EVALUATION OF METHODS FOR SCREENING TARO (*COLOCASIA ESCULENTA*) GENOTYPES FOR RESISTANCE TO LEAF BLIGHT CAUSED BY *PHYTOPHTHORA COLOCASIAE*

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ABSTRACT

Three screening methods; spray-on, stick-on and leaf-disc were compared to reliably identify moderate or partial resistance against taro leaf blight caused by Phytophthora colocasiae. *Each of the method was tested on susceptible, moderately resistant and resistant cultivars at three, four and five months of plant age. No significant differences ($P \geq 0.05$) were observed among inoculation methods and plant ages for the area under disease progress curve (AUDPC) of the susceptible and resistant cultivars. Using the leaf-disc method, twenty-six elite taro genotypes from third recurrent breeding cycle were screened and six moderately resistant genotypes, which are likely to be associated with polygenic and durable resistance were advanced to the fourth breeding cycle for further evaluation.*

Key words: Taro, leaf blight, breeding cycle, polygenic, inoculation

INTRODUCTION

Taro leaf blight (TLB) caused by *Phytophthora colocasiae* Racib. is considered to have originated in Southeast Asia where it coevolved with its principal host taro (*Colocasia esculenta* L.). It is now widely distributed throughout the tropical regions of the world. The disease entered the southern Pacific countries including Papua New Guinea (PNG) and Solomon Islands during the Second World War. Since then, the disease continued to spread in PNG. In 1976 a severe epidemic occurred on the islands of Manus, and in 1988 the disease occurred for the first time in Milne Bay and devastated the entire taro crop. TLB remains a major constraint to taro production in PNG (Gunua *et al.* 1998) and significant reduction in corm yields as high as 50% has been reported (Cox and Kasimani 1988; Jackson *et al.* 1980).

Early efforts to combat TLB in PNG included the use of cultural practices and chemicals. However, these efforts were not sustained because most farmers could not afford the costs of chemicals, equipment and labour. In this realization, the National Agricultural Research Institute (NARI) of PNG initiated a breeding programme in 1993 to develop cultivars resistant to TLB. The original programme was based on the use of resistant sources that had characteristics of major gene resistance and were therefore, likely to be vulnerable to breakdown from single mutations in the pathogen. Recently, the programme shifted its focus on recurrent population breeding and is discarding the genotypes with hypersensitive reactions likely to be associated with single major gene(s), as it is very difficult to select polygenic resistance in the presence of major gene(s). From progeny to progeny, it is possible to accumulate minor genes, which are likely to be polygenic and durable. Since, there is no identifiable indicator of such, as the hypersensitive flecks in major gene resistance, there is an important need for identifying reliable screening method that will help selecting for polygenic resistance.

The components of such resistance, like lesion diameter, latent period and area under disease progress curve can be useful criteria for its identification, but demands a reliable and quantified screening technique. TLB is normally irregularly distributed within a field and within the same genotype. One part of the field may have severe disease and another part may have much lower disease. During dry daytime conditions, but with heavy night dews, it is possible for one plant to be severely diseased (due to new infections arising from old lesions on the same plant) but for adjacent plants to have little disease. This characteristic cause serious problems when selecting resistant progeny under natural conditions, as it is not possible to distinguish between resistant plants and those that have simply escaped infection. This again demands the identification of a reliable screening method for making the breeding programme more efficient and successful.

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In the past, different TLB screening methods have been used to assess the host response. Ivancic *et al.* (1996) carried out resistance tests on taro using screen-house tests, shade-house tests and tests on detached leaves without recommending any effective and reliable method for use. Recently, Fullerton *et al.* (2000) screened taro varieties using spraying, field inoculation of plants with paper tabs and *in vitro* inoculation of leaf discs to determine the variability in lesion development in each of the methods. In a separate study, Fullerton *et al.* (2001) identified some of the factors causing variability in lesion development using the leaf-disc method. Thus, the screening techniques previously developed have not been fully exploited and have never been critically compared for reliability.

The objectives of the present studies were to i) determine the reliability, efficiency and practicality of three different screening methods for resistance to TLB, and ii) use the selected method to screen 26 advanced genotypes from the third breeding cycle to determine their responses to TLB.

MATERIALS & METHODS

Controls and breeding lines

The evaluation of screening methods was carried out on three controls - Numkowec, Ph-21 and Bangkok, which are respectively, susceptible, moderately resistant and resistant to TLB isolate named Bubia-isolate. The preferred method was used to screen 26 advanced lines selected from Cycle 3 breeding progeny.

Field layout and maintenance

Each of the 29 genotypes (three controls and 26 breeding lines) were replicated three times as single plants in a randomized complete block design. The plants were spaced at 0.6 m between and within rows for inoculations and intercultural convenience. The total plot size was 2.4 x 17.4 m. Four rows of taro cultivar NT 03 were planted outside the plot as guard rows. The experiment was rain-fed without any fertilizer application and weeds were controlled manually. Taro hawk moth (*Hippotion celerio*) larvae which attack the leaves, were physically removed during weeding or whenever sighted.

Preparation of inoculum

Preparation of inoculation solution followed the method described by Fullerton et al. (2000). Triton stock solution was prepared by mixing 0.4 ml Triton X 100 with 400 ml boiled water. The stock solution was well shaken and kept in a fridge when not in use. To prepare inoculation solution, one part Triton stock solution was mixed with nine parts (v/v) of clean boiled water. This dilution was used for washing sporangia off the lesions for the preparation of sporangial suspension.

Preparation of sporangial suspension

Leaves with young lesions showing heavy sporulation were collected early in the morning. Immediately after collection, the leaves were sprinkled with water and placed in a plastic bag. The plastic bag with its top folded over without being sealed was then stored in a cool place at approximately 25°C until leaves were used later in the day. To prepare a sporangial suspension, either sporulating lesions or edges of large lesions were cut-off the leaves and sporangia were washed-off the lesions with a soft brush using the inoculation solution. A final concentration of 300 sporangia ml^{-1} was used in the suspension as suggested by Fullerton *et al.* (2000).

Inoculations

Plants were inoculated by three different methods (spray-on, stick-on and leaf-disc) on three successive occasions at plant age of three, four and five months. A total of nine plants per genotype were inoculated at each plant age (three plants per genotype for each of the three methods). After inoculating at three months, the plants were left for a period of approximately three weeks after which a similar procedure was repeated on new first leaf at four and five months.

Spray-on method

The spray-on method was carried out as commonly practiced for initial screening of large numbers of taro progeny in breeding programmes. The spore suspension was placed in a 250 ml hand-pumped sprayer and sprayed onto the second fully expanded leaf surface until wet as described by Wall and Wiecko (1998). The Triton wetting agent was added to the inoculum suspension to ensure that the droplets remained on the leaf. Inoculations of plants were done late in the afternoon after 1600 hours.

Stick-on method

The stick-on method was carried out as described by Fullerton *et al.* (2000). The name is derived from the fact that the inoculum is held on the leaves by sellotape. The sporangial suspension was poured into a beaker, and blotting paper tabs (previously cut with a standard office paper-punch) were added. Ten paper tabs successively drawn from the sporangial suspension were placed directly onto the upper surface of the taro leaf lamina of the second leaf

(counting from the top). Tabs were placed in two rows of five, one row on each side of the leaf at approximately 2.5 cm apart. Two strips of 50 mm sellotape were cut and a strip was carefully placed on each row of tabs while the sides were pressed firmly to ensure that the paper tabs were in contact with the leaf in addition to edges being properly sealed. Inoculation of plants was done in the afternoon, after 1600 hrs (Fullerton *et al.* 2000). The Sellotape strips and paper tabs were removed early next morning (Day 1). The position of each paper tab was marked by circling with a marker. The diameter of each lesion on the leaf was recorded on two, three and four days after inoculation.

Leaf-disc method

The leaf-disc assay in this experiment was carried out in the laboratory following the procedure initially described by Fullerton *et al.* (2000). Four leaf discs were collected from the fully expanded second leaf of three different plants of each genotype early in the morning while leaves were still turgid. In preparation of inoculation, a 10-cm square paper towel wetted with 10 ml of benzimidazole solution to retard senescence and Triton wetting agent was placed in a standard 127-mm^2 laboratory weighing tray. Four leaf discs were carefully laid on the paper towel in each tray and flattened with a cotton bud. Care was taken while placing leaf discs to avoid getting any of the preservation solution on top of the discs.

To inoculate leaf discs, the sporangial suspension was poured into a Petri dish and blotting paper tabs were added. Triton wetting agent was added to the inoculum suspension to ensure that tabs adhere to leaf discs. Using forceps, one tab was drawn and placed in the centre of each of the four leaf discs and each tray was wrapped in cling wrap film (Gladwrap®) and incubated at room temperature of approximately 25°C. Tabs were removed from discs 24 h after inoculation (Day 1) and trays were rewrapped. Lesion diameter was recorded on days two, three and four after inoculation. The rewrapping procedure was again repeated on days two and three following disease measurement, to preserve discs for the final reading on day four after which the discs were simply discarded.

Disease assessment and analysis

The rate of lesion development (mm) was recorded on days two, three and four after inoculation for each combination of inoculation method and plant age. When measuring and recording the lesion diameter, failed infections were recorded as 0 (missing value) and hypersensitive reactions (flecks) as 1 mm (Fullerton *et al.* 2000). Missing values or 0s, which were escapes, were eliminated from the final calculation of average lesion diameter per plant. Comparisons of inoculation method and effect of plant ages were carried out concurrently in the same field plot.

Data analysis was performed by estimating the Area Under the Disease Progress Curve (AUDPC), which represented the grid of epidemics in relation to time. The AUDPC was calculated by incorporating average lesion diameter per plant for days two, three and four (reading dates) into the following formula:

AUDPC = 0.5x((LD1+LD2)x(t2-t1)+(LD2+LD3)x(t3-t2)), where LD1, LD2 and LD3 were mean lesion diameter for days two, three and four, respectively; and t1, t2 and t3 represent first, second and third observation days after inoculations.

Intervals for separation of genotypes into resistance categories

Four intervals were used in separating the genotypes, according to the levels of disease observed. Each of the intervals represented the following resistance categories, namely highly resistant (HR), moderately resistant (MR), moderately susceptible (MS) and highly susceptible (HS), respectively. To get the range of values within each of the intervals, the minimum and maximum values were identified and the difference between the maximum and minimum value was calculated. The difference was divided by four and the value obtained would be the interval for the ranges. The calculated value is added to the maximum value of the previous range to get the maximum value for the next range.

Comparison of leaf-disc results with natural epidemics

A field assessment was conducted in a separate breeding trial at NARI, Bubia under natural infection. Assessment were based on the percent leaf area affected using the diagram developed by Gollifer and Brown (1974). Independent host responses were deduced from this trial such that leaf damage up to 10% was considered HR; 10-40% MR; 40-70% MS; and above 70% HS. The results were compared with the leaf-disc method to estimate a match frequency for two independent rankings of phenotypes.

RESULTS

Evaluation of screening methods

For all three methods, lesions ranging from 10-19 mm and 5-8 mm were observed on the susceptible cultivar

Table 1. Mean AUDPC for three check cultivars at plant ages of 3, 4 and 5 months (m)

Genotype	TLB Resistance	Mean AUDPC (Methods/ Age)								
		Stick-on			Spray-on			Leaf-disc		
		3m	4m	5m	3m	4m	5m	3m	4m	5m
Numkowec	Susceptible	27.53	18.46	10.24	18.94	13.83	11.83	22.54	26.29	16.57
Ph-21	Moderately resistant	7.99	2.96	2.27	4.10	2.43	2.10	13.93	10.29	8.58
Bangkok	Resistant	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	1.00

Numkowec and moderately resistant check Ph-21, respectively, however, hypersensitive-flecks were observed on resistant check Bangkok. Mean AUDPC of cultivar Numkowec and Ph-21 produced by spray-on method was lower relative to stick-on and leaf disc methods (Table 1). Separate analysis of variance of mean AUDPC on the three checks showed no significant difference ($P > 0.05$) between the methods of inoculation and plant ages for the susceptible and resistant checks. However, there was significant difference for methods ($P < 0.01$) and plant age ($P < 0.05$) for the moderately resistant check.

Selection of a screening method

Although, the susceptible and resistant cultivars showed no significant difference among the three screening methods, indicating that all methods are equally suitable for screening, however, there are variety of other factors, which can affect the choice and application of a particular method (Table 2). Therefore, the choice of a method for further use in screening the genotypes of cycle-3 population was largely based on the relative merits and demerits of each method (Table 2). As we intended to screen a

Table 2. Comparative advantages and disadvantages of the three different screening methods

Screening method	Advantages	Disadvantages	Remarks
Spray-on	- Easy to carry out - Less laborious - Less time consuming	- Least precise - Least technical - Symptom influenced by the environment - Inoculum amount not consistent	- Suitable for screening large populations where precision is not required and other techniques are impossible to use
Stick-on	- Inoculum amount is consistent and can be standardized	- Moderately precise - Laborious - Requires skilled persons - Time consuming - Symptom influenced by the environment - Variability in results within replicates	- Suitable only for screening small-medium size population where enough skilled persons are available
Leaf-disc	- Most precise. - Symptom not influenced by the environment - Control of incubation conditions	- Laborious - Time consuming - Highly technical	- Most precise and reliable for screening small number of genotypes

small population of 26 highly advanced and valuable breeding lines, the leaf-disc method was considered comparatively precise and reliable with little or no environmental variability and hence was used for further screening of these genotypes.

Evaluation of selected genotypes and interference of host response

A range of host response was observed in terms of lesion development when the 26 selected genotypes were inoculated with Bubia-isolate using leaf disc method. Progressive lesion diameter for days 2, 3 and 4 and AUDPC for each genotype is presented in (Table 3). All genotypes showed significantly (P<0.01) lower and higher AUDPC, when compared to susceptible (Numkowec) and resistant (Bangkok) checks, respectively. However, in comparison to the moderately resistant check (Ph-21), the genotypes C3-10, C3-17, C3-28, C3- 32 and C3-45 showed significantly (P<0.01) lower mean AUDPC and the remaining 21 showed significantly (P<0.01) higher AUDPC.

Host response was deduced for each of the genotypes (Table 4) based on the interval range of separation as outlined in Methods. Genotype C3-45 and the resistant check were grouped as HR; six genotypes and moderately resistant check as MR; 19 genotypes as MS; and only the susceptible check as HS.

Table 3. Mean lesion diameter and AUDPC calculated for 26 genotypes and three checks for days 2, 3 and 4 using leaf-disc method

Genotype	Lesion Diameter			AUDPC
	Day 2	Day 3	Day 4	
C3-3	1.47	6.57	14.53	14.57
C3-6	1.42	6.25	14.08	14.00
C3-10	1.00	4.00	8.92	8.96
C3-12	1.08	6.08	12.00	12.62
C3-14	1.25	5.25	12.50	12.13
C3-16	1.61	7.94	16.94	17.22
C3-17	1.08	3.52	8.67	8.40
C3-18	1.58	6.25	13.75	13.92
C3-19	1.64	7.21	16.14	16.10
C3-22	1.19	5.07	12.03	11.68
C3-26	1.42	6.77	15.42	15.19
C3-27	1.50	7.43	16.42	16.39
C3-28	1.00	2.64	7.50	6.89
C3-29	1.64	7.11	16.31	16.09
C3-30	1.33	6.25	13.25	13.54
C3-32	1.00	2.83	7.86	7.26
C3-33	1.50	5.17	11.77	11.81
C3-34	1.50	6.68	13.33	14.10
C3-35	1.33	5.67	12.75	12.71
C3-36	1.60	6.93	15.25	15.36
C3-40	1.25	6.08	12.33	12.87
C3-41	1.71	7.97	16.95	17.30
C3-42	1.33	6.25	12.50	13.17
C3-43	1.75	7.58	16.00	16.46
C3-45	1.00	1.92	4.17	4.51
C3-48	1.58	6.00	13.42	13.50
Numkowec	2.11	13.23	24.00	26.29
Ph-21	1.00	4.00	11.58	10.29
Bangkok	1.00	1.00	1.00	2.00
LSD (5%)				0.85
LSD (1%)				1.13

Table 4. Match coincidence of host response of 29 genotypes deduced from leaf disc method and field rating separately

Genotype	Host Response		Match coincidence
	Leaf-disc method[A]	Field rating[B]	
C3-3	MS	MS	✔
C3-6	MS	MS	✔
C3-10	MR	HR	✗
C3-12	MS	MS	✔
C3-14	MS	MR	✗
C3-16	MS	MS	✔
C3-17	MR	MR	✔
C3-18	MS	MR	✗
C3-19	MS	MS	✔
C3-22	MR	HR	✗
C3-26	MS	MS	✔
C3-27	MS	MR	✗
C3-28	MR	MS	✗
C3-29	MS	MS	✔
C3-30	MS	MR	✗
C3-32	MR	MR	✔
C3-33	MR	MR	✔
C3-34	MS	MR	✗
C3-35	MS	MS	✔
C3-36	MS	MS	✔
C3-40	MS	MS	✔
C3-41	MS	MS	✔
C3-42	MS	MR	✗
C3-43	MS	MR	✗
C3-45	HR	HR	✔
C3-48	MS	MS	✔
Numkowec	HS	HS	✔
Ph-21	MR	MR	✔
Bangkok	HR	HR	✔

[A]HR highly resistant (AUDPC 2 - 6); MR moderately resistant (> 6 -12); MS moderately susceptible (> 13 - 18); HS highly susceptible (>18).

[B] From a separate study under natural TLB epidemics at Bubia research station based on % leaf damage (< 10% HR; 10-40% MR; 40-70% MS; > 70% HS)

Comparison of Leaf-disc and field ratings

Independent host responses were also inferred for all genotypes from separate field ratings (Table 4). Field ratings were compared with the leaf-discs ratings and the comparison showed a slight difference in host response of certain genotypes. Nonetheless, there was similarity in host response in majority of the genotypes (Table 4). Percentage coincidence and difference observed on host response of genotypes under leaf-disc and field ratings showed 66% coincidence, while the remaining 34% showed no pattern.

DISCUSSION

The three methods tested in this study had been widely used in the past (Ivancic *et al.* 1996; Singh *et al.* 2000; Fullerton *et al.* 2000). However, these techniques have not been fully exploited and have never been critically compared for reliability. Our results indicated that there were no significant

differences among the three tested methods on the resistant and susceptible cultivars. This may imply that all three methods are equally suitable for screening breeding populations and advanced genotypes. However, practical merits and demerits of each of the methods would determine its suitability and should assist breeders in choosing a suitable method, taking into account the effect of the environment on disease development, precision, population size and the resources available. The present research has attempted to summarize the relative advantages and disadvantages of the three methods (Table 2), which will assist the researchers in decision making for selecting an appropriate inoculation method. It is apparent that leaf-disc method is the most precise, reliable and not influenced by environmental variability. The study also established that there are no additional advantages in performing inoculations at a specific growth stage post three months, although the results of four months apparently appeared better in terms of disease development. These findings are restricted to the 29 genotypes used in the present investigations and does not rule out that certain genotypes in taro may be associated with adult plant resistance as pointed by Singh *et al.* (2003).

The NARI breeding programme produces more than 10,000 seedlings in every recurrent cycle. Initial screening which is restricted to single plants is focused on negative selection i.e. discarding the highly susceptible and hypersensitive genotypes from the population. The second round of selection in the form of intermediate and advanced trials, identify only limited number of genotypes which are moderately resistant to TLB, and those which qualifify the selection criteria for yield and eating quality. In this context, it is recommended to the breeding programme that progenies selected from this study should be screened in the field only at four months because of limited resources. The genotypes from advanced selections need to be analyzed more critically. More importantly, they should not be influenced by environmental variability for a reliable estimate of components of polygenic resistance, like AUDPC. As such, leaf disc method should be used for elite genotypes before they are advanced to variety release trials.

The present study evaluated 26 advanced genotypes selected from third breeding cycle by the leaf disc method. The method differentiated host responses among 26 genotypes such that six genotypes were MR, 19 were MS, and one genotype was HR. The HR genotype (typifying hypersensitive reaction) was discarded from the parent population for creating Cycle 4 because of the possibility of association of this genotype with single major gene. Of particular interest are six moderately resistant genotypes (C3-10, C3-17, C3-22, C3-28, C3-32 and C3-33), which are likely to be associated with polygenic and durable resistance. These genotypes will be recommended for further varietal evaluations if they qualify other set selection criteria. If required, 19 moderately susceptible genotypes could also be included in the programme provided that they are further crossed with resistant parents to increase the number of minor resistant genes in the subsequent progeny.

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REFERENCES

COX, P. G. and KASIMANI, C. (1988) Control of leaf blight using metalaxyl. *Tropical Pest Management* **34**, 81-84.

FULLERTON, R. A. and TYSON, J. L. (2001) Biology and epidemiology of *Phytophthora colocasiae*. Pp. 8-14, *in* AusAID/SPC Taro Pathology and Breeding Workshop (5-7 November 2001), Alafua campus, Samoa. Secretariat of Pacific Community, Noumea, New Caledonia.

FULLERTON, R. A., TYSON, J. L., HUNTER, D. and FONOTI, P. (2000). Plant Pathology Progress Report. In: TaroGen Six- Monthly Report (2000). Secretariat of Pacific Community, Nournea, New Caledonia.

GOLLIFER, D. E. and BROWN, J. F. (1974) Phytophthora Leaf Blight of *Colocasia esculenta* in the British Solomon Islands. *PNG Agriculture Journal*, **25**, 6-11.

GUNUA, T., KOKOA, P. and DARIE, A. (1998) Effect of mixed planting of taro leaf blight resistant varieties on the disease and yield of a preferred susceptible taro variety. *PNG Journal of Agriculture, Forestry and Fisheries* **41**, 32 –36.

IVANCIC, A., KOKOA, P., GUNUA, T. and DARIE, A. (1996) Breeding approach on testing for resistance to taro leaf blight. Pp.93-96 *in* Proceedings of the Second Taro Symposium. GV Jackson, ME Wagih (eds). Cenderawasih University and Papua New Guinea University of Technology, Lae, Papua New Guinea.

JACKSON, G. V. H., GOLLIFER, D. E. and NEWHOOK, J. (1980) Studies on the taro leaf blight fungus *Phytophthora colocasiae* in Solomon Islands: Control by fungicides and spacing. *Annals of Applied Biology* **96**, 1-10.

SINGH, D. and OKPUL, T. (2000) Evaluation of 12 taro (*Colocasia esculenta* (L.) Schott) leaf blight-resistant clones for yield and eating quality in Papua New Guinea. *SABRAO Journal of Breeding and Genetics* **32** (1), 39-45.

SINGH, D., OKPUL, T., GUNUA, T. and HUNTER, D. (2003) Inheritance studies in taro cultivar Bangkok for resistance to taro leaf blight. *Journal of South Pacific Agriculture* **8**, 22-25.

WALL, G. C. and WIECKO, A. T. 1998. Screening of 29 taro cultivars (*Colocasia esxulenta*) propagated in vitro, for resistance to taro leaf blight (*Phytophthora colocasiae*). *Journal of South Pacific Agriculture*. **5**:9-12.