf wet weight. Leaf length had strong correlation with percentage of downy mildew infestation and had positive value. Wet weight did not correlate with percentage of the downy mildew infestation. Leaf dry weight correlated with percentage of downy mildew infestation and had negative value. Leaf moisture correlated rather strongly with percentage of the downy mildew infestation. Number of leaves per plant and plant height did not correlate with percentage of the downy mildew infestation, and these are presented in Table 3.

Correlation among characters showed relationship among values obtained by each character. Correlation among characters may has positive or negative values. Positive value means that the character is directly proportional to the change in value. While the negative correlation means that the character is inversely proportional to the change in value (Sudjana, 1992; Kustanto, 2023). Characters of leaf width and leaf length correlated with percentage of the downy mildew infestation in which the increase in sizes of the leaf width and leaf length can increase percentage of the downy mildew infestation on the sweet corn. Leaf wet weight did not affect the percentage of downy mildew infestation. Leaf dry weight moderately affected the percentage of downy mildew, which the increase of leaf dry weight will reduce the percentage of downy mildew infestation. Moisture content in leaf rather strongly affected the percentage of downy mildew infestation, in which the higher the moisture content in leaf will increase the infestation of downy mildew. Number of leaves per plant and plant height did not affect the percentage of downy mildew infestation. Characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining the selection criteria against downy mildew infestation on sweet corn. The production of sweet corn is affected by the production variable. In general, the influence of variety on production is in line with the effect of variety on disease intensity, in which the lower intensity of the disease, the higher yield will be produced (Ginting et al. 2020). Results of the research showed that cytoplasm constitution of the female parent affects the inheritance of downy mildew in the hybrids (Elmoghazy et al, 2018).

Table 3. Correlation among the observed variables

	LW	LL	LWW	LDW	LM	NLP	PH	% DMI
LW	1							
LL	0.23	1						
LWW	0.22	-0.32	1					
LDW	0.00	-0.48*	0.83**	1				
LM	0.71**	0.24	0.20	0.10	1			
NLP	0.60**	-0.23	0.47*	0.29	0.20	1		
PH	-0.49*	0.00	0.10	0.02	-0.58**	-0.38*	1	
% DMI	0.57**	0.79**	-0.18	-0.51	0.32	0.05	-0.19	1

Notes: LW: Leaf Width, LL: Leaf Length, LWW: Leaf Wet Weight, LDW: Leaf Dry Weight, LM: Leaf Moisture, NLP: Number of Leaves per Plant, PH: Plant Height, % DMI: Percentage of Downy Mildew Infestation.

Conclusions

Based on results of the research, some conclusions are drawn as follow:

1. Inbred lines of HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04, and HSJM 01 are categorized as highly resistant genotypes. HSJM 09 is categorized as resistant inbred line. HSJM 10 and HSJM 03 inbred lines are categorized as moderately resistant genotypes.
2. Characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining the selection criteria against downy mildew infestation on sweet corn.

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