Growth and resistance of F1: the results of kenaf interspecific crosses to root-knot nematode (*Meloidogyne incognita*)

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Abstract. The aim of interspecific crosses between kenaf and close relatives is to obtain superior varieties of kenaf resistant to nematodes. This study aims to determine F1 inter-specific kenaf crosses resistance compared to its parents to *M. incognita* infection. The evaluation of kenaf genotype resistance was determined by gall index. The growth of F1 genotypes resulted from crossing SRRH023 x Kin2, and crosses of Kal II x Kin2 showed that it was higher than his close relative parents and smaller than the kenaf and *M. incognita* resistant. The F1 of kenaf inter-specific crosses with its close relatives was resistant to *M. incognita* nematodes.

1. Introduction

Root-knot nematode (*Meloidogyne* sp.) is an important disease and infects many kenaf plants. Kenaf plants are generally susceptible to root-knot nematodes (RKN) of *M. arenaria, M. javanica*, and *M. incognita* [1,2]. One trait of a plant being infected with RKN is forming a lump or root-knot on the roots. The RKN infection in the roots affected the plant to grow stunted, then the heavy attack can cause death. The lost yield of kenaf which is up to 19% [1], 32% - 67% [3]. Tahery [3] described that due to RKN infection, kenaf stems weight decreased to 0-3 tonnes per hectare. Root-knot nematode control is generally carried out by using nematicides. However, this control method is less economical and not environmentally friendly. Another effort that can be made to control RKN is by using resistant varieties. The use of resistant varieties is the most appropriate way to resolve the problem of RKN. Success in obtaining the superior varieties which resistant to root-knot nematodes requires genetic sources of resistance.

In general, kenaf (*Hibiscus cannabinus* L.) plants are not resistant to RKN. However, kenaf's close relatives of wild species such as Kal II (*H. radiatus*), SRRH023 (*H. acutosella*) are known to contain resistance genes to RKN [1,4]. Inter-specific crosses between *H. radiatus* (2n=4x=72), *H. acutosela* (2n=4x=72) as the recipient of *M. incognita* resistance and kenaf (2n=2x=36) as donors with high production, is expected can produce the superior varieties of kenaf and resistant against *M. incognita*. According to the results of Setyo-Budi [1] studies, the inter-specific crosses of kenaf and *H. radiatus* planted on RKN endemic land in the Blitar - East Java area showed that no one could stand it.

In 2017 an inter-specific cross was carried out between Kal II x kenaf and SRRH023 x kenaf, which obtained fertile F1 seeds. A total of 6 F1 genotype populations are expected to contain genes for resistance to *M. incognita*, so it is necessary to test their resistance by including both parents (P1 and P2) through resistance tests against *M. incognita* infection. The purpose of this study is to determine the growth and resistance of F1 from inter-specific crosses of kenaf with its close relatives compared to its parents against the root-knot nematode caused by *M. incognita*. 
2. Methodology

2.1 Experimental materials

The genetic material used in this study is presented in Table 1.

| Number | Genotypes name | Generation     |
|--------|----------------|----------------|
| 1.     | Karangploso 1 (KR1) (Hibiscus cannabinus L) | Kenaf parent (P1) |
| 2.     | Karangploso15 (KR15) (Hibiscus cannabinus L) | Kenaf parent (P2) |
| 3.     | Kenaf indoaqrubun 2 (Kin2) (Hibiscus cannabinus L) | Kenaf parent (P3) |
| 4.     | Kal II (Hibiscus radiatus) | Radiatus parent (P4) |
| 5.     | SSRH023 (Hibiscus acetosella) | Acetosella parent (P5) |
| 6.     | Kal II X KR1      | F1 (P4xP1)     |
| 7.     | Kal II X KR15     | F1 (P4xP2)     |
| 8.     | Kal II X Kin2     | F1 (P4xP3)     |
| 9.     | SSRH023 X KR1     | F1 (P5xP1)     |
| 10.    | SSRH023 X KR15    | F1 (P5xP2)     |
| 11.    | SSRH023 X Kin2    | F1 (P5xP3)     |

In addition, in this study, sandy soil was used as a growing medium, formaldehyde, plastic mulch, polybags 30 cm x 30 cm, lime, fungicide, urea and phonska fertilizers. The tools used are the nematode counting dish, binocular microscopes, centrifuge, counter, meter and caliper.

2.2 Experimental design

Inter-specific crosses between Kal II and SRRH023 as resistant parents, while kenaf as potential parents were carried out in Karangploso Experimental Garden, Indonesian Sweetener and Fiber Crops Research Institute (ISFRI) Malang in 2017, and obtained fertile F1 offspring. The semi-field study was conducted to test the resistance of offspring and its parents using a randomized block design with 3 replications. All genotypes were planted in polybags on 5 kg medium, which consisted of sandy soil (sandy) 55%, dust (silt) 36%, and clay 17% sterile. The sterilization of the planting medium using a 4% formalin solution.

The experiments were carried out by infecting kenaf plants aged 15 days after planting with M. incognita nematodes in a population of 40 2nd stage juveniles /100 ml of soil [5]. At 75 days after planting, plant growth was carried out by measuring the plant height, stem diameter, shoot, and root wet weight. Furthermore, the plants were dismantled for observation of the final population (Pf) of the nematode. In addition, to determine and classify the level of plant resistance to RKN infections, it was carried out based on the root-knot index. The root-knot index is calculated based on the scoring of the number of root-knot formed by the Canto-Saenz method, which has been done by Suparmana and Lisnawati [6] and Supriyono and Suhara [5] that is: scale 0 = no root-knot, 1 = 1-15 root-knot, 2 = 16-35 root-knot, 3 = 36-50 root-knot, 4 = 51-100 root-knot, and 5 = more than 100 root-knot (Table 2).

| Number of root-knot | Index of root-knot | Resistance level         |
|---------------------|--------------------|--------------------------|
| 0                   | 1                  | Immune (I)               |
| 1-15                | 2                  | Resistant (R)            |
| 16-35               | 3                  | Moderately Resistance (MR) |
| 36-50               | 4                  | Moderately Susceptible (MS) |
| 51-100              | 5                  | Susceptible (S)          |
| >100                | 6                  | Highly Susceptible (HS)  |

Analysis of the reproductive factor (R) of larvae used the method of Canto-Saenz [7] and Sasser [8], which has been done by Adegbite [9] with the equation:

Factor R = Pf/Pi

Note: R = reproductive factor of M. incognita larvae in the soil; Pf = RKN larval population in a final soil; Pi = RKN larval population in the initial soil.
The observed data on plant growth were analyzed using variance based on a randomized block design using SPSS.16 software. If the results of the analysis of variance show a significant difference, the data is then further tested using the Duncan Multi Range Test (DMRT) at the 5% confidence level. The result of calculating the number of root-knot at the root was carried out by scoring the root-knot number. Furthermore, scoring the number of root-knots is used as the basis for classifying the level of resistance, as Canto-Saenz[6] studied in Table 2.

4. Results and Discussion

4.1 F₁ Plant Growth

Based on the F₁ of the six sets of inter-specific crosses between kenaf x Kal II or crosses between kenaf x SRRH023 showed that all individual F₁ plants exhibit characters that are close to kenaf parents as female parents, both upright plant habitus, shape, leaf color, and stem color. In some genotypes, the results of crossing kenaf x Kal II and kenaf x SRRH 023 produced short plants with thick leaves. According to Arangzeb [10] the presence of thick leaf fenomes in the inter-specific crossing of kenaf x H. sabdariffa was might be due to a spontaneous allohexaploid.

The growth of F₁ plants resulting from crossing Kal II and SRRH023 with kenaf was based on plant height, stem diameter, shoot and root weight. Based on the results of the analysis of variance on plant growth, it was shown that the growth of F₁ plants resulting from crossing Kal II and SRRH023 with kenaf was significantly different from the two parents (Table 3). The F₁ growth resulting from crossing Kal II x KR1, Kal II x KR15, SRRH023 x KR1, and SRRH023 x KR15 showed smaller plant growth than the two parents. The F₁ growth resulting from crossing Kal II x Kin2 and SRRH023 x Kin2 showed better growth than the other F₁ and the parents of Kal II and SRRH023. The F₁ growth resulting from crossing Kal II x Kin2 and SRRH023 x Kin2 were smaller than the kenaf parents KR1, KR15, and Kin2.

Table 3. Effect of nematodes on the growth of F₁ plants resulting from crossing Kal II and SRRH023 with kenaf.

| Genotype       | Stem Height | Stem Diameter | Fresh weight of Shoot | Fresh weight of root |
|----------------|-------------|---------------|-----------------------|---------------------|
| KR1            | 159.83 c    | 1.09 d        | 344.28 abc            | 70.33 ab            |
| KR15           | 178.78 cd   | 1.37 e        | 422.28 c              | 107.17 bc           |
| KIN2           | 191.28 cd   | 1.42 e        | 406.11 bc             | 106.39 bc           |
| KAL II         | 114.92 b    | 0.60 ab       | 334.28 abc            | 55.72 ab            |
| SRRH023        | 84.47 a     | 0.50 a        | 280.44 abc            | 74.44 abc           |
| KAL II x KR1   | 84.61 a     | 0.78 c        | 299.44 abc            | 65.17 ab            |
| KAL II x KR15  | 108.61 b    | 0.68 bc       | 326.67 abc            | 68.67 ab            |
| KAL II x KIN2  | 125.34 b    | 0.69 bc       | 343.45 abc            | 82.78 abc           |
| SRRH023 x KR1  | 66.67 a     | 0.67 bc       | 201.39 a              | 49.56               |
| SRRH023 x KR15 | 81.78       | 0.68 bc       | 260.06 ab             | 62.11 ab            |
| SRRH023 x KIN2 | 119.58 b    | 0.69 bc       | 360.45 bc             | 125.11 c            |

Note: The numbers followed by the same letter in the same column show no significant difference according to Duncan's Multiple Range Test at the 5% level.

Based on the F₁ plant growth variable, it shows that the genes for height, stem diameter, shoot and root weight in kenaf were more dominant than its close relatives (Kal II and SRRH 023). Kal II and SRRH 023 have short and small stems and lower weight. This study’s results were agreed with the results...
of Setyo-budi [11] which stated that the growth variables in the form of plant height, stem diameter, stem weight from F1 crosses of kenaf x Kal II show closer to the properties of kenaf. This is important because the selection process for future generations will be easier.

4.2 Plant response to root-knot nematode infection

4.2.1 The number of root-knot

The observation of the number of root-knot was carried out at 75 days after planting by counting each root-knot in the root system. The observation of the number of root-knot is based on root-knot symptoms found in each plant root system. Root-knot is a symptom indicator of root damage caused by *M. incognita*. The presence or absence of root-knot on kenaf roots indicates a genotype of resistance or susceptibility to root-knot nematodes. The variance analysis results on the number of root-knot showed a significant difference between the tested genotypes (Table 3).

The formation of root-knot in the roots of the kenaf genotype tested showed that all genotypes responded to *M. incognita*. Plants resistant to nematodes are characterized by the nematodes' failure to form feeding sites in the host after the invasion and abortive to thrive [12]. A similar study was conducted by Setyo-budi [1] by testing the resistance of F1 crosses of kenaf x Kal II to *M. incognita*. The results showed that Kal II parents were resistant genotype, while kenaf parents showed highly susceptible, then F1 showed susceptible - slightly susceptible.

Based on the root damage values in Table 4, there is a fairly high variability of resistance traits among the tested genotypes. From those tables, it can be seen that Kal II and SRRH023 female parents were seen as resistant parents, even though *M. incognita* nematodes still infected the plants. On the other hand, based on the root damage index of three kenaf parents: KR1 and Kin2 showed very highly susceptible. Meanwhile, the KR15 kenaf parents showed moderately resistance. The six F1 offspring showed resistance to root-knot nematodes caused by *M. incognita*. Thus, it can be said that the results of crossing Kal II and SRRH 023 with kenaf resulted in F1 which was resistant to root-knot nematodes caused by *M. incognita*.

### Table 4. The gall index, R factor with the level of resistance to root-knot nematodes.

| Genotype  | Number of root-knot index | Reproductive factors | Resistance level       |
|-----------|---------------------------|----------------------|------------------------|
| KR1       | 113.00 c                  | 6.03 c               | Highly Susceptible (HS) |
| KR15      | 34.80 b                   | 2.26 b               | Moderately Resistance (MR) |
| Kin 2     | 109.47 c                  | 6.89 d               | Highly Susceptible (HS) |
| Kal II    | 5.83 a                    | 0.12 a               | Resistant (R)          |
| SRRH023   | 0.17 a                    | 0.01 a               | Resistant (R)          |
| Kal II x KR1 | 14.11 a | 0.24 a               | Resistant (R)          |
| Kal II x KR15 | 10.39 a | 0.22 a               | Resistant (R)          |
| Kal II x Kin 2 | 14.17 a | 0.18 a               | Resistant (R)          |
| SRRH x KR1 | 2.17 a                    | 0.09 a               | Resistant (R)          |
| SRRH x KR15 | 3.22 a | 0.17 a               | Resistant (R)          |
| SRRH x Kin 2 | 1.22 a | 0.11 a               | Resistant (R)          |

Note: The numbers followed by the same letter in the same column show no significant difference according to Duncan's Multiple Range Test at the 5% level.

The resistance of kenaf to root-knot nematodes can be categorized into two types of resistance: pre-infection and post-infection resistance. Pre-infection resistance that is nematodes cannot enter plant roots because of the presence of toxic chemicals in the root tissue that can neutralize the formation of giant cells as active resistance [13,14]. Meanwhile, post-infection resistance means that the nematodes are able to enter roots but abortive to thrive [15]. Kenaf plants are generally susceptible to highly susceptible to root-knot nematodes [2,16].

Resistance of kenaf plants and their close relatives might be more influenced by post-infection resistance [17,18]. This is based on the analysis results of the salicylic acid and phenols content in plant
roots. Kenaf plants or their close relatives in various tests, showed that plants continued to be infected by RKN [1, 17,18]. However, based on the analysis results of the content of salicylic acid and phenol in the roots, the plants' roots were infected with the amount of salicylic acid and phenol have increased compared to control. In addition, Parnidi [18] explained that in plants infected by RKN, the total amount of lignin in the roots also increased compared to control plants. Phenolic secondary metabolites such as salicylic acid, phenol, and lignin play a role in plant resistance from pathogens [19, 20]. One of the reactions of plant tissue to pathogenic infections is an increase in phenolic compounds. These compounds can inhibit hydrolysis enzymes, including pectolytic enzymes, which are produced by pathogens.

The study of Jenkins [21] showed that the cotton root infection by M. incognita caused the formation of terpenoids aldehyde in infected cotton root endodermal stele. In resistant plants, the formation of these substances is faster than the susceptible plants. Karsen[22] reported that highly susceptible host plants allow nematode larvae to enter roots, developing to grown, and produce large numbers of eggs. In contrast, resistant plants suppress their development and do not allow reproduction. It was assumed that the reaction of compound formation for plant defense formed more slowly. The resistance to nematodes does not mean it can withstand the roots' invasion of nematode larvae [23]. However, precisely after the larvae intervention in the roots, there will be restrictions on food induction (feeding site) for larvae, or the destruction of feeding structures early in larval development so that the larvae do not develop or die because they cannot eat.

4.2.2 Reproductive factors

The reproductive factor, which reflects the ability of root-knot nematodes to reproduce in the root tissue, is shown in second instar larvae in the soil. The second instar juvenile reproductive factor in the growing medium was obtained by comparing the final population with the initial population.

The results of the statistical analysis of the nematode reproductive factors on the growing media showed a significant difference in Duncan's Multiple Range Test at 5% level (Table 3). If the reproductive factor is high, it means that the genotype is a good host for nematode development. Canto-Saenz[6] described that if the R-value is < 1, M. incognita larvae are underdeveloped or unable to develop. Conversely, if the R-value is > 1, it can be ascertained that M. incognita larvae reproduce.

The sooner or the later and the higher or the smaller of reproduction, the larvae depend on the host plant's resistance. From the value of the R factor, it can be claimed that Kal II and SRRH 023 as female parents have the ability to restrain the reproductive rate of M. incognita larvae to be slower (resistant) than the three male parents (kenaf: KR1, KR15, and Kin 2). The nematode reproduction factors in the planting medium showed that the F1 offspring from the interception cross between kenaf and its close relatives were not suitable hosts for RKN. This is based on the plant's ability to withstand the slower reproductive rate of M. incognita larvae.

The inter-specific cross between H. radiatus x H. cannabinus and H. acutella x H. cannabinus to transfer this resistance gene successfully obtained F1 offspring that inherited the character or resistance from the male parent (H. radiatus) or (H. acutella). This is reflected in the R factor and root damage results, which has the same resistant properties as its close relatives (Kal II and SRRH023). The interspecific cross between H. cannabinus x H. radiatus produced F2 that was resistant to RKN[24] In addition, according to Arangzeb [10], the results of crossing H. acutella x F1 (H. Radiatus x H. cannabinus) are plants that were resistant to RKN and not branched.

4. Conclusion

Genotype Kal II and SRRH 023 are close relatives of kenaf, which have resistance to root-knot nematodes. The results of crosses between Kal II, SRRH 023 with the kenaf produce F1 offspring (Kal II x KR1, Kal II x KR15, Kal II x Kin2 and SRRH 023 x KR1, SRRH023 x KR15, and SRRH023 x Kin2) that are resistant to RKN caused by M. incognita. The growth of F1 Kal II x Kin2 and F1 SRRH023 x Kin2 has better plant growth than the other F1 and is resistant to RKN.
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