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Comparison of methods for evaluation of Cassava cultivars for resistance to African Cassava Mosaic disease

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Abstract

African cassava mosaic disease (ACMD) caused by several related viruses is a major constraint to cassava production in Africa and the utilisation of resistant cultivars is considered the most effective and sustainable way of controlling the disease.

In cassava breeding programmes, screening of cassava breeding lines is conducted by exposing clonal plants to natural infections and recording over a time period, disease incidence (DI) and intensity of disease symptoms (index of symptom severity, ISS). A scale of 1-5 is used to score for severity of infection and this approach is the standard procedure to evaluate resistance.

Several models have been utilised for assessment and categorisation of disease resistance in cassava, each with a slightly different emphasis. For evaluation of breeding lines, the use of an enlarged rank-sum method has been effectively used in measuring different aspects of yield stability. A method for disease assessment which is based on relating yield reductions to the "area under the disease progress curve", AUDP, is also proposed and providing a very tight correlation between the cumulative level of disease and the resulting yield loss.

Both methods: rank-sum and AUDPC, were compared for calculation of disease resistance levels in cassava and the confidence of both methods to reveal similar assessments was determined using the Spearman rank correlation.

Twenty-three improved Cassava lines from the International Institute of Tropical Agriculture (IITA) and two local land races were evaluated for resistance to ACMD by recording DI and ISS over a period of fourteen weeks under high disease pressure. By applying rank-sum and AUDPC for calculating disease resistance, it was found that both methods result in a comparable assessment of the resistance levels of cassava with a Spearman rank correlation of >99%, indicating an high confidence of the tests to reach similar results. However, compared to rank-sum, AUDPC models do not require a

cumbersome ranking of DI and ISS scores. Hence, this method was found most appropriate for assessing resistance to ACMD in cassava breeding lines.

Introduction

Cassava (*Manihot esculenta* Crantz), a major tropical root crop (Hahn *et al*., 1989), is an important source of energy for over 200 million people in Africa (Dahniya, 1994). It is used directly for human consumption in the producer countries where it is processed on a small scale.

There are several constraints to high yield and African cassava mosaic disease (ACMD) had been singled out as a major biological constraint that can reduce yield by about 50% (Guthrie, 1990). The use of resistant cultivars is one of the most reliable ways of controlling the disease (Thresh and Otim-Nape, 1994). These resistant cultivars were developed through interspecific hybridization and repeated back-crossing to restore consumer qualities (Hahn *et al*., 1989).

However, differential symptom expression by cassava varieties has been observed suggesting genetic variability for resistance to African cassava mosaic begomovirus (ACMV) (Ssemakula et al., 1997). Resistance was first assessed by exposing batches of clonal plants to infection and recording the number of months before each plant developed symptoms (Thresh *et al*., 1994). Recording procedures were also revised to take account of both the incidence of the disease and intensity of symptoms (Jennings, 1957).

Progenies are evaluated using a scale ranging from no symptom (score 1) to severe leaf mosaic and distortion (score 5) (Fauquet and Beachy, 1989; Guthrie, 1990; IITA, 1990). Several selections made in this way have been categorised as 'highly resistant', 'resistant' or 'moderately resistant' to ACMD.

Three categories of model for disease assessment were reported by Burdon (1987): Critical-point model provides an estimate of yield loss incurred for any given level of disease at a given time; Multiple-point model estimates losses from a disease progress curve that consists of many separate assessment points; and an intermediate model, relates yield reductions to the cumulative area under the disease progress curve (AUDPC).

The first model is potentially a poor predictor as it is based on the assumption that all disease curves reaching the same level of severity at the same time will cause the same crop loss, and this is frequently not the case. AUDPC models avoid this weakness and incorporate the effects of variations in both the duration and severity of disease epidemics. The second model is inappropriate in a situation where relatively little is likely to be known about the physiological response of the host to the disease. AUDPC models have no *a priori* knowledge requirements. Moreover, on many occasions, AUDPC models have shown very close correlation between the cumulative level of disease and the resultant disease loss (Van der Plank, 1963; James and Teng, 1979; Scott and Griffiths, 1980).

Nagarajan and Muralidharan (1995) also indicated the AUDPC as one of the means of quantifying host resistance. Wilcoxon *et al*. (1975) quantified this AUDPC as A-value. Also, the use of an enlarged Rank-sum method has been effectively used in measuring the different aspects of yield stability of some cassava clones apart from integrating yield with stability (Mba and Dixon, 1995). This paper aims to determine the relative resistance of some newly developed cassava cultivars to ACMD and compare the efficiency of the two methods of evaluation.

Materials and methods

A field trial was established in May 1997 with 25 cassava genotypes (23 improved and 2 local cassava cultivars) in a randomised complete block (RCB) design with four replications at the research farm of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Table 1). IITA is located to the north of Ibadan at latitude 7^{o} 31' N and longitude 3^{o}45' E and 210 m above sea level in the forest-savanna transition agroecological zone. The soil type is Ferric Luvisols. The mean annual rainfall is 1400-mm spread between April and October after which there is usually a 5-month period of dry weather. The minimum temperature is 20-23 °C and the maximum 27-37 °C (Jagtap et al., 1994). Stakes of the genotypes measuring 25 cm were planted at the beginning of the rains (April 1997). Each plot consisted of 40 plants in four rows (ridges of height 30 cm and length of 10 m) spaced 1 m apart. Plant spacing was 1 m x 1 m giving a plant population of 10 000 plants per hectare. The genotypes were grown for 12 months under rainfed conditions and no fertilizer or herbicide was applied during the course of the experiment. Plots were hand-weeded when necessary.

Disease Incidence (DI) and Index of Symptom Severity (ISS) were assessed on all cassava plants of each of the clones for 14 weeks on a fortnightly basis commencing in the eighth week after planting (WAP). In scoring for DI, each plant was first tagged using ribbons of a different colour for each of the lateral stems of a plant. The DI was scored for each leaf on a lateral stem. Scoring was done fortnightly for 14 WAP. The incidence per stem of a plant was calculated as:

$$\frac{\text{Total number of diseased leaves per stem}}{\text{Total number of leaves per stem}}$$

In scoring for ISS, all leaves on each plant stem were scored in five classes of symptom severity with class five showing the most severe symptoms (Terry, 1976; IITA, 1990). This ISS for each plant was computed as follows (Fauquet et *al.*, 1987; IITA, 1990):

$$\text{ISS} = \frac{\text{Sum of severity scores for all leaves on a plant}}{\text{Total number of leaves on the plant}}$$

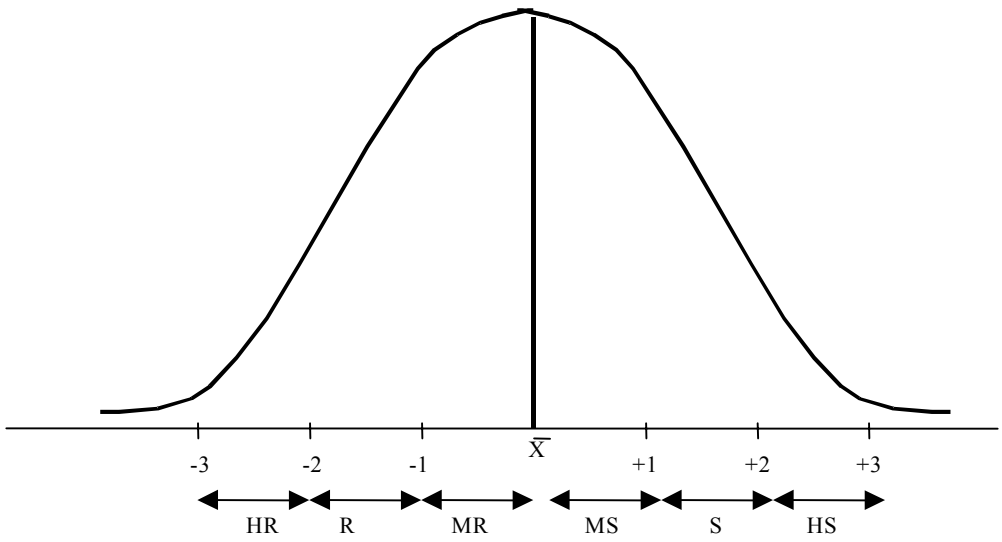
The means of DI and ISS for each clone were calculated at the end of the observation and ranked in descending order. For each rank position in each clone, DI and ISS ranks were added together after which the grand mean of the rank position ($\overline{X}$) for all the clones was calculated. Deviation from this rank position grand mean ($\overline{X}$ n-$\overline{X}$) was calculated for each clone. In calculating the area under the disease progress curve (AUDPC), the disease index (DIn), which is a product of DI and ISS for each clone, was first calculated.

Then, the AUDPC was calculated for each clone in seven successive evaluations (i.e., fortnightly for 14 weeks) using the formula below (Nagarajan and Muralidharan, 1995):

$$\text{A-value} = \sum_{i-1}^{k} \frac{1}{2}(S_i + S_{i-1})\, x\, d$$

Si = the disease index at the end of every fortnight; K= the number of successive evaluations of disease; d = interval between two evaluations, (i.e., 2 weeks).

The A-values for all the cassava genotypes were then ranked. The grand mean ($\overline{X}$) of the ranks and deviations from this rank grand mean ($\overline{X}$ n-$\overline{X}$) were calculated. Deviation to the right-hand side of the mean distribution curve was rated moderately susceptible, susceptible or highly susceptible while that to the left was rated moderately resistant, resistant or highly resistant (Figure 1).



X = grandmean, HR= highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible

Figure 1. Mean distribution curve for evaluating resistance status.

Results

Plants of clone ISU shared the highest DI (82.6) and ISS (3.09); clone M94/0121 had the lowest DI (36.4) and ISS (1.36) (Table 1).

Plants of clones TMS 30572, 82/00058, 94/0270, and 91/02322 showed a DI above 70% while the DI for clones 92/0326, 92B/00061, 92/0325, 92/0342, 92/0427, and M94/0177 was above 60%. A DI just slightly above 60% was found in clone TME 1 (60.1) and M94/0192 (60.6). The DI for clones TMS 4(2) 1425, 92B/00068, 94/0237, 92/0398, M94/0461 and 91B/00455 was observed to be above 50% while that of clone

91/0234 (50.3%) was just slightly above 50%. The DI for plants of clones 91/02327, 94/0239 and M94/0583 was between 45.7% and 40.9% while clone M94/0121 was within a lower range, as earlier mentioned.

Table 1: Relative resistance of some newly developed cassava cultivars to African cassava mosaic disease (ACMD) as determined by the Rank-sum and AUDPC

Clones	DI	ISS	Rank-sum	di	AUDPC	dii
ISU	82.6(25)	3.09(25)	50	+2.4(HS)	3705.03(25)	+2.4(HS)
TMS30572	79.7(24)	2.50(24)	48	+2.2(HS)	2978.35(24)	+2.2(HS)
82/00058	72.8(22)	2.37(23)	45	+1.9(S)	2600.54(23)	+2.0(HS)
94/0270	76.4(23)	2.27(22)	45	+1.9(S)	2543.03(22)	+1.8(S)
91/02322	70.8(21)	2.01(19)	40	+1.4(S)	2181.34(21)	+1.6(S)
92/0326	68.4(20)	2.04(20)	40	+1.4(S)	2095.09(20)	+1.4(S)
92B/00061	68.1(19)	1.91(18)	37	+1.1(S)	2045.28(19)	+1.2(S)
92/0325	67.0(18)	1.90(17)	35	+0.9(MS)	1928.63(18)	+1.0(S)
4(2) 1425	58.2(10)	2.06(21)	31	+0.5(MS)	1724.79(17)	+0.8(MS)
92/0342	62.1(16)	1.80(16)	31	+0.5(MS)	1709.00(16)	+0.6(MS)
92/0427	63.9(17)	1.72(13)	30	+0.4(MS)	1693.10(15)	+0.4(MS)
M94/0177	61.9(15)	1.74(14)	29	+0.3(MS)	1690.88(14)	+0.2(MS)
TME-1	60.1(13)	1.79(15)	28	+0.2(MS)	1591.65(13)	0
M94/0192	60.6(14)	1.69(11)	25	-0.1(MR)	1419.08(10)	-0.6(MR)
92B/00068	59.7(11)	1.71(12)	23	-0.3(MR)	1570.24(12)	-0.4(MR)
92/0057	60.0(12)	1.62(10)	22	-0.4(MR)	1470.66(11)	-0.2(MR)
94/0237	57.0(9)	1.61(9)	18	-0.8(MR)	1416.12(9)	-0.8(MR)
92/0398	52.5(8)	1.54(8)	16	-0.99(MR)	1336.85(8)	-1.0(R)
M94/0461	51.3(7)	1.52(7)	14	-1.2(R)	1159.80(5)	-1.6(R)
91/02324	50.3(5)	1.50(6)	11	-1.5(R)	1194.07(7)	-1.2(R)
91/02327	45.7(4)	1.45(5)	9	-1.7(R)	1029.52(4)	-1.8(R)
91B/00455	51.0(6)	1.42(3)	9	-1.7(R)	1161.98(6)	-1.4(R)
M94/0583	40.9(2)	1.43(4)	6	-1.99(R)	903.53(3)	-2.0(HR)
94/0239	41.5(3)	1.41(2)	5	-2.1(HR)	900.17(2)	-2.2(HR)
M94/0121	36.4(1)	1.36(1)	2	-2.4(HR)	739.66(1)	-2.4(HR)

DI = Mean disease incidence and rank scores; ISS = Mean index of symptom severity and rank scores; Rank-sum = Addition of the rank scores of DI & ISS; di = Deviation from the grand mean of the Rank-sum & resistance category; AUDPC = Area under the disease progress curve and rank scores; dii = Deviation from the grand mean of the rank scores of AUDPC values & resistance category; HS = Highly susceptible; S = Susceptible; MS = Moderately susceptible; HR = Highly resistant; R= Resistant; MR = Moderately resistant.

Variations in ISS among clones follow the same pattern as in DI, with the ISS value of clone ISU being the highest (3.09) and that of M94/0121 being the lowest (1.36). No sharp difference (0.01) was observed between the ISS values of clones 91B/00455 (1.42) and M94/0583 (1.43). But there was a reasonable difference (10.1%) between their percentage means of DI (Table1). Also the difference in ISS values of clones 92B/00068 (1.71) and M94/0192 (1.69) was not remarkable (0.02). But the percentage means of DI for the former is lower than for the latter.

Clone ISU showed the highest AUDPC (3705.03) while the lowest value was observed in clone M94/0121 (739.66). There was a remarkable difference between the AUDPCs of clones 94/0270 and M94/0121 as well as between 91/0237 and ISU (Figure 2).

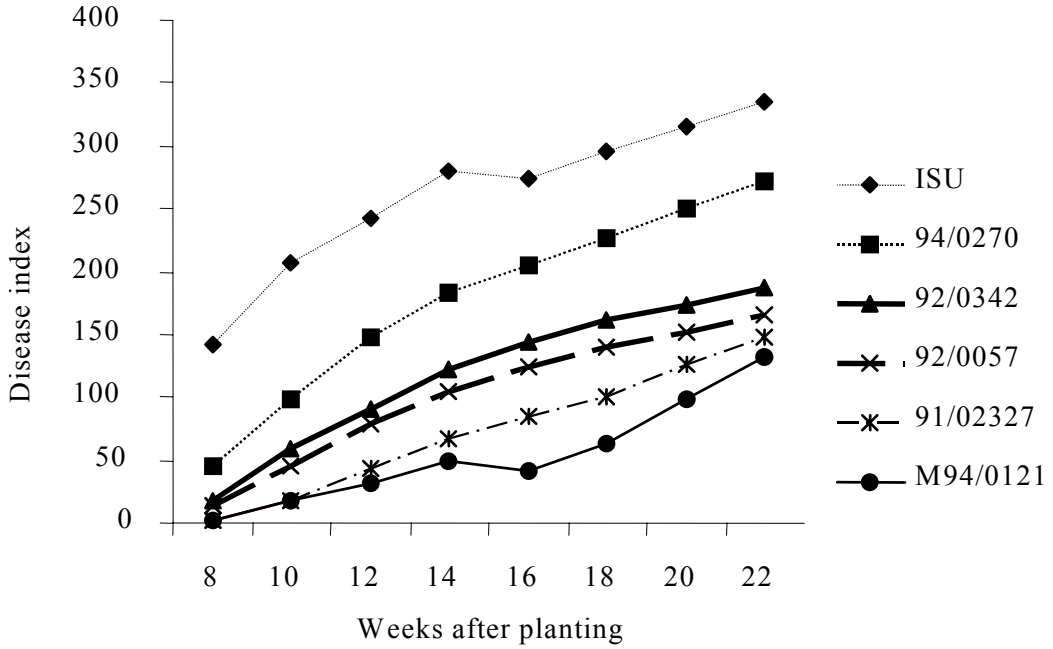


Figure 2. Cassava mosaic disease progression on 6 cassava clones between 8 and 22 weeks after planting

Generally, low Rank-sum and AUDPC values were observed in clones 92/0398, M94/0461, 91/02324, 91/02327, 91B/00455, M94/0583, 94/0239, and M940121 and the highest values were observed in clones ISU, TMS 30572, 82/00058, 94/0270, 91/02322, 92/0326, and 92B/00061.

With the Rank-sum method, the rank position (i.e., the addition of ranks of DI and ISS) in clone ISU showed the highest deviation to the right of the grand mean of the rank position ($\overline{X}$) (+2.40) followed by clone TMS 30572 (+2.20) (Table 1). These were the only two clones with deviation ($\overline{X}$ n- $\overline{X}$) ranging from +2 to +3. Also a deviation to the right of $\overline{X}$ ranging from +1 to +2 was observed in clones 94/0270 (+1.90), 82/00058 (+1.90) and 91/02322 (+1.10). A deviation between 0 and +1 was observed in clones 92/0325 (+0.90), 92/0342 (+0.50), 4(2) 1425 (+0.50), 92/0427 (+0.40), M94/0177 (+0.30) and TME-1 (+0.20). Apparently, there was no clone without symptom expression of the disease.

With the AUDPC method, the deviation range was almost the same for all clones as observed under the Rank-sum method except for 82/00058 (+2.00) deviation range +1 to +2, 92/0323 (+1.00) range 0 to +1, 92/0398 (-1.00) range 0 to +1,and M94/0583 (-2.00) range –1 to -2

Spearman rank correlation of the two methods showed a significant correlation at 99% confidence level (r = 0.99).

Discussion

The highest scores were for clone ISU with DI 82.6 and ISS 3.09 and imply that clone ISU is highly susceptible to ACMD. This agrees with the report of Hahn *et al.* (1989) on ISU (a local cassava cultivar) which is susceptible to ACMV with a score of 3.4. Also clone ISU showed the highest AUDPC (3705.03) while clone M94/0121 showed the lowest (739.66). This indicates further that clone ISU is the most susceptible of all the clones to the virus infection while clone M94/0121 is the least susceptible. Clones ISU and TMS 30572 were the only clones with a deviation range between +2 and +3 under the two methods of evaluation, Showing that they are highly susceptible (HS). Also a deviation range to the right of $\bar{X}$ from +1 to +2 was observed in clones 94/0270, 82/00058, and 91/02322 with the Rank-sum method, Showing that they are susceptible (S). Clones 92/0325, 92/0342, 4(2) 1425, 92/0427, M94/0177 and TME-1 with Rank-sum method, showed a deviation within the range of 0 and +1, signifying moderate susceptibility (MS).

The AUDPC method produced results contrary to those from the Rank-sum method for clones 82/00058 (range +1 to +2, HS), 92/0325 (range 0 to +1, S), 92/0398 (range 0 to –1, R) and M94/0583 (range –1 to –2, HR). Previous evaluation had observed a low incidence of ACMD for TMS 30572 and categorized TMS 4(2) 1425 as partially resistant, considerably less susceptible than Ebwanateraka and other local varieties (Thresh et al, 1994). This present study found the percentage means of DI with above 70 for TMS 30572 (resistance category HS) and near 60 (58.2) for TMS 4(2) 1425 (resistance category MS). See Table 1. This change in resistance categories could probably be due to an increase in DI at successive plantings of the same stock, because cuttings of these cultivars were usually obtained from cuttings (that might have been infected) of the previous growing seasons for the past 2 decades. This agrees with the report of Fargette and Vie (1995), that without reversion and cutting selection, the DI increased in successive plantings of the same clonal stock and ultimately reached 100%, whatever the degree of host resistance.

Spearman rank correlation of the ranks of the methods showed a significant correlation at 99% confidence level ($r = 0.99$). This implies that the two methods of evaluation are identical. and therefore, similar results were obtained. But the AUDPC is less cumbersome in use than the Rank-sum method.

There was no clone without symptom expression of the disease. This indicates that immunity as a form of resistance (Russell, 1978) was not observed in any of the clones screened. This study showed that resistant clones are reliable in limiting the incidence and symptom severity of ACMD. Farmers at local levels are therefore advised to adopt the use of these resistant cultivars to forestall problems due to the disease.

References

Burdon, J. J. (1987). Effects of pathogens on individual plants. In: *Diseases and plant population biology*. eds R. S. K. Barnes, H. J. B. Birks, E. F. Connor, J. L. Harper and R. T. Paine, pp. 8 - 27. Cambridge University Press, Cambridge, UK.

Dahniya, M.T. (1994). An overview of cassava in Africa. *African Crop Science Journal* 2:337-343.

Fargette, D. and Vie, K. (1995). Simulation of the effects of host resistance, reversion, and cutting selection on incidence of ACMV and yield losses in cassava. *Phytopathology* 85: 370 -375.

Fauquet, C., Fargette, D. and Thouvenel, J.C. (1987). Impact of African Cassava Mosaic Disease on the Growth and Yield of Cassava. In: *The International Seminar of African Cassava Mosaic Disease and its Control,* Technical Centre for Agricultural and Rural Co-operation, Wageningen, The Netherlands.pp. 20-25.

Fauquet, C. and Beachy, R. N. (1989). *Cassava Virus and Genetic Engineering. International Cassava Trans Project.* CTA, Wageningen, The Netherlands. 30 pp.

Guthrie, E. J. (1990). *Controlling African cassava mosaic disease.* CTA, Wageningen, The Netherlands. 20 pp.

Hahn, S. K., Isoba, G. G. J. and Ikotun, T. (1989). Resistance to cassava mosaic breeding in root and tuber crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. *Crop Protection* 8: 147-148.

IITA (International Institute of Tropical Agriculture) (1990). *Cassava in Tropical Africa: A Reference Manual.* IITA, Ibadan, Nigeria. 208pp.

Jagtap, S.S, Alabi, R.T., Roberts, A.B.C. and Adeleye, O. (1994). IITA weather Bulletin. International Institute of Tropical Agriculture, Ibadan, Nigeria. 90 pp.

James, W. C. and Teng, P. S. (1979). The quantification of production constraints associated with plant disease. *Applied Biology* 4: 201 - 267.

Jennings, D.L. (1957). Further studies in breeding cassava for resistance *East African. Agricultural Journal* 22: 213-219.pp

Mba, R. E. C. and Dixon, A. G. O. (1995). Correlation studies among yield and various stability parameters and an enlarged Rank-sum method for identifying high yielding stable cassava clones. In: *Proceedings of the sixth Triennial Symposium of the ISTRC-AB*, pp 261 - 266. Malawi.

Nagarajan, S. and Muralidharan, K. (1995). Disease Dynamics and Analytical Epidemiology. In: *Dynamics of Plant Diseases*. 120-135. pp

Russell, G. E. (1978). *Plant Breeding for Pest and Disease Resistance.* Butterworths, London (1987). 229 pp.

Scott, S. W. and Griffiths, E. (1980). Effects of controlled epidemics of powdery mildew on grain yield of spring barley. *Annals of Applied Biology* 94: 19-31.

Ssemakula, G. N., Baguma. Y. K., Otim-Nape, G. W., Bua, A. and Ogwal, S. (1997). Breeding for resistance to mosaic disease in Uganda. *African Journal of Root and Tuber Crops* 2 (1&2): 36 - 45.

Terry, E. R. (1976). Description and evaluation of cassava mosaic disease in Africa, In: *The International Exchange and Testing of cassava Germplasm in Africa* (eds. Terry, E. R. and Maclntyre, E.), International Development Research Centre, Ottawa, Canada, pp. 53 - 54.

Thresh J. M., Otim-Nape, G. W. and Jennings, D. L. (1994). Exploiting resistance to African cassava mosaic virus. The Impact of genetic variation on sustainable agriculture. *Aspects of Applied Biology 39: 51*-59.

Thresh J.M. and Otim-Nape, G.W. (1994). Strategies for controlling African cassava mosaic geminivirus. *Advances in Disease Vector Research* 10: 215-236.

Wilcoxson, R. D., Skovmand, B. and Atif, A. H. (1975). Evaluation of wheat cultivars for their ability to retard development of stem rust. *Annals of Applied Biology* 80: 275 – 281.

Van der Plank, J. E. (1963). *Plant diseases: Epidemics and Control.*: Academic Press. New York USA. 349pp