Analyzed of Single Nucleotide Polymorphisms (SNPs) of Sengon (*Falcataria falcata*) Tolerant and Intolerant to Boktor Pests (*Xystrocera festiva*)

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Abstract. Sengon is fast growing legume tree that is widely cultivated by community, however threathened by pest attacks, especially Boktor stem borer. This study aims to evaluate the growth of Sengon progeny testing planted in Kediri, under Perhutani State Forest Company, and analyze the relationship between Single Nucleotide Polymorphisms (SNP) markers and the phenotypes regarding their tolerance and intolerance to Boktor pests. The sample consisted of tolerant (R28, R29, R30, R33, and R47) and intolerant (S13, S21, S22, S24, and S25) accessions, with 4 individual replicates for each accession. All samples were genotyped using 4 SNP primers, i.e AAI1, AAI2, TI1 and TI2 applying the High-Resolution Melting (HRM) method at the Forest Genetics Laboratory and Advance Research Laboratory of IPB University. Phenotypic data were analyzed using the Mann-Whitney test, while SNP genotypes were assessed using Chi-square test, visualized in a Manhattan plot, and Principal Component Analysis (PCA). The disease severity of tolerant and intolerant accessions were 84.33% and 79.64% respectively, which were not significantly different. Three SNP primers gave X^2 value higher than 5.99 indicated their association with the tolerant and intolerant traits. Therefore, SNP markers can be used to assist the selection program of sengon that is tolerant to Boktor pests.

1 Introduction

Sengon (*Falcataria falcata*) is a legume tree native to Indonesia that belongs to the Fabaceae family (Nuroniah *et al*. 2021). Sengon is also called a Multi-Purpose Tree Species (MPTS) because it has high economic and ecological value. According to Prematuri *et al*. (2020) sengon is a very important and profitable species in community forest plantation because of its very fast growing (Ohorella *et al*. 2023). Sengon wood is widely used for various purposes such as fiberboard, raw materials for the pulp and paper industry, carpentry, particle board, wood veneer, matches, and so on which have the potential to improve a country's economy (Supriatna *et al*. 2017). Sengon can fix nitrogen and the harvest period is also relatively short (Krisnawati *et al*. 2011; Putra *et al*. 2015).

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Based on economic value and ecological value, Sengon is very prospective for future development. However, the production of cultivated sengon plants in 2022-2023 tends to decline (BPS 2023), because sengon is susceptible to pests and diseases attacks. The main pest that generally attacks sengon plants is Boktor stem borer (*Xystrocera festiva*), which can reduce the productivity of sengon plantations. According to Haneda *et al*. (2021), Boktor attacks Sengon plants aged 3 years and over and destroys the inner skin (sapwood). This pest can cause tree death, and stem breakage, and reduce the productivity and quality of the wood produced. Supriatna *et al*. (2017) stated that 50% of Sengon plants were attacked by Boktor pests which caused a decrease in production yields of carpentry wood. Based on research by Darwiati and Anggraeni (2018), the percentage of Boktor pest attacks on Sengon plants was 56.66%, the highest attack intensity was 90%, and the lowest attack intensity was 40%. Several control methods have been carried out, including biological, physical, chemical, thinning, and so on. However, these control methods have not been effective, and to overcome this, genetic control is being carried out through a tree breeding program by looking for superior seeds from tolerant sengon accessions. Tree breeding is an activity to improve plant characteristics that are inherited in new populations with new genetic traits, namely tolerance to pests and diseases. These populations were selected using molecular markers. The molecular markers used to select superior trees resistant to boktor pests should be very spesific such as SNPs. Previously, research was conducted by Siregar *et al*. (2019) to fingerprint sengon accessions resistant to both Boktor pest and gall-rust disease using microsatellite markers in the form of Simple Sequence Repeat (SSR), however, the markers were not specific, as it failed to distinguish the resistant from the susceptible accessions. The SNP can detect genetic diversity that occurs with a frequency of around one to13 SNPs in every 1000 nucleotides of genomic DNA (Putri and Wathon 2018). These markers are also able to distinguish complex genetic traits and understand genome evolution (Wellenreuther *et al*. 2019; Mageiros *et al*. 2021; Nugroho *et al*. 2024). One method used to detect differences in DNA sequences and produce changes in the melting curve is the High-resolution Melting (HRM) method via Real Time-Polymerase Chain Reaction (RT-PCR). This curve varies due to changes in the base arrangement and length of the nucleotide bases. This research aimed to evaluate the growth of Sengon progeny testing planted in Kediri under Perhutani State Forest Company, and analyze the relationship between SNP markers with the tolerance and intolerance traits to Boktor pests.

2 Metode

2.1 Time and Place

The research was carried out from November 2022 to October 2023 at the Forestry Genetics and Molecular Laboratory, Department of Silviculture, Faculty of Forestry and Environment IPB, and the IPB Advance Research Laboratory. Sampling was carried out in September 2022 with accessions of 21-month-old sengon plants after planting at the Pandantoyo RPH, Perum Perhutani, Kediri FMU (Figure 1).

Fig. 1. Sengon collection location at Perum Perhutani RPH Pandantoyo, Kediri FMU, East Java

2.2 Tools and Materials

The tools used are nitrogen cylinders, water baths, tubes, tips and micropipettes, analytical balances, vortexes, centrifuges, measuring cups, Erlenmeyer cups, desiccators, microwaves, horizontal electrophoresis devices, agar molds and combs, UV transilluminators, Implen Nanophotometer machines NP80, Real Time-PCR machine. The materials used were 10 samples of Sengon accessions in each of the 4 experimental plots, silica gel, liquid nitrogen, 70% alcohol, water, extract buffer solution, 10% cetyl-trimethyl ammonium bromide (CTAB) solution, 26% polyvinylpyrrolidone (PVP), β-mercaptoethanol, Sodium chloride (NaCl) 5 M, chloroform isoamyl alcohol (CIA) 24:1, phenol, cold isopropanol, ethanol 96% cold nuclease-free water, TE buffer, agarose gel, TAE buffer 1x, DNA marker 100 bp, and red nucleic acid stain gel.

2.3 Procedure

Sampling. The Sengon leaves that have been taken are placed in a plastic clip containing silica gel to prevent moisture and attack from fungi which will cause the sample to rot. The plastics were given accession numbers, tolerant (R28, R29, R30, R33, R47) and intolerant (S13, S21, S22, S24, S25) each with 4 replicates from each experimental blocks, so the total number were 40 samples. The numbering of each Sengon accession sample is as in Figure 2.

R 28-1

Information :
R : Tolerant (R)/Intolerant (S)
28 : Family number
1 : Block number

Fig. 2. Example of sengon accession numbering

Tolerance is observed based on the severity of the gall rust disease attack, evaluated using scoring and attack severity as in Table 1 and calculated using the formula (Nugroho 2021):

$$\text{DSI} = \frac{\sum(ni\ x\ vi)}{N\ x\ Z}\ x\ 100\%$$

Information :
DSI = disease severity intensity (%)
ni = number of plants attacked with score-i
vi = disease scoring value (0 to 5)
N = the number of trees observed in a plot
Z = the highest score used in the scoring classification

Table 1. Scoring the severity of the rust attack on sengon plants (Lelana *at al*. 2017)

Score	Description
0	No disease
1	Attacks on leaves, twigs, and branches <10%
2	Attacks on leaves, twigs, and branches are 11-20%
3	Attacks on leaves, twigs, and branches as much as possible 21-50%
4	Attacks on leaves, twigs, and branches were>50%, there were no attacks on stems
5	Attack on the main stem

Disease incidence was calculated using the formula from Baskorowari *et al.* (2012); Nugroho *et al*. (2023) as follows:

$$\text{Disease Incidence (\%)} = \frac{The\ number\ of\ plants\ attacked\ in\ one\ measuring\ plot}{The\ number\ of\ all\ plants\ in\ one\ measuring\ plot} \times 100\%$$

Meanwhile, plant mortality (death) can be calculated using the formula from Nugroho *et al*. (2023) as follows:

$$\text{Mortality (\%)} = \frac{The\ number\ of\ dead\ plants\ in\ a\ measuring\ plot}{The\ number\ of\ all\ plants\ in\ a\ measuring\ plot} \times 100\%$$

DNA isolation used a modification of the CTAB method (Doyle & Doyle 1987). The leaves were weighed at 0.08 g with an analytical balance, put in a plastic clip, and placed in the freezer. Heat the 1000 µl extract buffer that has been put into the tube in a water bath at 65℃ for 45-60 minutes. Samples stored in the freezer were treated with liquid nitrogen and then ground with a mortar and pestle until smooth. The sample was put into the heated extract buffer, adding 50 µl of 26% PVP and 10 µl of mercaptan. The sample is turned around and vortexed until everything is evenly. Then incubated at 65℃ for 1 hour, the tube was inverted every 10 minutes. Cool the sample for 15 minutes, and add 750 µl CIA solution and 20 µl phenol into the cooled sample tube. The tube was turned slowly and homogenized using a vortex, and centrifuged for 10 minutes at a speed of 10,000 rpm. Take the supernatant and transfer it to a new 2 ml tube, add 750 µl CIA solution, invert the tube slowly, homogenize with a vortex, and centrifuge again for 10 minutes at a speed of 10,000 rpm. The second supernatant was transferred to a 1.5 ml tube and added 600 µl cold isopropanol and 300 µl NaCl. The tube is flicked with a finger until a white thread appears which is usually called DNA. The sample is incubated for 24 hours at a temperature of -20℃ to precipitate the DNA. Carry out the washing process with a sample centrifuge for 10 minutes at a speed of 10,000 rpm. The liquid is slowly discarded until only the DNA pellet remains, add 300 µl of 96% ethanol, centrifuge, and carry out the washing process 2 times. The tube was dried in a desiccator for 15 minutes, added 33 µl of TE buffer, and incubated in the refrigerator for approximately 24 hours. The quality of the DNA is tested using electrophoresis and a UV transilluminator. Pour 33 ml of TAE 1x solution into the Erlenmeyer, weigh 0.33 agarose

powder, and pour into the Erlenmeyer. The solution is stirred and placed in the microwave for 1 minute or until dissolved. Add 1 µl of red nucleic acid stain gel as a dye. Pour the liquid into the electrophoresis tray which already contains the agar mold and comb. Wait until the gel freezes and transfer the gel to an electrophoresis bath containing 1x TAE solution. Prepare paper film for mixing 3 µl DNA template with 1 µl dye loading and homogenize with a micropipette. Place the mixture into the agarose gel well that has been formed with a comb. Insert a 100 bp DNA ladder marker in the edge of the gel well. Then, connect the electrophoresis device to a power supply powered by 100 volts for 28 minutes. The results of electrophoresis can be seen with the help of a UV transilluminator lamp and a wavelength of 260 nm, the results can be photographed using a cellphone or camera. DNA quantity was tested using an Implen NP80 Nanophotometer and TE buffer blank. Clean the optical hole with 1 µl TE buffer, wipe with tissue, close the hole, and click the measure blank menu on the computer. Open the cover and clean it again with tissue. Insert 1 µl DNA sample into the optical hole and click the measure sample menu. Save and name the sample whose quantity has been tested. Clean the tool again with the TE buffer and press the "off" button when finished. The primers were validated using an RT-PCR machine according to the tolerance properties of Sengon as shown in Table 2. The primers were validated on 40 DNA samples using the HRM method.

Table 2. Primary list of SNPs

Primary name	Primer code	Position of SNPs	Sequence 5'-3'
Alpha-amylase Inhibitor	AAI_1	575 (C>T)	F: TATCCCAATCCGACGACAA R: CGGCTGACGAGAGTGAAG
	AAI_2	944 (G>T)	F: GGTGTCCTACAGAAGTGTGTCC R: CGACAGGAAGAGCATTACCA
Trypsin inhibitor	TI_1	771 (A>G)	F: TTGTGACTTGTGTGTTGATGTG R: CCCATCGGTTCTCTCTCTCT
	TI_2	850 (G>A)	F: AGGTAAGTGTGGACATGTTGGT R: ATGCCCAGGGATGTTTATTC

The letters in the SNPs position column are the nucleotides thymine (T), adenine (A), cytosine (C), guanine (G). C>T means that there is a mutation from cytosine to thymine or a change in the reference base to an alternative base. F = primary forward, R = primary reverse. Source: Nugroho *et al.* (2021)

Table 3. Scoring the severity of the rust attack on sengon plants

Reagent	Volume (µl)
2x SensiFAST HRM Mix	10
10 µM *Forward* Primer	0,8
10 µM *Reserve* Primer	0,8
H_2O	6,4
100ng/ µl *Template* DNA	2
Volume total	20

Source: Nugroho *et al*. (2021)

Mix all the compositions, and carry out RT-PCR through DNA amplification stages, with details of initial denaturation (one cycle at a temperature of 95℃ for 60 seconds), and denaturation (at a temperature of 95℃ for 5 seconds) and annealing/extension (at a temperature of 60 ℃ for 25 seconds) for 40 cycles. Continue the melt curve process with details of denaturation (one cycle at 95℃ for 10 seconds), annealing (one cycle at 60℃ for 60 seconds), high-resolution melt (one cycle at 95℃ for 15 seconds), and annealing (one cycle at 60℃ for 15 seconds). The amplification and melt curve processes were carried out with a ramp rate of 0.3%.

3 Data Analysis

3.1 Mann-Whitney Test

The Mann-Whitney test was carried out to see the difference in attack severity between tolerant and intolerant Sengon accessions with the following hypothesis:
H0: There was no difference between tolerant and intolerant sengon accessions
H1: There are differences between tolerant and intolerant sengon accessions

3.2 Chi-square Test

The Chi-square test is carried out using the following formula:

$$X^2 = \sum_{i=1}^{p} \frac{(|Oj - Ej| - 0{,}5)2}{Ej}$$

Information :
Oj : observation value in class-i
Ej : expected value in class-j; j=1, 2.

3.3 Manhattan Plot

The chi-square test results with each primer were visualized by Manhattan plot using RStudio. The x-axis shows the location of the primer on a particular contig, while the y-axis shows the p-value with a -log10 scale.

3.4 Principal Component Analysis (PCA)

Data from 40 samples of Sengon accessions with Trypsin Inhibitor (TI) and Alpha-Amylase Inhibitor (AAI) primers were then visualized using PCA to determine whether samples were separated or not separated.

4 Results

4.1 Attack severity, attack incidence, and mortality

The data used in this study were from gall rust disease attacks, as the progenies are still relatively young, that Boktor pest has not found yet. Previous study showed that susceptibility to Boktor pest is significantly correlated with susceptibility to gall rust disease (Nugroho *et. al.* 2021). Damage to Sengon trees due to gall rust disease in the Pandantoyo RPH, FMU Kediri, East Java is relatively high. The average percentage of severity of attacks that have been carried out is around 81.98%, the percentage of incidence is 100%, and the percentage

of mortality is 47.79% (Figure 3). The average attack severity values for tolerant and intolerant accessions were 84.33% and 79.64% respectively.

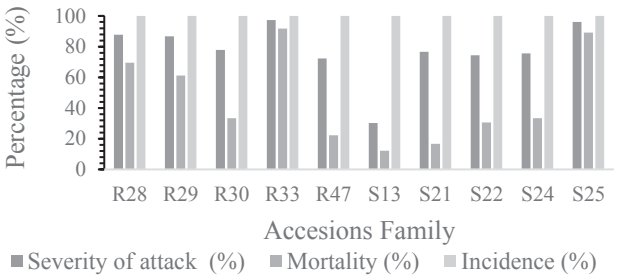

Fig. 3. Percentage of attack severity, disease incidence, and mortality in each progeny, R = tolerant progeny; S = intolerant

The difference between tolerant and intolerant sengon accessions can be evaluated using the Mann-Whitney test using attack severity data. The results of the Mann-Whitney test can be seen in Table 4.

Table 4. Mann-Whitney test results

Parameter	Accession	
	Tolerant	**Intolerant**
Average (%)	84,33	79,64
p-value	1,302601911	

The results of the Mann-Whitney test stated that the p-value was > α (0.05), so H0 was accepted, meaning there was no difference between tolerant sengon accessions and intolerant sengon accessions. This stated that the attack severity score was not able to separate tolerant sengon accessions from intolerant sengon accessions because these sengon accessions were still relatively young (21 months old after planting). Young sengon trees are easily attacked by disease because gall rust infects sengon trees when they are still young (Rahayu *et al*. 2009; Syakirin 2014).

4.2 DNA Quality and Quantity of Sengon Accessions

Quality DNA determines success in molecular analysis. The quality of DNA is measured for purity using electrophoresis with the help of a UV transilluminator lamp and produces DNA bands. The thickness of the band is influenced by the density of the sample, in this study there were varying DNA bands Figure 4. The electrophorogram produces good bands, smears, and no bands.

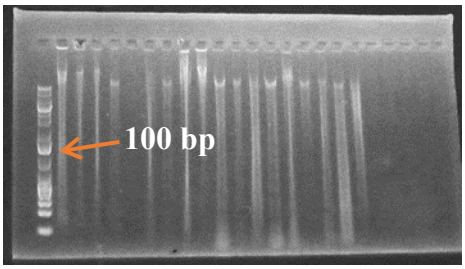

Fig. 4. Electrophoregram of sengon accession and 100 bp DNA marker as control

4.3 Difference plot High-resolution Melting

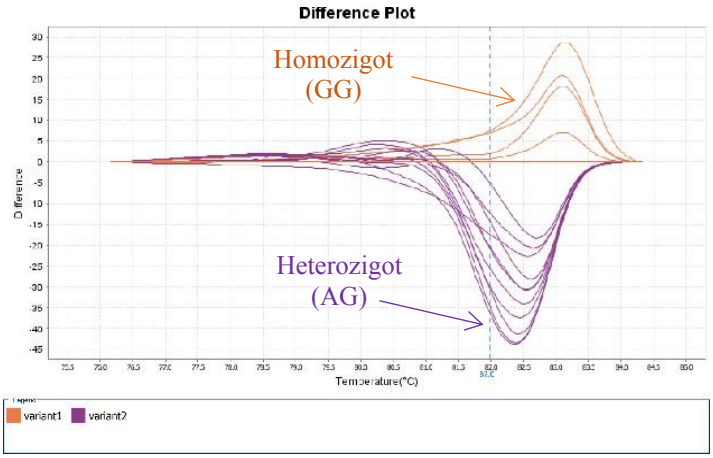

Fig. 5. HRM curves of each SNP primer TI_1

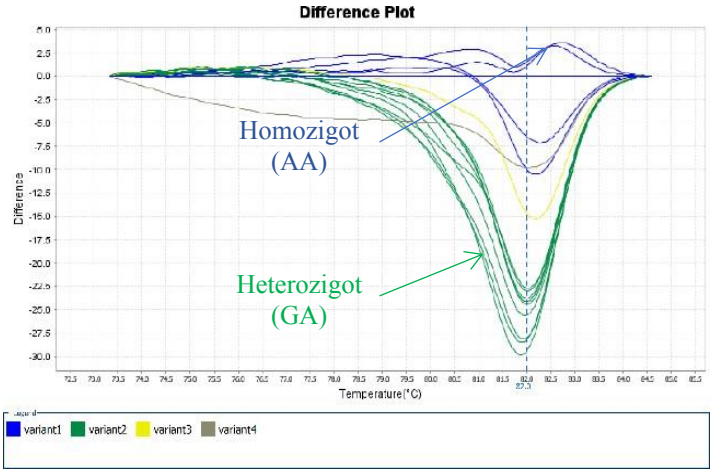

Fig. 6. HRM curves of each SNP primer TI_2

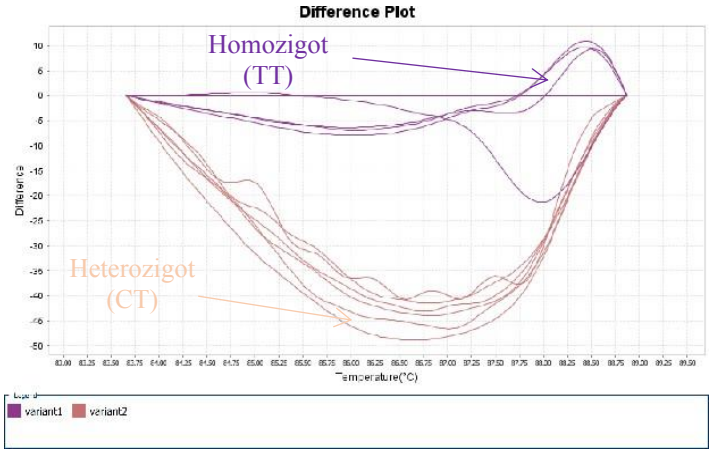

Fig. 7. HRM curves of each SNP primer AAI_1

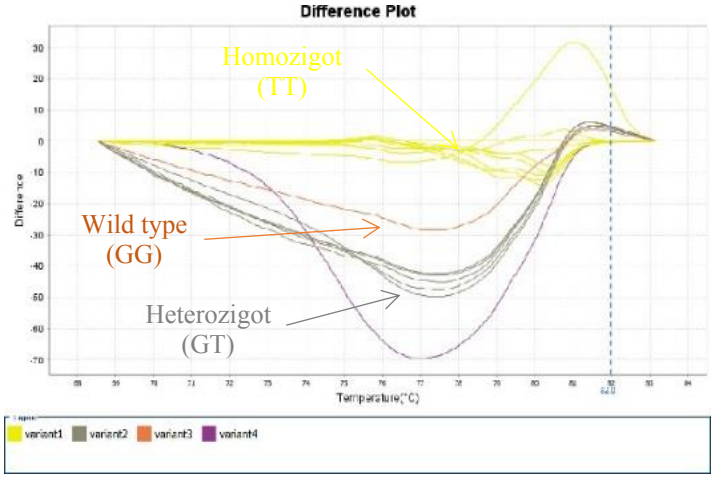

Fig. 8. HRM curves of each SNP primer TI_2

4.4 Relationship between SNPs and Sengon Resistance Traits

Table 5. Mann-Whitney test results

Mendel's Ratio	Primary	X^2 Count	-log 10 p value	X^2 Table
	AAI_1	4,70	1,02	
1:2:1	AAI_2	39,50*	8,57	5,99
	TI_1	22,70*	4,929	
	TI_2	54,30*	11,79	

4.5 Significance of SNPs with Sengon Resistance Traits

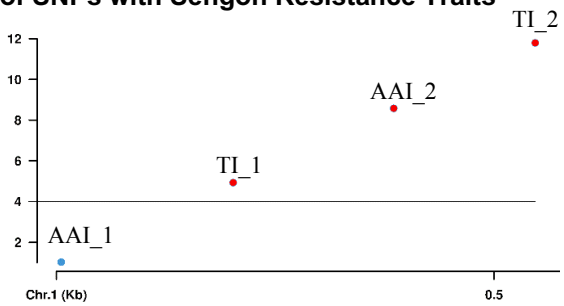

Fig. 9. Visualization of Manhattan plot of primary significance

4.6 Genetic Markers

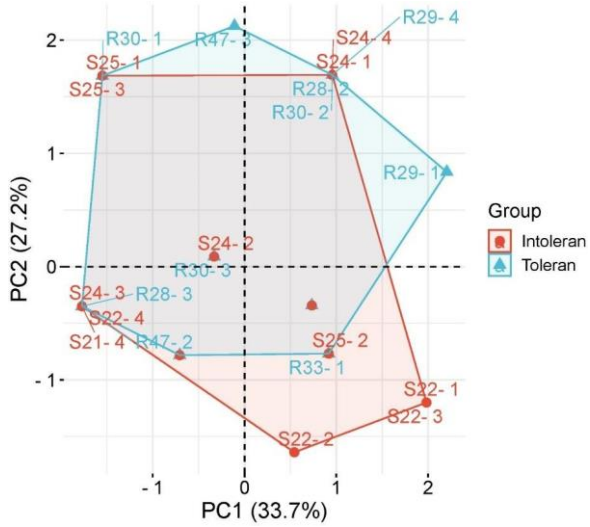

Fig. 10. PCA visualization of sengon accessions against TI and AAI primers

Table 6. Mann-Whitney test results

Accession	Accession number	AAI_1	AAI_2	TI_1	TI_2
Toleran	R29-1	CC	GG	AG	AG
	R47-3	CT	GT	AA	AG
Intoleran	S22-1	CC	GG	AG	GG
	S22-2	CC	GG	GG	GG
	S22-3	CC	GG	AA	GG

5 Discussion

5.1 Atack severity, attack incidence, and mortality

The data used in this study were from gall rust disease attacks, as the progenies are still relatively young, that Boktor pest has not found yet. Previous study showed that susceptibility

to Boktor pest is significantly correlated with susceptibility to gall rust disease (Nugroho *et al*. 2021). Damage to Sengon trees due to gall rust disease in the Pandantoyo RPH, FMU Kediri, East Java is relatively high. The average percentage of severity of attacks that have been carried out is around 81.98%, the percentage of incidence is 100%, and the percentage of mortality is 47.79% (Figure 3). The average attack severity values for tolerant and intolerant accessions were 84.33% and 79.64% respectively.quations should be centred and should be numbered with the number on the right-hand side.

5.2 DNA Quality and Quantity of Sengon Accessions

Quality DNA determines success in molecular analysis. The quality of DNA is measured for purity using electrophoresis with the help of a UV transilluminator lamp and produces DNA bands. The thickness of the band is influenced by the density of the sample, in this study there were varying DNA bands Figure 4. The electrophorogram produces good bands, smears, and no bands. The resulting bands vary because the freshness levels of the samples are different. According to Siregar and Diputra (2013), the thickness of the ribbon can be influenced by the level of freshness of the leaf sample used. Samples that are still fresh will produce thicker bands than samples that are less fresh. Smear bands can be caused by degradation or impurities in the form of chemicals contained in the extracted DNA solution. DNA that does not produce bands is because the sample is no longer fresh or contamination occurred during extraction. This contamination can be observed in the ratio A260 and A280. Storing samples for too long can be a factor causing damage to leaf cells and the DNA contained therein will also be damaged (Siregar and Diputra 2013). Damaged DNA usually undergoes degradation by showing a smear on the DNA band (Cefhiandra 2023). Another factor that causes DNA smear bands is that drying leaves at high temperatures can cause DNA to be degraded and fragmented (Sembiring *et al.* 2023). DNA whose quality is known will be tested for quantity using the Implen NP80 Nanophotometer based on the thickness of the ribbon. The thickness of the band will affect the DNA concentration. According to Siregar and Diputra (2013), high DNA concentrations are influenced by the thickness of a band. Different DNA band thicknesses will produce different concentrations as well. Good DNA will show an absorbance ratio value of 260/280 nm with a good DNA purity range of 1.8 – 2.0. Contaminated DNA will show a purity value below 1.8.

5.3 Difference plot High-resolution Melting

The difference plot curve (Figure 5,6,7,and 8) shows the occurrence of a mutation because there are different melting points on the curve. When a mutation is identified, the type of allele pair (homozygous or heterozygous) and the type of nitrogen base become a reference. Homozygous alleles are characterized by alleles that have a low DNA melting point and weak binding. Heterozygous alleles have a high DNA melting point and strong binding. The melting point of DNA is related to base bonds. The higher the base content, the higher the melting point of the DNA (Darmawati *et al*. 2023). There are 4 types of base bonds in DNA, namely adenine (A), cytosine (C), guanine (G), and thymine (T). Difference plot on primer TI_1, there is a homozygous allele pair which is characterized by a change in the nitrogen base adenine (A) to guanine (G) to GG, both bases are the same, the curve is upward and colored orange. Heterozygous allele pairs have unequal bases (AG), the curve is downward and colored purple. Difference plot on primer TI_2, there is a change in the base guanine (G) to adenine (A) resulting in a homozygous allele pair (AA), the curve is upward and colored blue. Bases that do not change or remain constant will produce a heterozygous allele pair (GA), the curve is downward and colored green. Difference plot on primer AAI_1, there is a change in the base Cytosine (C) to thymine (T) resulting in a homozygote (TT), the curve is

upwards, and is colored purple. Primer AAI_1 is said to be heterozygous, if there is no base or permanent change (CT), the curve is downward, and the color is purple to pink. The AAI_2 primer changes the base guanine (G) to thymine (T) resulting in a homozygous allele pair (TT), the curve is upwards, and the color is yellow. Allele pairs in which no base change (GT) occurs are called heterozygous allele pairs, the curve is downward and is colored grey. This difference plot also visualizes the results of the analysis of the differences between wild-type DNA and charged DNA. Wild type is a sample that has a reference base based on the results of SNPs calling, for example in the AAI_2 primer the wild type occurs, and the curve is gray, shown in Figure (8). TI_2 load is a sample that experiences a change in the bases of a gene or chromosome (has alternative bases).

5.4 Relationship between SNPs and Sengon Resistance Traits

The results of HRM analysis (Figure 5, 6, 7, and 8) show that the TI_1 primer produces the allele pair AA, AG, and GG. Primer TI_2 produces AA, GA, and GG allele pairs. Primer AAI_1 produces the allele pair CC, CT, TT. Primer AAI_2 produces the allele pair GG, GT, TT. The results of chi-square test of the alleles distribution in the samples presented in Table 5 shows that the calculated X2 value for primers TI_1, TI_2, and AAI_2 is greater than X2 Table (5.99) so that H0 (there is no relationship between SNPs and tolerant or intolerant traits in Sengon plants) is rejected (Table 5). This value shows that these alleles do not segregate freely and it is suspected that there is a relationship between the primers used and the tolerant properties of Sengon progenies, which can be seen from the calculation results of -log10 (p-value). The p-value of each primer is greater than 0.05, meaning that the primer has a significant effect on the tolerant properties of the Sengon accession (Table 5).

5.5 Significance of SNPs with Sengon Resistance Traits

The chi-square test results were visualized using a Manhattan plot (Figure 9). Based on the results of the Manhattan plot visualization, primers AAI_2, TI_1, and TI_2 passed the threshold line, meaning that these primers had a high significance value between SNP markers and the tolerant nature of the sengon accession compared to primer AAI_1. The higher the -log 10 (p-value) value, the more significant the relationship between SNPs markers and the tolerant nature of Sengon accessions (Nugroho *et al*. 2023). Primer AAI_1 has the lowest association significance value, this primer is below the threshold line and has the lowest p-value of the 4 primers. The p-values for each primer AAI_1, AAI_2, TI_1, and TI_2 are 1.02, 8.57, 4.92, and 11.79. The threshold line is a line that has been set (0.05) or a hypothetical value of 95%.

5.6 Genetic Markers

The genetic marker used in this research is SNP which uses a single variation in one base pair in DNA. TI (Trypsin Inhibitor) SNP markers derived from TI gene sequences have a relationship with the TI gene that influences the properties or function of the proteins encoded. SNPs located in the trypsin inhibitor primer will affect the primary expression or protein characteristics, thereby changing a single base in the DNA sequence and affecting the structure or function of the protein. The association of SNPs primers from Alpha-Amylase Inhibitor (AAI) gene also involves genetic variations at one nucleotide that can affect the structure, expression, and function of the gene. The relationship between SNP genotypes and the phenotypes can be analyzed using Principal Component Analysis (PCA). The results of the PCA analysis are visualized to see the relationship among samples regarding their genotypes and category of tolerance (Figure 10). TI and AAI genes play an important role in

the growth of Sengon plants because the proteins encoded can inhibit the activity of amylase and proteinase enzymes in the insect digestive tract (Siregar *et al.* 2021; Karray *et al.* 2022; Nugroho *et al*. 2024). This SNP was able to differentiate resistant accessions from susceptible accessions using PCA visualization and convex distribution of sample points in quadrants with the results presented in Figure 10. The results of the PCA visualization show samples that are close together, meaning they have similar SNP genotypes. Some tolerant sengon accessions can be separated from intolerant ones. However, there are still overlapping tolerant and intolerant accessions, meaning that the genotyping results from the 4 primers have not been able to separate tolerant and intolerant accessions as a whole. Separate tolerant sengon accessions are R29-1 and R47-3, while susceptible accessions S22-1, S22-2, and S22-3. The results of this PCA analysis were used to group tolerant sengon accessions from intolerant sengon accessions based on genotyping. The whole accessions are categorized into three group, namely tolerant accessions, intermediate accessions, and intolerant accessions. Separated tolerant and intolerant sengon accessions are presented in Table 6 with their each genotype. The AAI_1 primer has a CC allele pair that is dominant in the intolerant accession compared to the tolerant accession, meaning that a change from C to T could result in more tolerant individual The AAI_2 primer has a dominant GG allele pair in the intolerant accession compared to the tolerant accession, which indicates a change from homozygotes of G into heterozygotes could lead to more tolerant. The TI_2 primer in the tolerant accession does not have a GG allele pair that the intolerant accessions have. Those results showed that the SNP primers can separate tolerant and intolerant Sengon accessions against Boktor pests.

6 Conclusions and Suggestions

6.1 Conslusions

The phenotypic assessment showed high levels and not significantly different of disease attacks in both tolerant and intolerant Sengon accessions at the 84.33% and 79.64% respectively. Changes in the genotypes of the SNP markers can result in changes of the degree of tolerance of sengon accessions against Boktor pest. Therefore, SNP markers can be used to assist the selection program for sengon that is tolerant to Boktor pests.

6.2 Suggestions

Observations of this Sengon accession need to be carried out at the mature age of the tree to analyze attacks by the Boktor pest and gall rust disease in the adult phase and in larger numbers of samples. The number of Sengon accessions in this study was very small, so the results were not yet clearer. If there are more samples, a relationship between SNP markers and the tolerant nature of Sengon accessions will be more clearly seen.

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