

A promising breeding strategy for tomato resistance to *Cucumber Mosaic Virus* based on genetic analysis

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Abstract. Infection of CMV can reduce tomato yield. One of the controlling methods to solve the problem by using a resistant variety. However, the existence of resistant variety was still not available yet. Therefore, we needed the breeding program for resistance tomato to CMV to obtain a new variety. Information of previous genetic analysis results can be used to determine a promising strategy. Two tomato genotypes namely R8 51-12 as resistant and RG-57 as susceptible accession were crossed reciprocally to obtain F₁, F_{1R}, BC_{1.1}, BC_{1.2}, F₂. Then, all of the breeding materials were inoculated by strain CMV-2 and observed on symptom disease and virus concentration. Genetic analysis results showed that there was no maternal effect and the resistance controlled by two major genes as recessive, but there was the epistatic effect on disease symptom as a selection parameter. Based on these information, the tomato's breeding strategy were to use a susceptible genotype as female parent, while a resistant genotype as male parent. Phenotypically disease symptom selection can be conducted in the middle or late generation. However, the selection should be supported by serologically using the Elisa test. The breeding program could be addressed as a F₁ hybrid variety.

1 Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the important vegetables economically in Indonesia. Tomato has many functions, not only as a fresh consumption, but also as a raw material for the food industry, cosmetics, and pharmacy [1]. In every year tomato consumption demand increases in line with the growing population number and industries. Statistical data informed that tomato demand increased by 5% for the last five years (2015-2020) [2].

Tomato's productivity has to be increased to be able to fulfill the consumer's demanding. Nowadays tomato's productivity in Indonesia is around 15.00 t ha⁻¹ [2]. However, it is still low compared with other countries such as Spain, Japan, and China. One factor causal agent that can reduce tomato productivity is a disease caused by *Cucumber Mosaic Virus* (CMV). Tomato yield could decrease in the range of 30-60% by CMV infection, even until 100% if an infection occurred in the seedling stage [3].

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Resistant variety can be used to overcome this problem as a control method because easy, cheap, safe, and can be implemented in tomato cultivation at any time [4]. However resistant variety with preference consumer's attribute is still not available yet until now. Theoretically, the resistant variety can be obtained through the evaluation of selected parents. The screening evaluation results of inoculated 14 germplasm tomato genotypes by strain CMV-2 showed a resistance variability level [5]. The CMV incubation period to the germplasm tomatoes in the range 7 – 27 days after inoculated. There were two resistant genotypes namely: R8 51-12 and R8 110-11. This information is critical in implementing a tomato's resistance breeding program against CMV. Thus, it is better to comprehend genetic analysis of the resistance character as a preliminary study to determine a promising breeding strategy. The information includes maternal effect existence, number and action of resistance genes, genetic component's role, and their heritability.

Therefore, the purpose of this research was to comprehend genetic analysis of the tomato resistance against CMV to determine a promising breeding strategy. It is expected some tomato resistant varieties will be obtained in the future.

2 Material and methods

The study was conducted at Indonesian Vegetables Research Institute (IVEGRI), Lembang from April to December 2017. The study was carried out in a screen house to avoid other viral infections, while serological detection in a virology laboratory. The used genetic material were two pure tomatoes lines with criteria resistant (R8 51-12 with disease intensity $\leq 10\%$) and very susceptible (RG-57 with disease intensity $> 50\%$). Previously both of them were inoculated by strain CMV-2 as the most virulent. Strain CMV-2 was known to infect tomato plants in a wide range and caused the most severe symptoms [3]. Research activities included sowing, planting, crossing, screening, and parameter observation as follows:

2.1 Sowing

The tomatoes seeds were sown in plastic cups containing a mixture of soil: sand: sterile manure with comparison 2: 1: 2. Fertilization and pest-disease protection were applied at seedling phase two weeks after sowing using leaf fertilizer (1.5 g/l) and pesticides. Watering was done twice a day in the morning and afternoon.

2.2 Planting

After 35 days the seedlings were transferred to polybags (ϕ 40 cm) with media soil: sand: sterile manure = 1: 1: 1 referred volume. Inorganic fertilizer was applied at planting using NPK (15-15-15) as much of 3 g/polybag. At two weeks after planting was applied subsequent fertilization 1 g urea and 4 g TSP. The next subsequent fertilization was applied at one and two months after the first subsequent fertilization with NPK supply (3 g/plant). Plant maintenance included pest-disease protection by pesticides spraying one a week and plant watering every day in the morning.

2.3 Crossing pollination

Tomato is a self pollination crop, thus emasculation should be done to support succes crossing pollination. Usually the crossing activity was carried out in the morning (09.00-11.00). Female parent's flower was selected in condition still buds, but it's size already full.

At this stage the stigma was predicted already receptive, but the anther was still not yet broken out. Emasculation was done by removing all stamens carefully using small tweezers.

Two pure tomatoes lines were crossed with genotype R8 51-12 as a male and RG-57 as a female parent, then be obtained the F₁ population. The reciprocal crossing was also carried out to obtain the F_{1R} population. After that the F₁ population was planted, some plants occurred self-pollination to get F₂ population and some others were carried out backcrossing with resistant (R8 51-12) and susceptible parents (RG-57) to get a BC_{1.1} and BC_{1.2} populations respectively. Thus, the obtained seed material consisted of resistant (P₁) and susceptible parents (P₂), F₁, F_{1R}, BC_{1.1}, BC_{1.2}, and F₂ populations.

2.4 Screening

Preparation before screening included multiplication inoculum strain CMV-2 in tobacco plant (*Nicotiana tabacum* cv *Xanthi* nc) for four weeks inside rearing insect box. Inoculum was prepared by grinding the infected leaf tobacco added phosphate buffer (0.01 M, pH 7) with comparison 1 : 3 (weight/volume) [6]. Then the extract was filtered and be used as an inoculum.

Inoculation strain CMV-2 was carried out in the seedling stage as a critical point (\pm two weeks after sowing) by rubbing cotyledon's leaf surface using carborundum (600 mesh) to occur micro wounds with a cotton bud carefully, thus the inoculum can come into the cell without damage it. Reinoculation was carried out a week later to ensure that all plants occurred infection.

The minimum number of each population P₁, P₂, F₁ and F_{1R} were ten plants based on the assumption they had been high homozygote. But for each population BC_{1.1} and BC_{1.2} were 25 plants. Especially for F₂ population number was determined based on the formula [7]:

$$n = (\log F) / (\log q) \quad (1)$$

n = minimum number of the needed plants;

F = level 0.05;

q = failure probability level to obtain the desired genotype.

If resistance to CMV controlled by three genes, so the minimum number for F₂ population is $n = \log 0.05 / \log (63/64) = 191$ plants.

2.5 Observation

Observations were conducted visually on some parameters i.e., incubation periods, disease symptoms index, disease intensity, and virus concentration level.

2.5.1 The Incubation periods

The incubation period was calculated as days number of beginning inoculation until occurred the first symptoms in each tested plant, then averaged. Observation's time were limited until 25 days after inoculation as the highest incubation period to occur the systemic symptoms [8].

2.5.2 Disease symptom

Disease symptom parameter was observed on the youngest leaves but were already fully developed. The scoring was as follows [9]: 0 = no symptom (healthy); 1 = < 10% leaf area

occurred mosaic; 2 = ≤ 50% leaf area occurred mosaic; 3 = > 50% leaf area occurred mosaic, but no malformation; 4 = occurred severe mosaic, malformation, stunted to death.

2.5.3 Disease intensity

Disease intensity indicated damage rates in the total population due to pathogen infection. Disease intensity was calculated based on observation disease symptoms scoring until 25 days after inoculation calculated by the formula as follows [10]:

$$DI = (\sum (n \times v) / N \times V) \times 100\% \quad (2)$$

DI = disease intensity;
n = plants number in each disease scoring,
v = disease scoring in each observed plant;
N = total number of observed plants;
V = the highest disease scoring; Criteria of plant resistance:
DI = 0 (immune); DI = x ≤ 10% (resistant); DI = 10 < x ≤ 20% (rather resistant);
DI = 20% < x ≤ 30% (rather susceptible); 30% < x ≤ 50% (susceptible);
DI = x > 50% (very susceptible).

2.5.4 Virus concentration

Parameter virus concentration level was carried out by *enzyme linked immunosorbance assay* (Elisa) test indirectly with the reflected virus concentration by A₄₀₅ nm value. Elisa test was conducted on fully youngest leaf at 25 days after inoculation (DAI) [6].

2.6. Data analysis

Data analysis was carried out on the existence maternal effect, dominance degree, estimation of number and action genes, and heritability value.

2.6.1 Maternal effect

The existence of maternal effect was determined based on the t test at 5% level of mean population F₁ and F_{1R} with same population (n_{F1} = n_{F1R}) by the formula as follows [11]:

$$T_{cal} = (x'F_1 - x'F_{1R}) / Sd' \quad (3)$$

x'F₁, x'F_{1R} = mean of F₁ and F_{1R};
Sd' = standard deviation of range two means; If t_{cal} ≤ t_{tab} 0.05 it's mean not significant

2.6.2 Dominance degree

The dominance degree was calculated to estimate action of the resistance genes based on potential formula ratio (hp) as follows [12]:

$$hp = (F_1 - MP) / (HP - MP) \quad (4)$$

hp = potential ratio;
F₁ = average resistance value F₁;
HP = average resistance value of resistant parent;

MP = mean resistance of two parents; the dominance degree:

hp = 0 (there is no dominance); hp = 1 or hp = -1 (full dominance or full recessive), $0 < hp < 1$ (semi dominance); $-1 < hp < 0$ (recessive with unfull gene action); $hp > 1$ or $hp < -1$ (overdominance).

2.6.3 Estimation of number and action genes

Estimation of number the resistance genes was analyzed using the Mendelian genetic approach by comparison the observed F₂ population phenotypic frequency with the expected phenotypic ratio and tested fit properly with chi square test. A joint scaling test was carried out to determine action of genetic component [13].

2.6.4 Heritability value

Heritability is an indicator to determine what difference performance of a character caused by genetic or environment factor. There are two heritability types namely: broad (h^2_{bs}) and narrow (h^2_{ns}) sense heritability. These parameters can be calculated with formula as follows [14] [15]:

$$h^2_{bs} = [s^2F_2 - (s^2F_1 + s^2P_1 + s^2P_2) / 3] / s^2F_2 \quad (5)$$

$$h^2_{ns} = [2s^2F_2 - (s^2BC_{1.1} + s^2BC_{1.2})] / s^2F_2 \quad (6)$$

s^2P_1 , s^2P_2 , s^2F_1 , s^2F_2 are variance of population sets P₁, P₂, F₁, F₂ respectively.

Criteria of heritability value: $h^2 < 0.2$ (low); $0.2 \leq h^2 \leq 0.5$ (moderate); $h^2 > 0.5$ (high) [16].

3 Results and discussion

3.1 Genetic analysis of tomato resistance to CMV

Plant response to virus infection relate with a plant susceptibility, whereas symptom's occurrence depend on interaction between pathogene, plant host, and environment. Bos [17] stated that both of pathogene and plant host depended on genetic, whereas action and their reaction were modified by environment. Based on observation mosaic systemic was found as the most disease symptom after inoculation by strain CMV-2, whereas the symptom after 18 days was only local necrotic.

Tomato plant resistance category to CMV infection can be determine according to some indicators as the observation parameters i.e., disease intensity, incubation periods, and virus concentration level [17]. This case was caused by any correlation significantly among parameters. There was positive correlation ($r=0.98$) between disease intensity with virus concentration level, whereas disease intensity with incubation time and between virus concentration level with incubation time were negative correlation ($r=-0.94$ and $r=-0.98$ respectively).

In general, the resistant plants showed some indicators such as a low disease symptom index, a low virus concentration level, and a long duration for incubation periods [17]. This case was proved by the P₁ parent (R8 51-12) as a genotype with the best resistance level if compared with other genotypes because it had the lowest of disease symptom index and virus concentration level. The situation was contrast for the P₂ parent (RG-57) as the most susceptible genotype. As for position the resistance level of the genotype F₁, F_{1R}, BC_{1.1}, BC_{1.2} were between both of parent's average (Table 1).

Table 1. Mean of disease intensity, incubation time, virus concentration level, and resistance category of each population sets to CMV infection as a result of hybridization between genotype R8 51-12 with RG-57

Population Sets	Disease Intensity (%)	Incubation periods(DAI)	Virus concentration	Resistance categories
P ₁ (R8 51-12)	7.00	19	0.1125	Resistant
P ₂ (RG-57)	75.50	12	0.1850	Very susceptible
F ₁	45.15	13	0.1745	Susceptible
BC _{1.1}	22.00	15	0.1455	Rather resistant
BC _{1.2}	51.20	14	0.1655	Susceptible
F ₂	26.10	14	0.1570	Rather susceptible

Note: DI = 0 (immune); DI = x ≤ 10% (resistant); DI = 10 < x ≤ 20% (rather resistant); DI = 20% < x ≤ 30% (rather susceptible); 30% < x ≤ 50% (susceptible); DI = x > 50% (very susceptible).

Table 2. Means of disease symptom index and virus concentration level on population set F₁ & F_{1,R} and result of T and F test

Population sets	Disease symptom Index	Virus concentration level
F ₁	1.90 ± 0.40	0.1745 ± 0.0070
F _{1,R}	1.80 ± 0.40	0.1715 ± 0.0070
F _{1,U}	1.85 ± 0.35	0.1755 ± 0.0070
T _{cal} (F ₁ vs F _{1,R})	0.20 ^{ns}	0.6185 ^{ns}
F _{cal} (s ² F ₁ vs s ² F _{1,R})	1.05 ^{ns}	1.0890 ^{ns}

According to T test at 5% level against means of F₁ and F_{1,R} for disease symptom index and virus concentration level showed there was no a different significantly both of them (Table 2). It's mean there was no maternal effect inside it [18]. This indicated that the involved genes for resistance to CMV were inside nuclear cell. Therefore, it was usually the progeny's segregation referred to Mendelian analysis. Both of male and female parents shared genetic contribution were equal to their progenies, so resistance level population sets F₁ was same with F_{1,R}. Result of F test showed that population set variance F₁ and F_{1,R} for disease symptom index and virus concentration level was homogenous because F_{cal value} > F_{tab} at 5% level. At the end data F₁ and F_{1,R} could be combined to become one (F_{1,U}).

The formula's estimation potential ratio (hp) can give information that the dominance value of resistant genes for disease symptom index was -0.138 and -0.800 for virus concentration level. Therefore, it was indicated that the resistance gene is recessive according to negative sign. However, the action is not complete because of potential values between -1 and 0 [12]. Thus, the expression of disease symptoms is more dominant than the virus concentration level.

The F₂ phenotypic frequency distribution graph for the disease symptom index and virus concentration level did not show a normal distribution. This indicated that the major gene played a role in controlling resistance and susceptibility trait of tomato plants to CMV infection. But if we refer to the fact that genes expression was not completely resistant as a previous result analysis, the existence of a role modifier genes as a minor gene a little bit change expression quantity that controlled by main major genes [19].

Mendelian genetic analysis was carried out to estimate major genes number that control tomato resistance to CMV. In this case be used by comparison the observed F₂ population phenotypic frequency with the expected phenotypic ratio and tested fit properly with chi square test. The chi squared test result for disease symptom index showed two appropriate ratios, namely 7: 9 and 27: 37 (Table 3). However, the most suitable ratio was 7 : 9 because the probability value was higher [18]. The phenotype with a ratio of 7 : 9 turns out to be a genotype controlled by two major genes and there was an epistatic recessive duplicate

interaction. There were two resistant genes as recessive to cover disease symptoms controlled by susceptible gene as dominant [19].

The chi squared test results for virus concentrations level showed three ratios correctly in comparison phenotype, namely 1 : 15; 9 : 55; and 1 : 6 : 9 (Table 3). However, the most appropriate ratio was 1 : 6 : 9 because the probability value was the highest. Therefore, based on comparison phenotypic ratio, it turns out that two major genes controlled the expression of virus concentrations level and there was a semi-epistatic interaction. The two genes at different loci were independent and did not act on each other, but if they collab together in a genotype, they will interact with each other to add cumulatively until appearing expression a character [19].

Table 3. The results of the chi square test (c^2) the observed ratio by the expected ratio for the disease symptom index and the level of virus concentration in the F₂ population

Parameter	Observed	Expected	Ratio	λ^2_{cal}	λ^2_{tab}	P
	R M S	R M S				
Disease symptom index	86 - 106	84 - 108	7 : 9	0.0476	3.84	0.9536
	86 - 106	81 - 111	27 : 37	0.4324	3.84	0.7341
Virus concentration level	18 - 174	12 - 180	1 : 15	2.6889	3.84	0.2478
	18 - 174	27 - 165	9 : 55	3.1138	3.84	0.0679
	18 76 104	12 72 108	1 : 6 : 9	2.6655	5.99	0.2731

Note: Resistant if scoring symptomless (0), moderate if symptom scoring 1 and 2; susceptible if symptom scoring 3 and 4

Joint scaling test result confirmed epistasis prediction occurrence on disease symptom expression (Table 4). This was indicated by genetic incompatibility with an additive-dominant model (m[a][d]). It's means that there was an interaction between loci [13]. It was known that the fittest proper genetic model was m[a][d][dd] after testing a four-component genetic model at 1% level because it had the smallest c^2 calculate value.

According to genetic model (m[a][d][dd]) after dividing genetic component value with standard error (Table 5), finally it was known that genetic components played a role in disease symptom expression were additive [a], dominant [d], and interactions dominant-dominant [dd]. But for virus concentration level, the genetic was still following an additive-dominant model (m[a][d]). This indicated that occurred the interaction to express this parameter was not inter, but intra loci only. Thus, only genetic components additive [a] and dominant [d] played a role (Table 5). However, mutual interactions inter loci could be cumulative [13].

Table 4. C^2 value of the combined scale test for the index of disease symptoms and the level of virus concentration

Parameter	Genetic model				λ^2_{tab}	
	m[a][d]	m[a][d][aa]	m[a][d][ad]	m [a][d][dd]	0.05 (3)	0.01 (2)
Disease symptom index	35.21*	7.50 ^{ns2)}	15.47*	7.15 ^{ns2)}	7.815	9.21
Virus concentration level	5.37 ^{ns 1)}	-	-	-	7.815	-

Note: ns¹⁾: It is not significantly at 5% level; ns²⁾: It is not significantly at 1% level; *: It is significantly at 5% level

Table 5. The estimated value of the genetic component for the index of disease symptoms and the level of virus concentration in the most suitable genetic model based on the joint scaling test

Parameter	Model	Genetic component					
		m	[a]	[d]	[aa]	[ad]	[dd]
Disease symptom index	m[a][d][dd]	1.56±0.13*	-1.34±0.12*	-2.30±0.60*	-	-	2.57±0.81*
Virus concentration level	m[a][d]	1.46±0.03*	-0.33±0.03*	0.23±0.06*	-	-	-

Note : $T_{tab(0.05)} = 1.96$; * : It is significantly at 5% level

Table 6. Broad (h^2_{bs}) and narrow (h^2_{ns}) sense heritability value of disease symptom index and virus concentration level

Heritability value	Parameter	
	Disease symptom index	Virus concentration level
h^2_{bs}	0.46	0.62
h^2_{ns}	0.18	0.58
$(h^2_{ns} / h^2_{bs}) \times 100\%$	39.13	93.55

In the Table 6 could be known that broad sense heritability value (h^2_{bs}) for disease symptom index (0.46) included in moderate ($0.2 \leq h^2 \leq 0.5$), while for virus concentration level in high category ($h^2 > 0.5$) [16]. This case indicated that the variability of disease symptom index phenotypically influenced by genetic and environment, while virus concentration level caused more by genetic factor. Matthews [20] stated that environment factors such as temperature influenced to occur virus symptom. The symptomless would occur if condition is low temperature, thus disease symptom index observation's result became bias.

Narrow sense heritability value (h^2_{ns}) for disease symptom index (0.18) included in low ($h^2 < 0.2$), while for virus concentration level (0.58) in high ($h^2 > 0.5$). This case showed that additive variance proportion contributed to total genetic variance was only moderate for disease symptom index (39.13%), while for virus concentration level was high (93.55%). Epistatic effect's occurrence proved by previous joint scaling test caused additive contribution to genetic variant was low for disease symptom index. Warner [15] stated one causing factor narrow sense heritability would be high if there was no epistasis or linked gene in a trait

3.2 Implementation of tomato breeding program for resistance to CMV according to result of genetic analysis

CMV is an RNA plant virus transmitted to other plants by more than 60 vector insects non persistently, especially *A. gossypii* and *M. persicae* [21]. Virus particles can be acquired and transmitted in a short time because they can move from one plant to another rapidly. Therefore, it is difficult to prevent plants from this virus through controlling vector insect.

The existence of various virus strains is one obstacle to develop breeding programs for resistance to viruses. The resistant response will occur if the plant's resistant host interacts with avirulent viruses, while the susceptible response will occur if the plant's susceptible host interacts with either avirulent or virulent viruses, or the plant's resistant host interacts with virulent viruses [22]. Plant host-virus interaction phenomenon impact a breeding program for resistance to a virus not for one new variety only.

The study result showed that there was no maternal effect both of male and female parents to their progenies. Therefore, both of them contributed a half of characters to their progenies [18]. Based on this information the tomato's breeding strategy for resistance to CMV was using a susceptible genotype with good agronomy's trait as female parent, while

a resistant genotype as male parent. In this case the agronomy's trait was expected only a little bit change, but already been improved on its resistance trait.

The genetic resistance character of a variety can be divided into vertical and horizontal resistant. A variety known as a vertical resistant if it is better resistant only to a certain strain, while a horizontal resistant does not have a different to all strains. In general, vertical resistant has a better quality than horizontal and controlled by a single as a major gene, while horizontal resistant controlled by polygenic as a minor gene in which each gene makes a little bit contribution to resistance character [23]. The study result showed that the resistance genes in tomato against *Cucumber Mosaic Virus* (CMV) were controlled by two recessive as major genes. Based on this information could be estimated that the genetic resistance character was vertical especially for pathogen strain CMV-2. Vertical resistant can be obtained if the selection process is carried out strictly, so the susceptible gene is eliminated. However, a vertical resistant variety should be arranged in using implementation because the trait was not long durable especially if exist a new strain.

The study results showed the existence of epistasis effect for disease symptom index parameter. This case would influence to selection effectiveness [16]. Informed also that proportion of additive variant contribution to totally genetic variants for disease symptom index was only moderate, while for virus concentration level was high. Therefore, the implemented breeding strategy was conducted phenotypically symptom selection in the middle to late generation. But the selection could be supported by serologically using Elisa test. Usually if a genetic variant caused more by dominant or epistatic variants, so their breeding program could be addressed for the F₁ hybrid variety.

4 Conclusions and recommendation

Genetic analysis results showed that there was no maternal effect and the resistance controlled by two major genes as recessive, but there was the epistatic effect on disease symptom as a selection parameter. Based on these information, the tomato's breeding strategy were to use a susceptible genotype as female parent, while a resistant genotype as male parent. Phenotypically disease symptom selection can be conducted in the middle or late generation. However, the selection should be supported by serologically using the Elisa test. The breeding program could be addressed as a F₁ hybrid variety.

References

1. Y. Silva, B.C. Borba, V.A. Pereira, *Int. J. Food Sci. Nutr. E* **70**, 2 (2018)
2. Direktorat Jenderal Hortikultura, Statistik Hortikultura: Produksi, Luas Panen dan Produktivitas Buah, Sayuran, Tanaman Hias dan Biofarmaka Tahun 2020 (Angka tetap) (Kementrian Pertanian, Indonesia, 2021)
3. A.S. Duriat, *IARD. J.* **18**, 3 (1996)
4. N. Wondim, R.G. Marchoux, E. Pochard. A. Palloix, K.G. Selassie. *J. Phytopathol. E.* **137** (1991)
5. A.W. Wulandari, N. Gunaeni, A.S. Duriat, *Penyaringan berbagai nomor tomat hasil silangan dengan tetua resisten terhadap sifat tahan virus mosaik (Cucumber Mosaic Virus)*, in Proceeding of Seminar Nasional Pemuliaan Berbasis Potensi dan Kearifan Lokal Menghadapi Tantangan Globalisasi, UNSOED, 8-9 July 2011, Purwokerto, Indonesia (2011)
6. S.K. Green, *Guideline for diagnostic work in plant virology, Tech. Bulletin.* 15. E. 2nd (2011)

7. B.T. Scully, W.T. Federer, *Breeding for virus resistance in vegetables* (Academic Press, New York, USA 2019)
8. G.N. Agrios, *Plant Pathology*. E. 2nd (Academic Press, New York, USA 1978)
9. R. Gaswanto, N. Gunaeni, A.S. Duriat. *J. Hort. E.* **19**, 4 (2009).
10. L.M. Dolores, *Management of pepper viruses*, in *Proceeding of AVNET-II Final Workshop*, AVRDC, Tainan, Taiwan (1996)
11. R.G.D. Steel, J.H. Torrie, *Principles and procedure of statistics, A biometrical approach*. E. 2nd (McGraw Hill Intl, London, 2011)
12. F.C. Petr, K.J. Frey, *Crop Sci. E.* **6** (2016)
13. K. Mather, J.L. Jink, *Biometrical genetics: the study of continuous variation*, E. 3rd , (Chapman and Hall, 2002)
14. R.W. Allard, *Principles of plant breeding* (John Wiley and Sons Inc, New York, USA 2010)
15. J.N. Warner, *Agron J. E.* **44**, 8 (2012)
16. A.R. Halloran, R. Knight, K.S.M. Whirter, D.H.B. Sparrow, *Plant breeding* (Brisbane, Australia, 2019)
17. L. Bos, *Pengantar virologi tumbuhan* (Gadjah Mada Univ. Press, Yogyakarta, Indonesia, 1994)
18. H. Suryo, *Sitogenetika* (Gadjah Mada Univ. Press, Yogyakarta, Indonesia, 1995)
19. L.V. Crowder, *Genetika Tumbuhan* (Gadjah Mada Univ. Press, Yogyakarta, Indonesia, 1993)
20. R.E.F. Matthews, *Plant virology*, E.3rd (Academic Press Inc. New York, USA, 2019)
21. A.J. Gibb, B.D. Harrison, *Cucumber Mosaic Virus, Description of plant viruses*, E. 1 (2017)
22. R.S.S. Frasser, *Plant resistance to virus* (CIBA Foundation Symposium, John Wiley and Sons, Singapore, 2007)
23. N. Panda. G.S. Khush, *Host plant resistance to insects* (International Rice Research Institute Los Banos, Laguna, Phippines, 1995)