Estimation of Inheritance of Kenaf Resistance Against Root Chole Nematodes (*Meliodogyne incognita*)

Parnidi[1], Aditya Aji Noftara[2], Mala Murianingrum[1], Taufiq Hidayat RS[1], Marjani[1]

[1] Sweetener and Fiber Crops Research Institute, Malang, Indonesian
[2] Agriculture Faculty - Brawijaya University, Malang, Indonesian

Abstract: Root puru nematodes (*Meloidogyne spp.*) on kenaf plants are known as one of the harmful plant-disturbing organisms. NPA infection in roots causes plants to grow stunted and production potential decreases. The inheritance of kenaf resistance to NPA *M. incognita* was carried out using a sixpopulation approach (P1, P2, F1, F2 BC1P1 and BC1P2). The genotype (Kenafindo Agribun 2 = Kin2) which was highly susceptible to NPA *M. incognita* was used as the female parent and the tolerant genotype (Karangploso 15 = KR15) as the male parent. The nature of kenaf resistance to NPA *M. incognita* was based on the variable number of shells, reproductive factors, number of 2nd instar juveniles, number of egg masses and number of eggs per egg mass. Based on the results of the study showed that the resistance of kenaf to NPA *M. incognita* showed that it was not influenced by female parents. The number of resistance genes is controlled by one gene. The character of kenaf resistance to NPA *M. incognita* was controlled by a partially positive dominant gene. The action of genes controlling resistance to NPA *M. incognita* was additive-dominant. The heritability value in the broad sense and the heritability value in the narrow sense for all variables of kenaf resistance to NPA *M. incognita* were high.

Keywords: Heritability; Number of puru; Reproductive factors; Kenaf crosses.

Introduction

Namatoda Puru Root (*Meloidogyne spp.*) in kenaf is known as one of the plant pest organisms (OPT) that causes plants to grow stunted, thereby reducing production potential. (Tahery et al., 2011; Wokoma et al., 2015; Adegbite, 2018). According to Lawrence & McLean (1992) the loss of yield of kenaf plants due to root-knot nematode infection reached 32% – 67%. Tahery et al. (2011) added that the population of root-knot nematodes of 5000/500 cm3 of soil could reduce plant height, stem diameter, stem weight and kenaf roots. As a result of root-knot nematode infection, the wet weight of kenaf stems can be reduced up to 3 tons per hectare. The population of forty juveniles/100 ml of soil in the initial inoculation treatment showed that it was able to reduce kenaf production (Supriyono & Suhara, 2007) and reduce kenaf fiber quality (Dalmadiyo et al., 1989). Plant damage due to root-knot nematodes increases with the number of nematodes infecting root tissue.

The use of resistant varieties is an appropriate way to suppress nematode population density and limit the damage threshold, thereby reducing crop yield losses (de Deus Barbosa et al., 2009; Davis & Stetina, 2016; Costa et al., 2017). Before assembling kenaf varieties that have resistance to NPA, it is very important to know about the information on genes controlling resistance to NPA.

Inheritance of kenaf resistance to root-knot nematodes until now there is not enough information. Several studies have been carried out on several plants to determine the genes controlling resistance to NPA. Some of the results of research on plant resistance to NPA include; Rosella resistance to NPA is controlled by monogenic genes with dominant gene action (Falusi, 2008); cotton resistance to NPA is controlled by two dominant genes (McPherson et al., 2004) and resistance of tobacco plants to NPA *M. incognita* race 2 is additive (Shahadati et al., 2017). The values of heritability in the broad sense and heritability in the narrow sense for the number of root cavities were 0.93 and 0.75, respectively, the number of egg masses 0.94 and 0.56 and the number of eggs per egg mass were 0.61 and 0.28, respectively. The research results of Wang et al. (2017) that the resistance of cotton to root-knot nematodes is controlled by chromosome 11. QTL 11 has a negative effect on the number and production of nematode eggs.
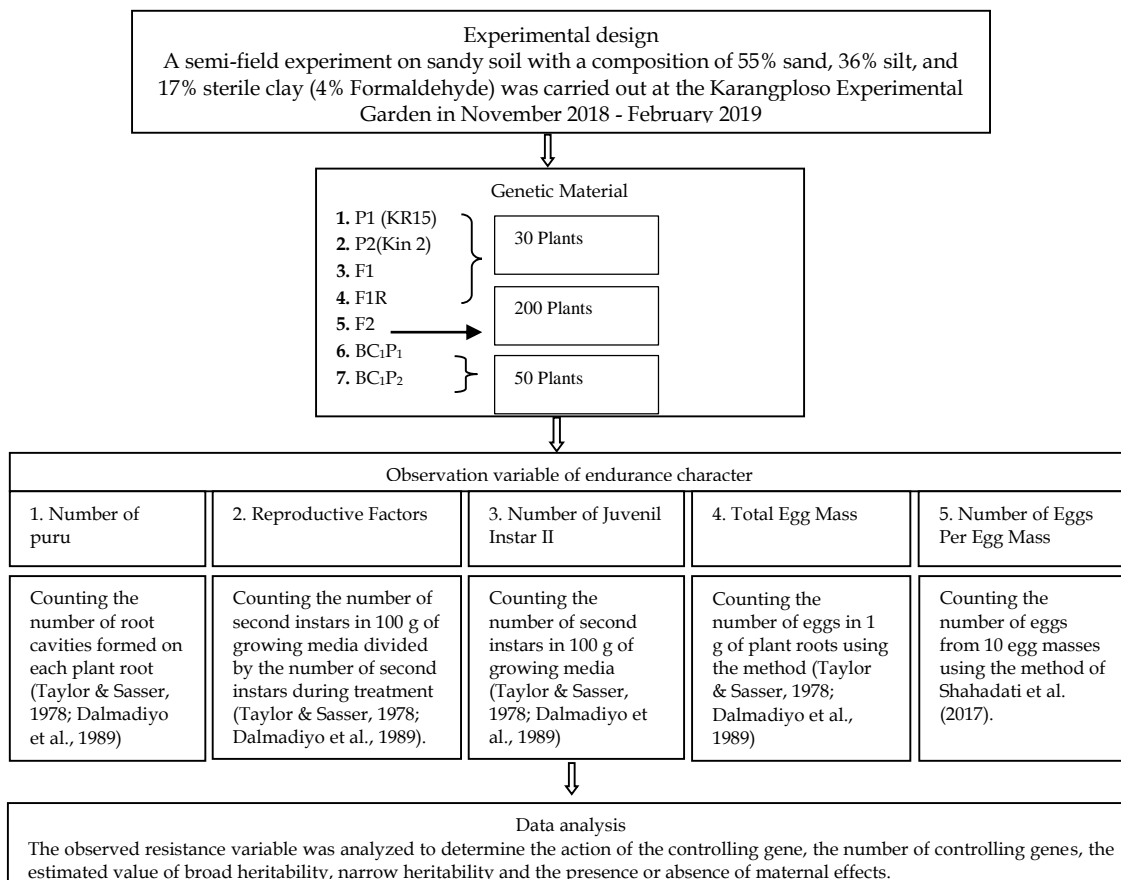
Analysis of the inheritance of kenaf resistance to NPA *M. incognita* has been carried out by several researchers by utilizing the source of resistance from close relatives of kenaf that have resistance to NPA *M. incognita*, including *H. sabdariffa, H. acetosella* and also *H. radiatus.* The availability of this information has not been able to provide much progress in breeding the resistance of NPA *M. incognita*. This is because there are constraints in the propagation of material for further study. Several obstacles in the inheritance of kenaf resistance to NPA *M. incognita* were caused by the high *incompatibilities* and sterility of the crosses between kenaf and its close relatives.

There is not enough information on the inheritance of resistance traits in intraspecific kenaf crosses. This is because the source of the gene for resistance to NPA in kenaf has not been obtained. Based on several research results, it is stated that the highest resistance of kenaf to NPA is tolerant (Yulianti & Supriyono, 2009). This has an impact on breeding methods which generally require a relatively long time because they must collect tolerant genes to obtain resistant genotypes.

Based on this, it is necessary to conduct a study on the inheritance of kenaf resistance to NPA in order to determine an effective and efficient breeding program strategy to obtain high yielding and NPA resistant kenaf genotypes. The purpose of this study was to estimate the inheritance of kenaf (*H. cannabinus L*) resistance to NPA *M. incogita.*

Method

Experimental design
A semi-field experiment on sandy soil with a composition of 55% sand, 36% silt, and 17% sterile clay (4% Formaldehyde) was carried out at the Karangploso Experimental Garden in November 2018 - February 2019

Genetic Material

1. P1 (KR15) **2.** P2(Kin 2) **3.** F1 **4.** F1R	30 Plants
5. F2	200 Plants
6. BC$_1$P$_1$ **7.** BC$_1$P$_2$	50 Plants

Observation variable of endurance character

1. Number of puru	2. Reproductive Factors	3. Number of Juvenil Instar II	4. Total Egg Mass	5. Number of Eggs Per Egg Mass
Counting the number of root cavities formed on each plant root (Taylor & Sasser, 1978; Dalmadiyo et al., 1989)	Counting the number of second instars in 100 g of growing media divided by the number of second instars during treatment (Taylor & Sasser, 1978; Dalmadiyo et al., 1989).	Counting the number of second instars in 100 g of growing media (Taylor & Sasser, 1978; Dalmadiyo et al., 1989)	Counting the number of eggs in 1 g of plant roots using the method (Taylor & Sasser, 1978; Dalmadiyo et al., 1989)	Counting the number of eggs from 10 egg masses using the method of Shahadati et al. (2017).

Data analysis
The observed resistance variable was analyzed to determine the action of the controlling gene, the number of controlling genes, the estimated value of broad heritability, narrow heritability and the presence or absence of maternal effects.

Maternal effects were tested by comparing the mean values of F1 and F1R with the t-test according to (Daryanto, 2016; Hapshoh, 2016) at the 5% level. The frequency distribution of the F2 population was tested by normality test using the Kolmogorov-Smirnov method.

The degree of dominance is calculated based on the formula of Petr & Frey (1966) namely hp = (F1 – MP) / (HP – MP); with hp = potential ratio, F1 = average value of F1, HP = average value of the highest elder, MP = middle value of the two elders. Based on the ratio potential value, the degree of dominance is classified as: HP = 0: no dominance, HP = 1 or HP = -1: fully dominant or recessive, 0 <HP< 1: partial dominant (incomplete positive dominant), -1 < hp < 0: partially recessive (imperfectly negative dominant), and HP > 1 or hp < -1: over dominant.

Estimation of the number of controlling gene pairs for each nematode resistance character used the calculation described by Lande (1981) namely $N = \mu_{P1} - \mu_{P2}/8(\sigma^2_{F2} - 2(\sigma^2_{F1} + \sigma^2_{P1} + \sigma^2_{P2})/4$ where μ_{P1} = Average of elders, Kin2, μ_{P2} = Average range of elders KR15, σ^2_{F2} = Variety of F_2, σ^2_{P1} = Variety of elders Kin2 σ^2_{P2} = Variety of elders KR15. Heritability in broad sense, calculated based on the formula (Acquaah, 2007) i. e. $h^2_{bs} = \sigma^2_{F2} - 1/3(\sigma^2_{P1} + \sigma^2_{P2} + \sigma^2_{F1})/\sigma^2_{F2}$ where h^2_{bs} = broad sense heritability, σ^2_{F2} = F2 variety, σ^2_{P1} = Kin2 parent variety, σ^2_{P2} = KR15 parent variety and σ^2_{F1} = F1 variety.

Narrow sense heritability is calculated based on the formula (Syukur et al., 2015) which is $h^2_{ns} = 2\sigma^2_{F2} - (\sigma^2_{BC1P1} + \sigma^2_{BC1P2})/\sigma^2_{F2}$. Where h^2_{ns} = narrow sense heritability, σ^2_{BC1P1} = population variance BC1P1, σ^2_{BC1P2} = population variance BC1P2, σ^2_{F2} = population variance

F2. The estimated heritability value is considered low if $h^2 < 20\%$, moderate if $20\% \leq h^2 \leq 50\%$ and high if $h^2 > 50\%$

The feasibility of the additive and dominant models was estimated by three genetic parameters, namely the mean (m), the amount of influence of the additive gene (d) and the amount of influence of the dominant gene (h) using the Joint Scaling Test method (Mather & Jink, 1982). If the action of the gene does not meet the additive-dominant model, a test is carried out to determine whether there is a non-allelic gene interaction using an epistatic model with six parameters (Mather & Jink, 1982).

Result and Discussion

The influence of the female elder

Tests for the influence of female parents were carried out to determine whether the resistance of kenaf to NPA *M. incognita* was influenced by female parents. To determine whether there was an influence of female parents on the resistance of kenaf to NPA *M. incognita*, a t-test was carried out on the F1 population with reciprocity. Based on the results of the comparison between the F1 population average and its reciprocity, it shows that there is no reciprocal effect as indicated by the probability value of t which is greater than 0.05.

The results of the homogeneity test of the resistance to root-knot nematodes using the F test on Kin2 x KR15 showed that the F1 and F1R variants were homogeneous. This can be seen from the calculated F value which is greater than the F table at the 5% level (Table 5). Thus, in the next analysis, the F1 and F1R data can be combined as F1 population data.

Table 1. The average value and standard deviation of the kenaf resistance character against the nematode M. incognita in F1 and F1R, the results of the test of different mean values and homogeneity of variance.

Population	IP	FR	J2	JMT	JTMT
F₁	70.27±3.48	5.41±0.01	35.77±0.65	11.73±0.45	402.43±9.45
F₁R	72.7± 6.2	5.71±0.36	34.01±1.60	12.87±1.30	403.8±16.0
Prob>(t)	0.19tn	0.55tn	0.62tn	0.33tn	0.84tn
Prob>(F)	0.93tn	0.70tn	0.73tn	0.73tn	0.77tn

Information: JP = number of shells, FR = reproductive factor, J2 = number of 2nd instar juveniles, JMT = number of egg masses and JTMT = number of eggs per egg mass. tn = not significantly different
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The character of kenaf plant resistance to NPA *M. incognita* was not affected by female parents (Table 1). This indicates that the resistance trait is controlled by *the nuclear genes*. (Hulupi, et al., 2007) stated that the resistance of Arabica coffee to *Radopholussimilis Cobb* showed that it was not influenced by female parents. The resistance properties of kenaf to NPA *M. incognita* showed similar to the resistance properties of chili plants to anthracnose or to aphids. According to Syukur et al. (2007); Hapshoh (2016) that the resistance of chili plants to anthracnose disease is not influenced by female

parents. Daryanto (2016) stated that the resistance of chili to aphids was also not influenced by female elders.

Analysis of the inheritance of resistance characters

Analysis of the inheritance of kenaf resistance characters against the nematode *M. incognita* included: normality test, degree of dominance and number of controlling genes, variance components, genetic model feasibility and heritability values.

Normality Test of Population Frequency F2

Analysis of the normality of the data in F2 was used to determine the number of genes controlling the resistance character of *M. incognita NPA*. The results of the normality test on all variables of kenaf plant resistance to NPA *M. incognita* showed that the F2 population frequency distribution was not normal (P < 0.10) (Table 2).

Wanda et al. (2014) stated that the distribution of F2 population data was not normal indicating that the character was controlled by a simple gene or a major gene. Furthermore (Barnawi et al., 2013) explained that the qualitative character of a plant is usually controlled by one or two genes, less influenced by the environment, so it is relatively easy to handle in breeding. The segregation pattern of the qualitative characters follows the Mendelian ratio and its modifications.

Table 2. The results of the normality analysis of the kenaf resistance variable against NPA using the Kolmogorov-Smirnov test.

Resistance Variable	Statistic	Df	Average value	Value of p
JP	3.22	200	71.77±6.62*	0.000†
FR	2.98	200	4.94±2.89*	0.000†
J2	1.56	200	38.99±1.37*	0.015†
JMT	2.87	200	9.50±4.96*	0.000†
JTMT	3.71	200	4.68±1.65*	0.000†

Note: Number of Puru (JP); Reproductive Factors (FR); Number of Juvenil Instar 2 (J2); Total Egg Mass (JMT); Number of Eggs per Egg Mass (JTMT) *= mean ± SD; = p<0.05 data is not normally distributed

Degree of Dominance and Number of Controlling Genes

Estimation of the degree of dominance was carried out by estimating the potential ratio (*hp*) of the median value of the two parents and F1 using the calculations described by Petr & Frey (1966). The potential value of the ratio of the results of the Kin2 x KR15 cross from the number of shells, reproductive factors, juvenile instar 2, number of egg mass and number of eggs per egg mass were 0.05, 0.05, 0.07, 0.15 and 0.01, respectively (Table 3).

Table 3. Value of potential ratio and number of genes controlling kenaf resistance to NPA *M. incognita.*

Population	The mean value of the resistance variable				
	JP	FR	J2	JMT	JTMT
P$_1$	109.47±4.96	8.89±0.01	55.90±0.65	19.61±0.65	561.98±8.65
P$_2$	34.80±3.32	2.26±0.01	22.16±0.42	5.89±0.32	282.21±7.32
F$_1$	70.27±3.48	5.41±0.01	37.77±0.65	11.73±0.45	390.58±9.45
MP	72.08	5.57	39.03	12.75	391.51
hp	0.05	0.05	0.07	0.15	0.01
Gene action	Partially positive dominant				
Number of genes	0.01	0.12	0.03	0.03	0.02

Note: JP: number of purua kar, FR: reproductive factor, J2= number of juveniles in the 2nd instar, JMT: number of egg masses, and JTMT: number of eggs per egg mass.

The degree of dominance (*hp*) on all of the evaluated resistance variables ranged from 0.03 to 0.55 (Table 3). If the hp value of a character is in the range of 0 and 1, it indicates that the character is controlled by the action of an imperfectly positive dominant gene (partially positive dominant). This agrees with the results of research by Setyo-budi (2009) which states that the inheritance of the kenaf genotype resistance to root-knot nematodes is controlled by the role of partially positive dominant genes. Bertrand et al. (1995) stated that the action of genes controlling resistance to the nematode *Meloidogyne* spp. that infects Catimor Arabica coffee is the action of a partially dominant gene. Hulupi et al. (2007) added that the resistance of Arabica coffee to *Radopholussimilis* Cobb was controlled by a partially dominant gene. According to Roberts et al. (1998) resistance of tomato varieties to *Meloidogyne* spp on the

character of the number of egg masses and the rate of formation of the bladder was controlled by genes with partially dominant gene action. The degree of partially positive dominance in the F1 genotype from the Kin2 x KR15 cross indicated a strong gene dominance event, and the inheritance was controlled by partial dominance.

Estimation of the genetic component

To determine the action of genes controlling the resistance variable to M. incognita NPA, a combined scale test was performed (Mather & Jink, 1982). Individual scale testing compares the t-count value with the t-table to state whether or not a genetic component is real (Singh & Chaudhary, 1979). Estimation values of genetic parameters of individual scale test (scaling test) of kenaf resistance variable to NPA M. incognita are presented in (Table 3).

Table 4. Predicted values of genetic parameters for individual scale test of resistance variables

Parameter	JP	FR	J2	JMT	JTMT
m	398.4	28.25	207.11	57.63	2233.11
[d]	37.33	3.32	16.87	6.86	109.30
[h]	6.99	1.34	2.84	2.84	101.48
C	265.21	21.01	129.39	39.85	1305.03
SE. C	193.43	13.83	67.09	43.70	671.35
t-count	1.37tn	1.52tn	1.93tn	0.82tn	1.94tn
t-table	1.96	1.96	1.96	1.96	1.96

C = 4 (mean F2) – (mean P1) – 2 (mean F1) – (mean P2) (Singh and Chaudary, 1979); m: mean value, d: additive value, h: dominance value, C: gene interaction value, SE C: standard error C. JP=Number of root cavities, FR= Reproductive factors, J2= number of 2nd instar juveniles, JMT=Number of egg masses and JTMT= Number of eggs per egg mass. ** = significant at 1% t-test level = 2.58 * = significant at 5% t-test level = 1.96, tn = not significant

Based on (Table 4) shows that the appropriate genetic model for the variable resistance to NPA *M. incognita* on all variables evaluated in the Kin2 x KR15 cross is the additive-dominant model (m[d][h]). This model is the most suitable model, this is because the value of t-count is smaller than t-table = 1.96.

The character of kenaf plant resistance to NPA *M. incognita* was controlled by one gene. This agrees with the results of Falusi (2008) which states that the resistance of rosella to SSP nematodes is controlled by one gene. Meanwhile, Shahadati et al. (2017) who conducted research on tobacco resistance to *M. incognita* NPA stated that tobacco plant resistance to *M. incognita* NPA was controlled by one gene, with a partially dominant effect. The number of controlling genes indicates the number of genes that are effective in controlling the expression of a character. The most suitable gene action model for the character of kenaf resistance to NPA *M. incognita* was additive-dominant (m[d][h]). Thus, it means that only additive [d] and dominant [h] gene action determines the diversity of kenaf resistance to NPA M. incognita. The value of the

additive variance is greater than the dominant variance, this indicates that the genetic variance is determined by the action of the additive gene.

The visible variety of appearance of a plant (phenotype) is the result of a combination of diversity due to genetic and environmental influences. The genetic diversity that is the focus of plant breeding is the diversity caused by the influence of additive gene action, the influence of dominant gene action and the influence of interactions between genes (Falconer, 1960).

Heritabilitas

Heritability is a reflection of the influence of genes (genotype) on the observed external appearance (phenotype). Heritability values in the broad sense (h2bs) and narrow sense (h2ns) on the results of the Kin2 x KR15 cross for the kenaf resistance variable to NPA *M. incognita* which included the number of lungs, reproductive factors, number of juveniles instar 2, number of egg masses and average number eggs per egg mass was high (Table 5).

Table 5. Variance components and heritability values of kenaf resistance to the nematode *M. incognita*.

Genetic parameters	JP	FR	J2	JMT	JTMT
Environmental Variety	881.32	3.54	50.95	38.70	3503.12
Variety of Additives	7628.57	41.91	1016.20	401.28	107262.94
Dominant Variety	4760.56	20.02	506.31	215.03	23303.85
Variety of Phenotypes	5885.74	29.50	685.63	293.10	62960.55
Variety of BC_1P_1 and BC_1P_2	7957.20	38.05	863.16	385.55	72289.63
h^2_{bs}(%)	56.11	66.69	80.44	63.01	86.95
h^2ns(%)	51.71	61.21	56.31	57.86	81.56
Proportion h^2_{ns}/h^2_{bs} (%)	92.15	91.78	70.00	91.83	93.80

Description: JP = number of shells, FR = reproductive factors, J2 = number of juveniles instar 2, JMT = number of egg masses and JTMT = average number of eggs per egg mass.

High additive effect has an impact on the effectiveness of the selection. In plant breeding, the selection of traits controlled by additive genes is expected to get a large and rapid selection progress. Selection of characters with high heritability can be done in the early generations. (Barmawi et al., 2013) said that a character that has a high heritability value can be

selected in the early generations (F2 and F3). The high value of heritability in the broad sense and in the narrow sense for the resistance of NPA *M. incognita* would be a good step because in the selection program high selection progress would be obtained.

The total phenotypic appearance seen from the heritability value in the broad sense of the variable number of shells (56.11%), reproductive factors (66.69%),

number of juvenile instar 2 (80.44%), total egg mass (63.01%) and average number of eggs per egg mass (86.95%) was influenced by genetic variation. In all variables of resistance evaluated, it was 51.71% on the number of shells, 61.21% on the reproductive factor variable, 56.31% on the variable number of juvenile instars 2, 57.86% on the variable number of egg masses and 81.56% on the variable average number of eggs per egg mass of the total variance was influenced by additive genes, and there was a difference of 4.40% in the number of shells, 5.48% in reproductive factors, 24.13% in the number of juvenile instars of 2, 5.15% in the number of egg masses and 5.39% in the average number of eggs per egg mass. This indicates the small role of the influence of non-additive gene action (dominant and interactions between genes) on the character of kenaf resistance to NPA *M. incognita.*

The magnitude of the influence of genetic variance from the total phenotypic appearance on all evaluated resistance variables indicated the small role of the influence of environmental variation (E=43.89% for the number of shells, 33.31% for reproductive factors, 19.56 for the number of juvenile instars 2, 36.99% for egg mass and 13.05% for the number of eggs per egg mass). This is consistent with the results of the estimation of the components of genetic variance that the environmental variance components have no effect on all resilience variables (Table 5).

The basis for the success of plant breeding work is largely determined by the amount of genetic diversity that can be passed from parents to offspring. The additive component is the only component of genetic variation that is passed from parents to offspring, and the narrow heritability value again shows this magnitude through the genetic proportion of total appearance as the portion that is completely passed on from parents to offspring (Falconer 1960; Poehlman & Sleper, 1995). From the point of view of breeding kenaf, which is a self-pollinating plant, selection should be made for additive effects in the hope of gathering superior genotypes; selection becomes ineffective if the superior genotype is determined by the influence of dominance and interactions between genes (Poehlman and Sleper, 1995).

Conclusion

Based on the results of the study, it can be concluded that the resistance of kenaf to NPA *M. incognita* is controlled by the genes present in the cell nucleus. Kenaf resistance to NPA *M. incognita* was controlled by a dimonan gene with heritability values ranging from 56.11 to 86.95.

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