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Testing of Selection Criteria for Screening of Oil Palm Genotypes Partially Resistant to *Ganoderma boninense* in Main-Nursery

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ABSTRACT

Resistance to basal stem rot (BSR) caused by *Ganoderma boninense* is now one of the main breeding objectives by many oil palm breeders. Many studies reported that resistance to *G. boninense* is partial. Two hundreds of DxP crosses between 16 dura lines and three pisifera palms were made for testing of their resistance to *G. boninense* in main-nursery. Ganoderma inoculums were prepared using rubber wood block (RWB) of 108 cm^3 size. Plot size was 20 seedlings $cross^{-1}$ and replicated five times in RCBD arrangement. The nursery was put in the screen house with 40% light transmission, 26–28^oC temperature and 70-80% relative humidity. Disease incidences (DI) were recorded for twelve months after inoculation (MAI). Internal symptoms were recorded at the last observation. DI data was analyzed using formula to obtain percentage of disease severity (DS), initial infection symptom (IIS), r and area under disease development curve (AUDPC). Seventh criteria: namely, IIS, DI at 7 MAI, DS, r, AUDPC, and fresh plant biomass weight (FBW) and fresh root weight (FRW) were used to select resistant genotypes to *G. boninense*. The results showed that: (1) there were differences in fastness of infection and severity of the disease; (2) variation in DS among tested crosses increase with time and reach maximum at the 12th MAI; (3) only four selection criteria had significant correlation FRW, namely FBW (0.74), AUDPC (-0.261), DS (-0.233), r (-0.205); and (4) four selection criteria which gave the closest results to FRW criterion consecutively were FBW (73%), AUDPC (65%), r (64%), DS (63%). It could be concluded that main-nursery screening could identify genotypes with lower r value. AUDPC and r values are suitable to be used as selection criteria for selecting oil palm genotypes partially resistant to *G. boninense* in main-nursery screening.

Keywords: basal stem rot, *Ganoderma boninense*, oil palm, partial resistance, planting material

INTRODUCTION

Basal stem rot (BSR) caused by *Ganoderma boninense* is one of the most important diseases of oil palm. Resistant planting materials are needed as an important component to be used in integration with other components to control this disease. No immune or complete resistant oil palm genotypes

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have been reported so far, but many researchers have found availability of genotypes with partial or incomplete resistance. Some resistant crosses have been selected and released for comercial planting as so call moderate resistant varieties to *G. boninense* (Turnbull *et al.* 2014; Rees *et al.* 2007; Purba *et al.* 2011). Quantitative resistance (QR) is defined as a resistance that varies in a continuous way between the various phenotypes of the host population, from almost imperceptible (only a slight reduction in the growth of the pathogen) to quite strong (little growth of the pathogen) (Do Vale *et al.* 2001, Van der Plank 1984). This resistance is often indicated with other terms such as partial, residual and field resistance or even (wrongly) with tolerance (Van der Plank 1984). QR occurs to most of our important pathogens at various levels in nearly all crops (Naher *et al.* 2013). Since this QR does occur in the cultivars grown, it is genetic material that is related to what the breeders' desire (Van der Plank 1984; Naher *et al.* 2013). For this type of resistance, breeders do not need to look for primitive genotypes from centers of diversity nor to related wild species (Tan *et al*. 2013). Faizah *et al.* (2022) studied the mechanisms of resistance and reported that Ganoderma infection-induced salicylic acid (SA) accumulation and lignification in resistant accessions promote the seedlings better root biomass, and concluded that oil palm seedlings have a synergistic physical, biochemical, and molecular defense. The desired genotypes with BSR disease resistance probably have specific physiological, biochemical, and molecular characteristics when interacting with *G. boninense* (Naher *et al.* 2013; Tan *et al*. 2013).

Selection for resistance implies measurements of plant resistance. Ideally one should measure the amount of pathogen present at a given moment compared with the amount present on or in an extremely susceptible cultivar (Do Vale *et al.* 2001, Naher *et al.* 2013; Parlevliet 1993). The larger the difference in amount, the larger the difference in susceptibility/resistance. It is normally not possible to measure the amount of pathogen, because the pathogen is either not visible or only partially so. However, one can evaluate the direct or indirect effects of the pathogen on the host even if the pathogen itself is not visible (Parlevliet 1989). The quantitative or partial resistance of a host cultivar cannot be assessed in absolute terms; it is always a relative measure compared with that of a well-known standard cultivar. This standard cultivar is often the most susceptible cultivar available (Breton *et al.* 2009). The amount of tissue affected is, in general, a good estimator of the amount of pathogen present. The objectives of this study were: (1) to test several criteria suitable for identifying genotypes to have certain level of resistance; and (2) to select the most appropriate criteria for selecting of oil palm genotypes partially resistant to *G. boninense* in main-nursery.

MATERIALS AND METHODS

Materials

Tested materials

Tested crosses were obtained by crossing 16 dura lines with different Ganoderma Disease Index (GDI) with three pisifera palms. The dura palms were 13 years old and grown under Ganoderma endemic field in Kisaran, North Sumatera. Disease incidence of the 16 dura lines ranged from 14.80% to 76.90% with the average of 31.94%. Based on GDI, 13 lines are categorized as resistant and 3 lines as susceptible. The DxP combinations of 200 tested

crosses are shown in Table 1. Majority of the tested crosses (94.5%) were the progenies of moderately resistant dura lines with GDI values ranging from 46 to 93. Other crosses involved three susceptible dura lines with the GDI values of 188, 191, and 241, respectively.

Table 1 Fourteen years old dura palms of sixteen lines with GDI crossed with three pisifera palms to obtain 200 tested cross.

No.	Dura Code	Resistance to *Ganoderma*			Number of DxP Crosses
		Disease incidence (%)	GDI	Category	
1	76	14.80	46	R	7
2	151	15.70	49	R	14
3	75	16.70	52	R	6
4	133	21.30	67	R	6
5	137	24.10	75	R	13
6	6	25.90	81	R	42
7	11	25.90	81	R	56
8	64	25.90	81	R	4
9	108	27.80	87	R	15
10	132	27.80	87	R	4
11	78	27.80	90	R	10
12	150	28.70	90	R	9
13	36	29.60	93	R	3
14	72	60.20	188	S	2
15	9	61.10	191	S	6
16	128	76.90	241	S	3
Total/ Av	16	31.94	100	I	200

Inoculum for artificial inoculation

Ganoderma isolates were collected from 16 collecting points inside the estates of Bakrie Sumatera Plantation (BSP) in Kisaran, North Sumatera. One of the most virulent isolates among the 16 isolates (S12) was used as source of inoculum for this screening. The inoculum preparation and inoculation methods were adopted and modified from the existing protocols (Rees *et al.* 2007; Breton *et al.* 2009; Rahmaningsih *et al.* 2018; Idris *et al.* 2004). The difference was that artificial inoculation background of using 1 month old seeds is to prevent death/abnormality of seedlings outside of pathogenic factors. made to one month old seedlings instead of germinated seeds. The inoculum was prepared on rubber wood blocks (RWB) of 6 x 6 x 3 cm (108 cm^3). There were 26,000 inoculums in RWB needed for making artificial inoculation. The treatment was arranged in a randomized block design (RCBD) with 5 replications. Plot size was 20 polybags per crosses.

Methods

Nursery screening

Germinated seeds were planted in small polybags (5 x 5 cm) and grown until two leaf-stage seedlings, and then transplanted to main nursery using polybags of 20 x 30 cm in size, suitable for placing pre-nursery (PN) seedlings and RWB inoculum in it. The seedlings used were in the two leaf-stage, healthy, and in normal size. The planting medium was a sterilized equal mixture of soil and sand (1:1).

Artificial inoculation

The inoculums were placed 1 cm below the seedlings. Seedlings were grown in screen house with 40% light penetration, ambient relative humidity ranging between 70−80% and temperature of 26−28 ºC.

Data Analysis

Initial infection symptom (IIS)

Initial infection symptom for every seedling was monthly recorded as appearance the first external disease incidence symptom (score 1) of twenty seedlings for each plot during monthly observation was used to estimate period.

Disease incidence (DI)

DI was calculated using the formula:

$$\text{Disease incidence} = \frac{a}{a+b}\ x\ 100\%$$

a: the number of diseased plants

b: the number of healthy plants

Disease severity (DS)

Disease severity (DS = X_t) was calculated using the formula:

$$\boldsymbol{DS}(\%) = \frac{\Sigma(AxB)}{\Sigma(nx5)}$$

A: the disease scale (0−5)
scale 1 disease percentage (1−5%); scale 2 (5−25%); scale 3 (25−50%); scale 4 (50−75%); scale 5 (75−100%).
B: the number of seedlings showing the corresponding disease scale per plot
n: the total number of plants
5: the maximal disease score

Rate of disease development (r)

$$r = \frac{2.3}{t2-t1}\left\{\log\frac{x2}{1-x2} - \log\frac{x1}{1-x1}\right\}$$

x1: the disease index at initial week time (t1)
x2: the disease index at subsequent week time (t2)

Area Under Disease Development Curve (AUDPC)

$$AUDPC = \Sigma\frac{X0+Xt}{2}\ x\ t$$

X_0 : previous disease incidence
X_t : subsequent disease incidence
t : interval time

Fresh biomass weight (FBW)

At the last observation (12 MAI), each seedling was dismantled and cleaned from the remaining inoculum and soil media using running water. After the bole and roots were clean and air dried, the fresh biomass per seedling was weighted using a digital scale.

Fresh root weight (FRW)

Average FRW of seedlings for every tested cross was measured by cutting roots from the bole of all seedlings and weighed, and then the result was divided by the number of seedlings per plot.

Comparing of selection criteria

The average value of DI for every selection criterion was used to determine the resistance and susceptibility responses against *G. boninense* infection by determining the GDI.

RESULTS AND DISCUSSIONS

Disease incidence (DI)

Figure 1 shows differential progress of DI among 200 tested crosses and MR control, and can be noted that: (1) the first disease symptoms was appeared on the 3rd MAI on the most susceptible cross (orange) and on the 6th MAI for the most resistant cross (blue), and on the 4th MAI for average of tested crosses (grey) and MR control (yellow), (2) all tested materials got infection but percentage of incidence varied among genotypes indicating there were differential degree of susceptibility, (3) the variation on percentage of incidence among tested crosses continuously increased up to the 7th MAI but steadily reduced there-after until all tested cross got 100% incidences on the 12th MAI. Selection for resistant crosses should be done the 7th MAI using DI as selection criteria.

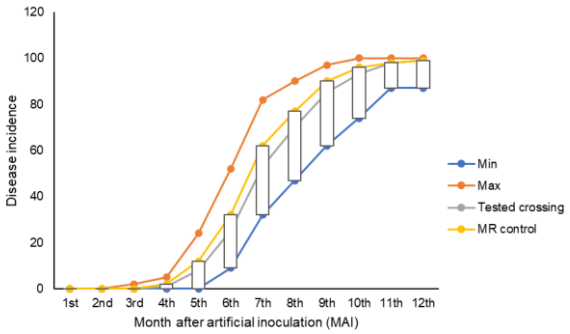

Figure 1 Monthly progress of disease incidence

Initial infection symptom (IIS)

Initial disease symptom (Figure 2) as the estimate of latent period (LP) for 200 tested crosses ranged from 4.4 months to 6.4 months with the average of 5.2 and 4.8 months of trial average and MR control cross, respectively. The frequency distribution was still closed to normal but with very small (6%) coefficient of variation. Since BSR is a root disease, initial symptom on upper surface might not be a good estimate of

latent period (Dickinson and Lucas 1977).

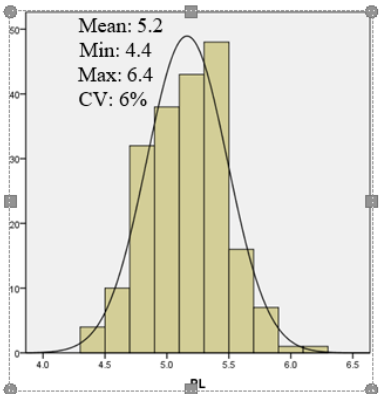

Figure 2 Frequency distribution of 200 tested crosses based on duration of IIS.

Disease severity (DS)

From Figure 3 it can be noted that: (1) percentage of DS continuously increased from the start of infection up to the last (the 12th) observation but the degree of severity varied among crosses, (2) differential variations of severity among crosses gave strong indication that the genotypes had different abilities to control the rate of disease infection (*r*), and (3) genotypes with low *r* values got low disease severity, perhaps due to the expression of certain genes for resistance.

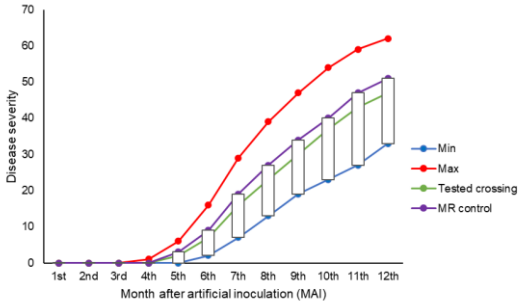

Figure 3 Differential progress of disease severity (DS)

Rate of disease development (r)

Figure 4a shows genetic variations based on r value of 200 tested crosses which ranged from 0.065 to 0.191 with the mean of 0.111 and 21% coefficient of variation (CV) for population. Differential *r* values among tested crosses were responsible for different degrees of disease severity (DS) and AUDPC values at 12 MAI. There was a big portion of genotypes which had lower *r* values than average crosses, indicating that this parameter is potential enough to be used as a selection criterion for selecting genotypes partially resistant to *G. boninense*.

Area under disease development curve (AUDPC)

Frequency of tested crosses based on AUDPC value (Figure 4b) also followed normal distribution with range from113.9 to 275.1, average of 180.6, and 17% CV. AUDPC gives better measurement because resistant genes express both latent period and *r* value combined together (Jeger and Roljani 2001).

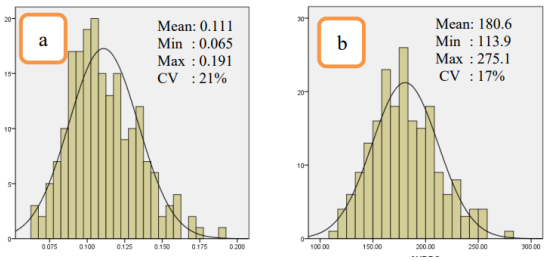

Figure 4 Frequency distribution of 200 tested crosses based on r value (a) and AUDPC value (b).

Figure 5 shows the progress of AUDPC value from 1st to 12th observations which ranged from the smallest (33.5, grey) to the highest (492.5, yellow) with the average of 180.6 for trial and 205.4 for the MR control cross. Crosses which had AUDPC values lower than the average and/or MR control (orange) could be selected as genotypes partially resistant to *G. boninense*.

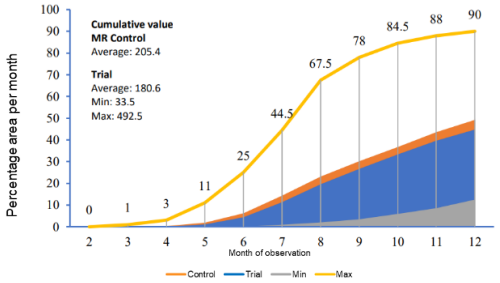

Figure 5 AUDPC of the most susceptible (orange), most resistant (grey), average (blue) of 200 tested crosses and MR control (red) for 12th MAI.

Fresh biomass weight (FBW)

Biomass weight is measured to see the ultimate effect of the disease on the growth and development of the infected genotype. Genotypes with higher biomass will give good indication that they have better resistance than genotypes with lower biomass weight. Figure 6 shows frequency distribution of 200 tested crosses based on FBW at 12 MAI ranging from 302.8 to 740.4 with the average of 563.8 g plant^{-1}. The distribution is closed to normal with 14% CV. Therefore, this parameter fulfills the requirements as a selection criterion.

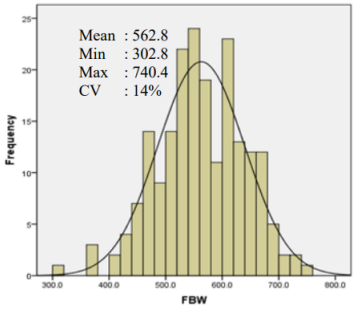

Figure 6 Frequency distribution of FBW.

Fresh root weight (FRW)

Figure 7 shows different appearances of root biomass per plot with different degrees of disease severity scores. Roots of seedling with score 1 looked very healthy and had very hight FRW indicating it had very small disease development rate and finally was able to recover. Seedlings with scores 4 and 5, on the contrary, looked very high infection and most of the roots were rotted. These distinct variations of disease severity among crosses gave very strong basis to make genetic improvement for BSR resistance by selecting genotypes which are able to limit the rate of disease development.

Frequency distribution of 200 tested crosses based on FRW per cross is given in Figure 8. FBW as a selection criterion looks very effective to differrentiate tested crosses with different levels of resistance as indicated by broader ranges from minimum to maximum in normal distribution curve and high CV. Based on GDI of FRW, 99 of the 200 tested crosses had FRW better than the average and were selected as genotypes partially resistant to *G. boninense.*

Figure 7 Appearance of root biomass of tested crosses with different scores of resistances to G. boninense at 12 MAI.

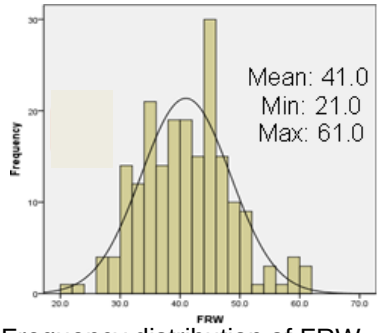

Figure 8 Frequency distribution of FRW.

Comparison among selection criteria

Selection for varietal improvement is only effective if there are a considerable genetic variations in the working population. Therefore, the most appropriate and suitable selection criteria for selecting resistant genotypes to *G. boninense* must be the ones that can differentiate the resistant from the susceptible ones. Three parameters, namely variability, correlation and results of selected genotypes are used in this study to test the effectiveness of tested criteria.

Variability

The existence of genetic variability within a population for the seven selection criteria was evaluated by calculating the range of minimum to maximum values and CV of GDI (Table 2). Based on CV and the range from minimum to maximum values, the most varied criterion consecutively was rate of

infection (*r)*, FRW, AUDPC, DI at 7 MAI, FBW, DS at 12 MAI and IIS.

Correlation

Since FRW is the most appropriate and reliable measurement for root disease severity, the degree of correlation of each criterion to FRW was calculated. There were four criteria (Table 3) with highly significant (P= 0.01) positive and negative correlations to FRW, namely FBW (0.7370), AUDPC (-0.260), DS (-0.233) and infection rate (-0.204). Two other criteria, DI at 7 MAI and IIS, had no correlation to FRW.

Selected progenies

Selected crosses which were better than the average DI (100) of the 200. Table 4 show only FRW gave the tested crosses varied from 96 to 116 crosses closest result to 100 minus 1 (99), followed by DS (101), AUDPC (104) and FBW (96). These deviations are related to the degree of normality of frequency distribution curves of the tested criteria. Similarity of selection results of the tested criteria to FRW ranged from the highest (72, FBW) to the lowest (45, DI). AUDPC, *r,* and DS gave fairly good results with the degree of similarity of 65%, 65%, and 63%, respectively. IS and DI gave very poor similarity to the FRW result because these criteria did not take infection rate (*r*) value into account.

Table 2 Comparison of variability parameters of seven tested selection criteria for selecting oil palm genotypes partially resistant to *G. boninense.*

Parameter	FRW	FBW	IS	DI 7 MAI	DS 12 MAI	(r)	AUDPC
Average	100	100	100	100	100	100	100
Maximum	149	132	120	154	133	172	152
Minimum	51	54	85	60	70	59	63
Range	97	78	35	94	63	113	89
Coefficient of Variation	18%	14%	6%	17%	13%	21%	17%

FRW=fresh root weight, FBW=fresh biomass weight, IIS=initial infection symptom, DI=disease incidence at 7th MAI, DS=disease severity at 12th MAI, *r*=rate of infection, and AUDPC=area under disease development curve.

Table 3 Correlation coefficients of six tested selection criteria to FRW and each other.

Criterion	DS	IS	AUDPC	r	FBW	FRW
Disease incidence at 7 MAI (DI)	.519**	-.226**	.648**	.424**	-.151*	-0.021
Disease severity at 12 MAI (DS)		-0.124	.927**	.954**	-.353**	-.233**
Initial symptom (IS)			-0.195**	0.049	0.223**	0.098
AUDPC				.890**	-.442**	-.260**
Infection rate (r)					-.341**	-.204**
Fresh biomass weight (FBW)						.737**

DI=disease incidence at 7th MAI, DS=disease severity at 12th MAI, DS=disease severity at 12th MAI, IS=initial symptom, AUDPC=area under disease development curve, *r*=rate of infection, FBW=fresh biomass weight, FRW=fresh root weight.

Table 4 Number of selected crosses partially resistant to *G. boninense* using GDI of seven selection criteria and percentage similarity results to FRW criterion.

Selected Cross and similarity	Selection Criterion						
	FRW	FBW	IS	DI	DS	r	AUDPC
Number of selected crosses	99	96	116	109	101	109	104
Number and percentage of similarity result to FRW criterion	99 (100%)	72 (73%)	55 (56%)	45 (45%)	62 (63%)	64 (65%)	64 (65%)

FRW=fresh root weight, FBW=fresh biomass weight, IS=initial symptom, DI=disease incidence, *r*=rate of infection, AUDPC=area under disease development curve.

CONCLUSIONS

Selection of oil palm for *G. boninense* resistance in the main-nursery screening was able to identify genotypes with three main categories, namely genotypes with longer initial infection or long latent period, small infection rate, and both long latent period and small infection. The latest is the most ideal category of partial resistant genotypes to be developed as an effective variety to control BSR disease of oil palm. Three criteria, namely AUDPC, r and DS could be used to select genotypes partially resistant to *G. boninense* in the main-nursery screening. AUDPC is the most suitable selection criterion because its value simultaneously considers both latent period and rate of disease development.

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