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APPLICATIONS OF AREA UNDER DISEASE PROGRESS CURVES IN ASSESSING RESISTANCE TO COWPEA APHID-BORNE MOSAIC VIRUS INFECTION IN GROUNDNUT

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ABSTRACT

Three methods of processing Area Under Disease Progress Curve (AUDPC) estimates were compared for consistency and reliability. Twenty groundnut cultivars were mechanically inoculated with *Cowpea aphid-borne mosaic virus* (CABMV) in a field trial during the 2015 cropping season in Minna, Southern Guinea Savanna agro-ecology of Nigeria. The trial was laid out in Randomised Complete Block Design (RCBD) with three replications. Seedlings were inoculated at 10 days after sowing. Disease severity was rated on 1 – 5 point scale based on percentage of leaf surface covered with symptoms. Symptom severity scores were subjected to AUDPC and further used for resistance class determination. There was no complete agreement among the three methods in allocating cultivars into resistance classes. Considering instances of general consensus, two (ICG – 01276 and ICG – 5195) cultivars were unanimously rated as resistant. Based on the principles employed in Methods 1 and 2, three (FDR7 – 67, ICGV – 91317 and ICGV – IS – 76855) cultivars were susceptible, six (ICG – 02189, ICG – 6654, ICG – IS – 13003, ICG – IS – 13986, SAMNUT 24 and SAMNUT 25) were moderately susceptible, and two (ICG – 01276 and ICG – 5195) were resistant. With Methods 2 and 3, one (FDR7 – 61) cultivar was unanimously placed in highly susceptible class, whereas two (ICG – 94169 and SAMNUT 14) were rated as highly resistant. Based on Methods 1 and 2, the probability of placing a highly susceptible genotype in either moderately susceptible or susceptible group is relatively low. If the purpose of the investigation is to identify only two classes of response (resistant and susceptible), all the methods are suitable in that there was 100 % agreement with respect to the cultivars found in each category.

Key words: AUDPC estimates; disease severity, groundnut cultivars; resistant classes; virus infection

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INTRODUCTION

Groundnut (*Arachishypogaea*L.) is a major legume crop at global level (Mensah and Obadoni, 2007). It is an important cash crop in subsistence and commercial farming systems, as well as an important food source (Izge*et al.*, 2007). The crop has been described as an excellent plant-based source of protein, and is high in various vitamins and minerals. In addition, groundnut is widely utilized for manufacturing pharmaceuticals, cosmetics, and lubricants (Ayele, 2010). Groundnut seeds and haulms are used as animal feed while the oilcakes are used as industrial raw material and fertilizer (Martey*et al.*, 2015). In traditional farming systems, groundnut is usually intercropped with cereal crops in order to enhance soil nitrogen status (Nyemba and Dakora, 2010). Studies have shown that about 31 viruses representing 14 genera threaten groundnut yield in different parts of the world. Among them, *Cowpea aphid-borne mosaicvirus* (CABMV) which causes significant yield reductions has been identified as one of the most economically important (Sreenivasulu*et al.*, 2008).

Plant viruses are of serious concern because of their deleterious impacts on crops' quantitative and qualitative values. *Cowpea aphid-borne mosaic virus* (CABMV) is one of the viruses inducing severe economic losses in groundnut (Alegbejo, 2015). Symptoms of infection in susceptible cultivars include leaf mottling, mosaic and stunting. From time immemorial, breeding for resistance has been considered the most effective strategy for managing plant viral diseases. Conventional breeding for virus resistance partly relies on visual assessment of plants' reactions after inoculation with severe strain (s) of the pathogen. Although serological (Enzyme-Linked Immunosorbent Assay) and nucleic acid-based techniques (Polymerase Chain Reaction) are more reliable and accurate, visual assessment provides a rapid method of evaluation and results are in most cases adjudged to be positively correlated with other techniques. For instance, Maruthi*et al.* (2003) demonstrated a positive correlation between visual assessment of tomato leaf curl virus disease severity scores and DNA hybridization signals. Earlier, Harrison (1956) posited that the virus content of a plant represents equilibrium between replication and degradation of the virus by the host system. Severity of virus infection in legumes is commonly rated on a 1 to 5-point scale (Arif and Hassan, 2002).

Application of Area Under the Disease Progress Curve (AUDPC) is becoming popular partly owing to the realization that a one-time assessment of disease infection does not give an objective information about the situation. For instance, readings made at the early stage of infection may underestimate the susceptibility of many cultivars, in the same way their resistance may be confounded if assessment is made at the later stage, owing to the natural senescence of lower leaves which may be difficult to distinguish from death induced by pathogens (Shaner and Finney, 1977). AUDPC is effective as it takes into consideration the effects of variation in both the time and severity of disease infection (Ariyo*et al.*, 2002). In addition, AUDPC estimates tend to reveal strong correlation between the cumulative level

of infection and the resultant disease loss (Scott and Griffiths, 1980). Effective and efficient plant disease management and breeding is greatly influenced by numerous factors including the successful identification of resistance sources and accuracy of resistance assessment (Pico *et al.*, 1998). In plant breeding, selection for disease resistance and desirable yield normally favours cultivars whose performance outweighs the population means. Therefore, further analysis of the AUDPC estimates is often undertaken. This paper compares three methods for determining resistant classes, based on AUDPC estimates from *Cowpea aphid-borne mosaic virus* infected groundnut cultivars. The ultimate goal was to ascertain the most appropriate method for determining resistance to the virus.

MATERIALS AND METHODS

Source of groundnut seeds

Twenty groundnut cultivars (FDR7 – 61; FDR7 – 67; ICG – 01276; ICG – 02189; ICG – 5195; ICG – 6654; ICG – 92267; ICG – 94169; ICG – IS – 13003; ICG – IS – 13986; ICGV – 91317; ICGV – IS – 76855; SAMNUT 10; SAMNUT 14; SAMNUT 21; SAMNUT 22; SAMNUT 23; SAMNUT 24; SAMNUT 25; SAMNUT 26) commonly grown in northern Nigeria were collected from the Institute for Agricultural Research (IAR), Samaru, Zaria.

Source of *Cowpea aphid-borne mosaic virus* isolate

The CABMV isolate used for infectivity was obtained from the virus culture at the Department of Crop Production, Federal University of Technology (FUT), Minna. Leaf tissue infected with CABMV was preserved on silica gel in a vial bottle and was recovered through mechanical transmission onto the plants (10-day old) of a susceptible cowpea cultivar (Ife Brown). Seeds were sown in 30 cm diameter plastic pots held under screenhouse conditions and seedlings inoculated with the virus at 10 days after sowing. Virus extract was prepared by grinding virus-infected leaf tissue with inoculation buffer pH 7.2 (0.1_M sodium phosphate dibasic, 0.1_M potassium phosphate monobasic, 0.01_M ethylene diamine tetra acetic acid and 0.001_M L-cystine per litre of distilled water) at the rate of 1 g of leaf in 1 mL, using cold mortar and pestle. Seedlings were inoculated by dusting the upper leaf surface with carborundum powder (600-mesh). A piece of cheesecloth was dipped in the extract and then used to rub the upper leaf surface. Leaves with virus symptoms were collected at 14 days after inoculation and preserved on silica gels for inoculating plants in the field trial.

Crop establishment and inoculation

Field trial was conducted at the Teaching and Research Farm, FUT, Minna (Latitude 6.44675 ^{0}E; Longitude 9.51715 ^{0}N; 220 m above sea level) during the 2015 cropping season. The trial was laid out in a Randomised Complete Block Design (RCBD) with three replications. Treatments consisted of twenty groundnut cultivars (FDR7 – 61; FDR7 – 67; ICG – 01276; ICG – 02189; ICG – 5195; ICG – 6654; ICG – 92267; ICG – 94169; ICG – IS – 13003; ICG – IS – 13986; ICGV – 91317; ICGV – IS – 76855; SAMNUT 10; SAMNUT 14; SAMNUT 21; SAMNUT 22; SAMNUT 23; SAMNUT 24; SAMNUT 25; SAMNUT 26). Each cultivar was sown in a 2-m long ridge at intra- and inter-row spacing of 15 and 75 cm, respectively. Seeds were sown on 11th August, 2015. Two seeds were sown per hole and seedlings thinned to one per stand after emergence. Seedlings were mechanically

inoculated with the virus at 10 days after sowing (Salaudeen and Aguguom, 2014).

Disease assessment and statistical analysis

Disease incidence was determined as percentage of the total number of plants exhibiting symptoms of CABMV infection after inoculation. Disease severity was measured as Symptom Severity Scores (SSS) at weekly intervals for five weeks, commencing from 2 Weeks Post-Inoculation (WPI). This was based on percentage of the topmost leaf surface covered with symptom, according to a 1 – 5 scale developed by Arif and Hassan (2002). In the scale, 1 = no symptoms (apparently healthy plant); 2 = slightly mosaic leaves (10 – 30 %); 3 = mosaic (31 – 50 %) and leaf distortion; 4 = severe mosaic (51 – 70 %), leaf distortion and stunting; 5 = severe mosaic (>70 %), stunting and death of plants. The data on disease severity were used to compute AUDPC estimates, as given by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i],$$

where:

Y_i = disease severity at the ith observation,

X_i = time (weeks) at the i*th* observation, and

n = total number of observations.

Statistical analysis was performed using Statistical Analysis System (SAS, 2008). Further processing of the AUDPC estimates was accomplished using the procedure of Ariyo*et al.* (2002) (Method 1), Goktepe*et al.* (2007) (Method 2), and Okechukwu*et al.* (2008) (Method 3). This was done in order to determine resistant classes of the tested groundnut cultivars.

In Method 1, the AUDPC estimates were used to determine the rank score, deviation of rank score from the grand mean of the entire scores and Standardized Rank Score (SRS). Rank scores (RS) were determined by arranging the AUDPC estimates in descending order and then assigning a score to each. Deviation of rank score was computed as the deviation of each rank score from the grand mean of the entire rank scores. Standardized rank score was the product of deviation and a constant value (0.2). Cultivars were assigned into resistance classes based on their SRS. Standardized rank scores falling to the right (positive) of the grand mean on the mean distribution curve were considered to be moderately susceptible, susceptible or highly susceptible while those falling to the left (negative) of the grand mean were placed in the moderately resistant, resistant or highly resistant category (Fig. 1). A cultivar was considered highly resistant to CABMV disease if its SRS was -3.0 to -2.1, resistant if it was between -2.0 and -1.1, moderately resistant if it fell between -1.0 and -0.1; moderately susceptible if it was between 0.1 and 1.0, susceptible if it fell between 1.1 and 2, and highly susceptible when it was ≥ 2.1.

In Method 2, the AUDPC estimates were used to calculate the Rank Scores (RS) as in Method 1. Normalized Rank Score (NRS) was computed as follows:

$$\text{Normalized rank score} = \frac{\text{Rank score}}{\text{The highest possible rank score}} \times 100$$

Resistance categories were based on the NRS. A cultivar was placed in the highly resistant class if its NRS was less than 11 %, resistant if between 11 and 25 %, moderately resistant if it was found in 26

– 50 %, susceptible if it fell between 51 and 75 %, and highly susceptible if greater than 75 %.

In Method 3, computation of the RS and deviation was accomplished as earlier described in Method 1 (Fig. 2). Standardized rank score was calculated as follows:

$$\text{Standardized rank score} = \frac{\text{Deviation}}{\text{Standard deviation of rank scores}} \times 2$$

A cultivar was considered to be highly resistant if its SRS was $\leq$ -3, resistant if fell between -3.0 and -2.1, moderately resistant if it was within -2 and 0; moderately susceptible if fell between 0.1 and 2, susceptible if it was within 2.1 and 3, and highly susceptible if it was greater than 3.

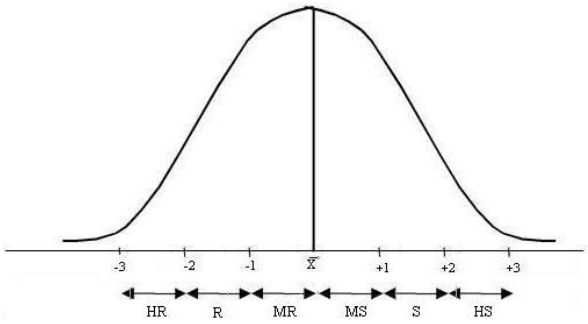

Fig. 1: Mean distribution curve for evaluating resistance status in groundnut cultivars inoculated with *Cowpea aphid-borne mosaic virus* in Minna, 2015. Adapted from Ariyo *et al.* (2002)

HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible;

S = susceptible; HS = highly susceptible

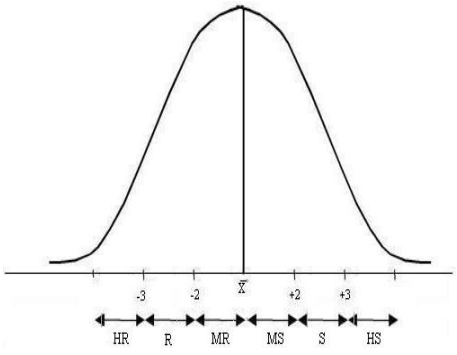

Fig. 2: Mean distribution curve for evaluating resistance status in groundnut cultivars inoculated with *Cowpea aphid-borne mosaic virus* in Minna, 2015. Adapted from Okechukwu *et al.* (2008)

HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible;
S = susceptible; HS = highly susceptible

RESULTS

All the inoculated plants elicited symptoms of CABMV disease. Symptom was first observed at 7 days post inoculation. This was mainly leaf mottling but by second week post inoculation (WPI), some leaves had already turned yellow. The intensity of symptoms varied among the cultivars from mild to moderate level throughout the period of evaluation. The resistant classes of the cultivars are presented in Table 1. AUDPC analysis indicated significant ($p<0.05$) differences for resistance to CABMV infection. Based on the method proposed by Ariyo*et al*. (2002), four (FDR7-61, FDR7-67, ICGV – 91317 and ICGV – IS – 76855) of the cultivars were susceptible, six (ICG – 02189, ICG – 6654, ICG – IS 13003, ICG – IS – 13986, SAMNUT 24 and SAMNUT 25) were moderately susceptible and 10 (ICG – 01276, ICG – 5195, ICG – 92267, ICG – 94169, SAMNUT 10, SAMNUT 14, SAMNUT 21, SAMNUT 22, SAMNUT 23 and SAMNUT 26) were resistant.

With the method of Goktepe*et al*. (2007), a total of seven (FDR7 – 61, FDR7 – 67, ICG – 6654, ICGV – 91317, ICGV – IS – 76855, SAMNUT 24 and SAMNUT 25) cultivars were highly susceptible, three (ICG – 02189, ICG – IS – 13003 and ICG – IS – 13986) were susceptible, six (ICG – 92267, SAMNUT 10, SAMNUT 21, SAMNUT 22, SAMNUT 23 and SAMNUT 26) were moderately resistant, two each were resistant (ICG – 01276 and ICG – 5195) and highly resistant (ICG – 94169 and SAMNUT 14). Based on the method of Okechukwu*et al*. (2008), one (FDR7 – 61) cultivar was highly susceptible, three (FDR7 – 67, ICGV – 91317 and ICGV – IS – 76855) were susceptible, six (ICG – 02189, ICG – 6654, ICG – IS – 13003, ICG – IS – 13986, SAMNUT 24 and SAMNUT 25) were moderately susceptible, six (ICG – 92267, SAMNUT 10, SAMNUT 21, SAMNUT 22, SAMNUT 23 and SAMNUT 26) were moderately resistant, two each were resistant (ICG – 01276 and ICG – 5195) and highly resistant (ICG – 94169 and SAMNUT 14).

There was no complete agreement among the three methods in allocating cultivars into resistance classes (Table 2). Considering instances of general consensus, two (ICG – 01276 and ICG – 5195) cultivars were unanimously rated as resistant. Based on the principles employed in Methods 1 and 2, three (FDR7 – 67, ICGV – 91317 and ICGV – IS – 76855) cultivars were susceptible and six (ICG – 02189, ICG – 6654, ICG – IS – 13003, ICG – IS – 13986, SAMNUT 24 and SAMNUT 25) were moderately susceptible. On the other hand, ICG – 01276 and ICG – 5195 were resistant. With Methods 2 and 3, one (FDR7 – 61) cultivar was unanimously placed in highly susceptible class, whereas two (ICG – 94169 and SAMNUT 14) were rated as highly resistant.

Table 1: Resistant classes inferred from Area Under the Disease Progress Curve (AUDPC) estimates after inoculating selected groundnut cultivars with *Cowpea aphid-borne mosaic virus* in Minna, 2015

			Method 1			Method 2		Method 3		
Groundnut Cultivar	AUDPC	RS	Dev	SRS	Class	NRS	Class	Dev	SRS¶	Class
FDR7 – 61	12.9	20	8.85	1.77	S	100	HS	8.85	3.11	HS
FDR7 – 67	12.8	19	7.85	1.57	S	95	HS	7.85	2.75	S
ICG – 01276	10.6	4	6.85	1.37	S	90	HS	6.85	2.40	S
ICG – 02189	11.1	13	6.85	1.37	S	90	HS	6.85	2.40	S
ICG – 5195	10.5	3	4.85	0.97	MS	80	HS	4.85	1.70	MS
ICG – 6654	11.2	15	3.85	0.77	MS	75	HS	3.85	1.35	MS
ICG – 92267	11.0	10	3.85	0.77	MS	75	HS	3.85	1.35	MS
ICG – 94169	10.4	2	1.85	0.37	MS	65	S	1.85	0.65	MS
ICG – IS – 13003	11.1	13	1.85	0.37	MS	65	S	1.85	0.65	MS
ICG – IS – 13986	11.1	13	1.85	0.37	MS	65	S	1.85	0.65	MS
ICGV – 91317	12.5	18	-1.2	-0.2	R	50	MR	-1.2	-0.40	MR
ICGV – IS – 76855	12.5	18	-1.2	-0.2	R	50	MR	-1.2	-0.40	MR
SAMNUT 10	10.9	8	-3.2	-0.6	R	40	MR	-3.2	-1.11	MR
SAMNUT 14	10.4	2	-3.2	-0.6	R	40	MR	-3.2	-1.11	MR
SAMNUT 21	10.9	8	-3.2	-0.6	R	40	MR	-3.2	-1.11	MR
SAMNUT 22	10.9	8	-3.2	-0.6	R	40	MR	-3.2	-1.11	MR
SAMNUT 23	10.9	8	-7.2	-1.4	R	20	R	-7.2	-2.51	R
SAMNUT 24	11.2	15	-8.2	-1.6	R	15	R	-8.2	-2.86	R
SAMNUT 25	11.3	16	-9.2	-1.8	R	10	HR	-9.2	-3.21	HR
SAMNUT 26	11.0	10	-9.2	-1.8	R	10	HR	-9.2	-3.21	HR

HR = Highly resistant; HS = Highly susceptible; MR = Moderately resistant; MS = Moderately susceptible; R = Resistant; S = Susceptible; RS = rank score (ranks ofAUDPC estimates after arranging in descending order); Dev = deviation of rank scores from the grand mean of the entire rank scores; SRS = standardized rank score (the product of deviation and a constant factor 0.2); NRS = normalized rank score (rank score divided by the highest possible rank score and multiplied by 100); SRS¶ = standardized rank score (deviation divided by standard deviation of the entire rank scores multiplied by 2)

Adapted from Ariyo*et al*. (2002) (Method 1), Goktepe*et al*. (2007) (Method 2), and Okechukwu*et al*. (2008) (Method 3)

Table 2: Comparison of resistant classes from groundnut cultivars inoculated with *Cowpea aphid-borne mosaic virus* in Minna, 2015

Resistance class	Method 1	Method 2	Method 3
Highly resistant		8, 14	8, 14
Resistant	3, 5, 7, 8, 13, 14, 15, 16, 17, 20	3, 5	3, 5
Moderately resistant		7, 13, 15, 16, 17, 20	7, 13, 15, 16, 17, 20
Moderately susceptible	4,6, 9, 10, 18, 19	-	4, 6, 9, 10, 18, 19
Susceptible	1, 2, 11, 12	4, 9, 10	2, 11, 12
Highly susceptible		1, 2, 6, 11, 12, 18, 19	1

Adapted from Ariyo *et al.* (2002) (Method 1), Goktepe *et al.* (2007) (Method 2), and Okechukwu *et al.* (2008) (Method 3)
Groundnut cultivar: 1= FDR7 – 61; 2 = FDR7 – 67; 3 = ICG – 01276; 4 = ICG – 02189; 5 = ICG – 5195; 6 = ICG – 6654; 7 = ICG – 92267; 8 = ICG – 94169; 9 = ICG – IS – 13003; 10 = ICG – IS – 13986; 11 = ICGV – 91317; 12 = ICGV – IS – 76855; 13 = SAMNUT 10; 14 = SAMNUT 14; 15 = SAMNUT 21; 16 = SAMNUT 22; 17 = SAMNUT 23; 18 = SAMNUT 24; 19 = SAMNUT 25; 20 = SAMNUT 26

DISCUSSION AND CONCLUSION

The symptoms observed on the infected plants were similar to those reported by Alegbejo (2015). The observation that all the inoculated plants elicited symptoms of CABMV disease indicates that there was no immunity against the virus in the evaluated cultivars. This finding corroborates the results published by Mundembe (2012). Leaf discolouration has a lot of implications on plants ability to photosynthesize. This is due to the fact that viruses usually hijack the chloroplast and physiology of the attacked plants. However, virus movement and accumulation in plants depends to a large extent on genetic background of the invaded plant and the outcome of their interactions determines the amount of the viral particles in the host (Salaudeen, 2016). The cultivars which produced mild symptoms at the later stage of evaluation could be described asbeing tolerant to infection. Studies have shown that tolerance as one of the well-known mechanisms for compensating the stresses imposed by parasites is elicited by reducing the deleterious impacts of parasite infection which could be manifested as alteration of host life-history characteristics (Agnew *et al.*, 2000).

There was a low level of agreement among the three methods for resistant class determination owing to the differences in the principles involved. For instance, whereas Methods 1 and 3 recognized six classes of resistance, only five were operative in Method 2. Although analysis based on Methods 1 and 3 resulted in six resistant classes, the procedures employed were not the same.

Because more than 50 % of the groundnut cultivars were ranked equally by Methods 1 and 2, it appears that there is a remarkable level of correlation between them. Similarly, Methods 2 and 3 are more closely related than Methods 1 and 3. In terms

of simplicity, Method 2 requires fewer steps compared to the other two. Method 1 appears to be less stringent in allocating cultivars into resistant classes relative to Methods 2 and 3, particularly with respect to the number of cultivars found in the resistant and highly resistant categories. Based on Methods 1 and 2, the probability of placing a highly susceptible cultivar in either moderately susceptible or susceptible group is relatively low. If the purpose of the investigation is to identify only two classes of response (resistant and susceptible), all the methods are suitable in that there was 100 % agreement with respect to the cultivars found in each category.

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