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Research Article
An Algorithm for Estimating Resistance Magnitude of Plants Against Disease Establishment and Pathogen Virulence Levels

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Abstract

Background and Objective: Host plant resistance approach is one of the highly sought techniques in mitigation of several plant diseases that cause huge losses. This is attributed to its ease of implementation and cost effectiveness especially in third world countries. However, the success of selecting stable resistant varieties is significantly affected by error prone qualitative approaches of screening, coupled with other techniques like computer imaging systems that are very expensive to acquire in resource poor countries. Therefore, this study sought to determine the quantitative levels of host plant resistance and pathogen virulence based on a series of combined models. **Materials and Methods:** A 7 step algorithm that integrates 3 plant parameters affected most by the disease, referred to as 'Omatec host plant resistance and pathogen virulence estimator algorithm was used. Post-experiment data of 3 napier grass varieties screened against napier stunt disease at International Centre for Insect Physiology and Ecology-Mbita, Kenya, was used to test the approach. **Results:** From the algorithmic outputs, Clone-13 variety (the susceptible check) had host plant resistance levels of 34.06% and was classified as having low resistance or susceptible. Ouma-2 and South Africa varieties used as the resistant checks had host plant resistance levels of 60.91 and 63.66%, respectively with a classification of high resistance or resistant. The pathogen virulence levels on Clone-13 variety was estimated at 65.94%, whereas, in Ouma-2 and South Africa varieties the impacted pathogen virulence levels were 39.09 and 36.34%, respectively. **Conclusion:** The algorithm outputs of resistance and pathogen virulence levels were consistent with the reported qualitative description of the varieties' responses amidst infection by napier stunt disease. The algorithm exhibited potential in complementing existing qualitative approaches of screening for resistance in a relatively cheap, reliable and accurate manner.

Key words: Host plant resistance, virulence, estimation, quantitative, napier stunt disease

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural resistance of plants against diseases is one of the most effective, cheap, easily and highly adapted technique for management of serious diseases of plants currently[1-4]. The technique relies on inherent resistance trait in some host plants to merit its use as a tool in the control of plant pathogens that have continued to cause huge economic losses due to low crop yields[1,2,5]. The trait varies in magnitude in different varieties in a continuum manner ranging from highly susceptible lower end to highly resistant at the higher end, due to genome differences brought about by agents of evolution[6]. It is classified into two forms namely; quantitative and qualitative resistance[1,7]. The qualitative resistance is encoded by a few or single gene and it is expressed by plants against a certain specific strain of a pathogen. The quantitative resistance is expressed by several multiple genes that are generally involved in the general growth of the plant. Moreover, it is expressed by plants against all strains of a particular pathogen though influenced by certain environmental factors[6].

Efforts to select resistant varieties to mitigate this situation of crop losses due to plant diseases are ongoing across the world[8]. However, many of the techniques used in screening are prone to errors due to their qualitative nature[9]. Further, modern techniques like computer imaging are expensive and unavailable for many researchers especially in the third world countries to use in screening quantitatively for resistance[9,10]. In addition, some plant diseases express limited symptoms in initial stages of infection to merit a good qualitative estimation of resistance[1,11,12]. This is a challenge towards successful mitigation of these plant diseases and management of the co-evolution of causative pathogens due to survival pressure resulting from use of highly resistant varieties only[13,14]. Therefore, this study sought to develop an algorithm that can quantitatively estimate the levels of host plant resistance and pathogen virulence levels in a plant under evaluation in a cheap, accurate, consistent and objective manner.

MATERIALS AND METHODS

Study period: This study was conducted between April, 2018 to March, 2019 at International Centre of Insect Physiology and Ecology (ICIPE) -Mbita and Masinde Muliro University of Science and Technology-Kakamega, Kenya.

Study approach: The study involved a designed 7 step algorithm which is referred to as Omatec host plant resistance and pathogen virulence estimator algorithm, which was used to estimate the virulence and host plant resistance levels of selected varieties of napier grass. The post-experiment data used in this study was from 3 selected varieties known as; Clone-13, South Africa and Ouma-2. These varieties were being used as test crops against a disease known as napier stunt disease at International Centre for Insect Physiology and Ecology at Mbita in Kenya. Clone-13 variety's data was purposively used as a positive control since it's susceptible to the disease, whereas, the other two varieties data was used due to their reported levels of resistance to the disease[8]. The disease is caused by a phytoplasma '*Candidatus* Phytoplasma oryzae' strain Mbita 1 transmitted by *Maiestas banda*[4,8,11,15].

Step 1

Measurement or selection of the plants' data to be evaluated by the algorithm: This step involved the identification of the data that was to be subjected in subsequent steps of analysis towards estimation of the virulence and host plant resistance levels of selected varieties. Three parameters captured on Table 4, were identified that are significantly affected by the napier stunt disease namely, plant height, chlorophyll levels and biomass levels[4,8]. The data was from an experiment that was hosted at the International Centre for Insect Physiology and Ecology in Mbita Kenya (ICIPE-Mbita) and was used to simulate how the algorithm works in subsequent steps 2-6.

Step 2

Determination of the magnitude of relative logarithmic index of divergence of the inoculated treatments (MRIDIT) from their respective controls (non-inoculated): This entailed the determination of magnitude in percentage of the relative logarithmic index of divergence of the inoculated treatment (MRIDIT) from the non-inoculated treatments using a modified Eq. 1, that integrates the 3 most affected parameters by the disease as described by Parry[1]. The use of natural logarithm (LN) in the model determining the MRIDIT value was to enable estimation of the logarithmic efficacy indices which are good estimators of efficiency in performance in such scenarios of plant analysis[1,16,17]. Secondly, logarithms can effectively be used in integrating different parameters regardless of their different units of measurement to obtain a holistic index that represents performance[2,12,18]:

$$\text{MRIDIT}\ (\%) = \left(\frac{\left(\text{LN}(\text{PHi} \times \text{CHi} \times \text{BMi}) - \text{LN}\left(\text{PHc} \times \text{CHc} \times \text{BMc}\right)\right)}{\text{LN}\left(\text{PHc} \times \text{CHc} \times \text{BMc}\right)} \right) \times 100 \quad (1)$$

where, MRIDIT was the magnitude of relative logarithmic index of divergence of inoculated treatments from their respective controls. Estimating the damage inflicted on the variety by the pathogen. The LN was the natural logarithm of the variables PHi, CHi and BMi that represented the mean plant height, chlorophyll mean levels and biomass mean levels of the individual variety's inoculated treatments. Whereas, the variables PHc, CHc and BMc represented the mean plant height, chlorophyll mean levels and biomass mean levels of the individual variety's non-inoculated treatments (control).

Step 3

Estimation of respective varieties integrated parameter damage levels (IPDL) impacted through pathogen infection: This step entailed estimation of damage levels in percentage based on the integrated parameters by taking in to consideration the MRIDIT value calculated in step two of the algorithm. Since, it measures the levels of divergence of the infected materials from their controls. The expected output was a negative value to demonstrate decline in performance due to infection[1,12]. Therefore, to remove the negative value from the value towards determination of integrated parameter damage levels; it meant multiplying by negative one as demonstrated in Eq. 2:

$$\text{Integrated parameter damage levels (IPDL)} = (-1 \times \text{MRIDIT}) \quad (2)$$

where, MRIDIT was the magnitude of relative logarithmic index of divergence of inoculated treatments as determined in step two above.

Step 4

Estimation of the integrated parameter growth levels (IPGL) based on individual variety's integrated parameters: The determination of integrated parameters growth levels (IPGL) of a variety was done to estimate the performance of an individual variety based on the integrated 3 parameters divergence from their respective controls (Plant height, chlorophyll levels and biomass levels). It meant subtracting the estimated damage levels percentage from 100% as illustrated in Eq. 3:

$$\text{IPGL} = 100 - \text{Estimated damage levels percentage} \quad (3)$$

where, IPGL is the integrated parameters growth levels. The estimated damage levels percentage value in the formula was determined from step 3.

Step 5

Estimation of each variety's relative performance levels of different parameters measured (RPL) after infection: This entailed the determination of the respective parameters relative performance potential of the individual variety's under infection, relative to the sum of their performance under infection by the disease and no-infection scenario. A concept that has been utilized effectively in evaluation of systems wellness especially ecosystems[19]. This was based on the premise that relative performance is a good measure of systems efforts in regeneration amidst varying environmental variables[20]. Therefore, by exploiting the same approach the infected varieties performance could be established quantitatively as illustrated in the Eq. 4:

$$\text{RPL} = \frac{\left(\left(\frac{\text{PHi}}{\text{PHi} + \text{PHc}}\right)\times 100\right) + \left(\left(\frac{\text{CHi}}{\text{CHi} + \text{CHc}}\right)\times 100\right) + \left(\left(\frac{\text{BMi}}{\text{BMi} + \text{BMc}}\right)\times 100\right)}{3} \quad (4)$$

where, RPL was the relative performance levels as a percentage of infected variety under study. The division by 3 was to obtain the mean percentage of the three different parameters measured. Whereas, the variables PHi, CHi and BMi represented the plant height levels, chlorophyll levels and biomass levels of an individual variety's inoculated/infected treatments. Whereas, the variables PHc, CHc and BMc represented the plant heights levels, chlorophyll levels and biomass levels of the individual variety's non-inoculated/non-infected treatments (control).

Step 6

Estimation of each variety's host plant resistance levels (HPRL) against the pathogen and virulence levels of the pathogen (PVL) impacted on the variety: This was the final step that involved the determination of the host plant resistance of a variety by simple averaging of 2 out puts as illustrated below in Eq. 5 and consequently virulence estimation by subtracting the host plant resistance levels from 100% using Eq. 6:

$$\text{Host plant resistance levels}\left(\text{HPRL}\right) = \frac{\left(\text{IPGL} + \text{RPL}\right)}{2} \quad (5)$$

$$\text{Pathogen Virulence levels (PVL)} = 100 - \text{Host plant resistance levels} \quad (6)$$

where, HPRL was the host plant resistance levels as a percentage. The division by 2 was to obtain the mean percentage of the 2 percentage values determined in steps 4 and 5 above of the algorithm.

Step 7

Interpretation keys for the estimated variety's host plant resistance levels (HPRL) against the pathogen: This step involved the generation of an interpretation key to aid in the interpretation of the final outputs from the algorithm. The key was modified for napier as described by Obura[21] and Kawube *et al*.[22].

Statistical analysis: The data generated was interpreted directly since the outputs were statistically descriptive percentages, where, they quantified the levels of host plant resistance of the evaluated varieties and pathogen virulence magnitude manifested by the germplasms against the pathogen's establishment.

RESULTS

The analysis of the results based on the algorithm, Clone-13 had the lowest levels of host plant resistance against the pathogen at 34.06%, followed by the Ouma-2 variety at 60.91%. South Africa variety had the highest levels of host plant resistance at 63.66% as demonstrated in Table 1. Basing on the interpretation key illustrated in Table 2 Clone-13 variety host plant resistance levels classification was described as having low resistance, since the value 34.06% lay between the range 25% to 49% as illustrated in Table 2. Whereas, Ouma-2 and South Africa varieties classification was high resistance based on their values falling within the range of 50-74% (Table 2).

Based on the data used the pathogen '*Candidatus* Phytoplasma oryzae' strain Mbita-1 damaged more Clone-13 variety with pathogen virulence levels of 65.94%. Whereas, Ouma-2 and South Africa varieties determined pathogen virulence levels were 39.09 and 36.34%, respectively as demonstrated in Table 1. The other values determined by the algorithm towards determination of host plant resistance and pathogen virulence levels are captured in Table 3 to enable validation by an independent individual or researcher.

DISCUSSION

The results indicated that Clone-13 had low host plant resistance levels to the pathogen '*Candidatus* Phytoplasma oryzae' strain Mbita-1(Table 1). This is consistent with the general qualitative description of the variety's response to the disease as generally susceptible though the level of host plant resistance has not been reported for this variety until these findings in this current study[4,22,23]. In the evaluations of Wamalwa *et al*.[4] Clone-13 variety had a validated disease incidence of 16.7%. The variety typical of many napier grass varieties is a vigorous grower when not diseased and generally exhibits moderately varying growth and biomass levels outputs in different agro-ecological zones due to genotype differences and agronomy practices

Table 1: Determined levels of host plant resistance levels (HPRL) of individual varieties and pathogen virulence levels (PVL) based on the algorithm

Varieties	Host plant resistance levels (%)	Pathogen virulence levels (%)
Clone-13	34.06	65.94
Ouma-2	60.91	39.09
South Africa	63.66	36.34

Table 2: Interpretation key for the determined host plant resistance levels by the algorithm

Range values (%)	Host plant resistance classification
From 0-24	Very low resistance (or) very susceptible
From 25-49	Low resistance (or) susceptible
From 50-74	High resistance (or) resistant
From 75-100	Very high resistance (or) very resistant

Table 3: Determined levels of other variables used in estimation of host plant resistance levels and pathogen virulence levels from the algorithm based on the data in step 1

	Values			
Varieties	MRIDIT	IPDL (%)	IPGL (%)	RPL (%)
Clone-13	-43.09	43.09	56.91	11.21
Ouma-2	-12.70	12.70	87.30	34.51
South Africa	-10.38	10.38	89.62	37.69

MRIDIT: Magnitude of relative logarithmic index of divergence of the inoculated treatments, IPDL: Integrated parameter damage levels, RPL: Relative performance levels, IPGL: Integrated parameter growth levels

Table 4: Data of 3 varieties mean performance in 2 trials of 24 weeks each of growth under *Candidatus* phytoplasma oryzae' strain Mbita 1 infection and their controls performance

	Napier stunt Inoculated treatments performance in 3 parameters			Non-inoculated (controls) treatments performance in 3 parameters		
Varieties	Plant height (cm)	Chlorophyll levels (SPAD)	Biomass levels (g)	Plant height (cm)	Chlorophyll levels (SPAD)	Biomass levels (g)
Clone-13	18±9.5	8±1.3	28±5.20	158±12.6	46±2.2	298±15.2
Ouma-2	98±7.8	28±2.5	204±16.4	188±14.2	58±3.6	352±18.5
South Africa	86±4.5	30±3.3	177±5.4	146±10.4	53±1.8	267±8.7

Data was means±standard deviation of the varieties' respective treatments each replicated ten times in each of the 2 trials of evaluation at International Centre for Insect Physiology and Ecology-Mbita, Kenya, data was used to test the algorithm levels of estimating host plant resistance and pathogen virulence levels

effect[24,25]. This scenario could explain why the variety was significantly damaged though not entirely by the pathogen at 65.94% virulence levels (Table 1). Thus, validating the likely use of the algorithm in estimating quantitatively host plant resistance levels.

The other 2 varieties, Ouma-2 and South Africa were equally consistent with their descriptive qualities in various evaluation reports, as generally being tolerant to the napier stunt disease[3,8]. However, no report of quantitative estimation on the host plant resistance level has been reported until these findings in the current study. This scenario can be attributed to their inherent gene properties that enable plants mount a defense against an infection[1,6,7]. Therefore, the quantitative estimation of this trait may open new horizons in the evaluation of plants for resistance against diseases and prediction of the types of resistance[6]. Moreover, when screening for possible presence of pathogen pathovars quantification of virulence and resistance will be a critical parameters as reported by Keane[6]. This is because different pathovars have varying impact on qualitative levels of resistance unlike quantitative resistance which is specific[1]. This is likely to complement the qualitative strategies existing especially in selection of resistance trait and generally plant improvement. The host plant resistance of an infected crop (napier grass) has been quantitatively estimated using a designed algorithm and differences in levels exist. Secondly, the estimation of resistance by the algorithm could be used to select stable and desirable plant cultivars that can be used to manage various plant diseases effectively to limit the process of co-evolution using host plant resistance approach.

CONCLUSION

Basing on the available reports of the varieties general response to the disease, the algorithm managed to give a quantitative value on their host plant resistance that seems consistent with the available qualitative information. Hence, the algorithm exhibits some potential of being applied in complementing screening efforts for resistance that use qualitative approaches, especially in third world countries where computer imaging technologies are limited. However, there is need for the algorithm to be adopted with other crops and different plant diseases to test its versatility in application. This will contribute in its improvement towards a very accurate, reliable, cheap and cost effective tool of analysis of host plant resistance and plant diseases' virulence levels.

SIGNIFICANCE STATEMENT

The article introduces a novel concept of estimating quantitatively the host plant resistance levels of plants towards pathogens management by use of an algorithm. The algorithm uses post experiment collected data of napier grass infected by an obligate pathogen' *Candidatus* Phytoplasma oryzae' strain Mbita 1. This approach will complement the selection approaches of resistant plants during screening for resistance. Further, the findings will help in giving direction on estimation of pathogen virulence levels which is an important variable in plant pathology.

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