COMPONENTS OF QUANTITATIVE RESISTANCE TO BLIGHT IN FOUR CASHEW CULTIVARS AND THEIR RELATIONS WITH FIELD ASSESSMENTS

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Abstract
Occurrence of blight on the four cashew clones named AC4, AC10, AZA2 and AZA17 was investigated under *in vitro* and field conditions, and the resistances of different clones to the disease were analyzed. AC10 (2.27×10^7) had the highest number of spores per unit area followed by AC4 (1.61×10^7), AZA17 (7.25×10^6) and AZA2 (6.67×10^6) respectively. AZA2 recorded significantly higher latent period (26 h) followed by AZA17 (23 h), AC10 (22 h) and AC4 (20 h). AC10 had the highest lesion size 3 days after inoculation followed by AZA17, AC4 and AZA2 respectively. AC10, AC4 and AZA17 genotypes had the highest number of lesions increase per day (2) while AZA2 revealed a low increase of lesions per day. Lowest mean rate of infection (r) was observed in AC4 which ranged between 0.10 and 0.29 whereas AC10 recorded higher rate of infections which ranged between 0.45 and 0.48. It shows that cashew clones AZA2 and AZA17 are more tolerant to cashew blight pathogen.

Key words: Cashew, blight, resistance, clones

1.0 Introduction

Whereas complete resistance has been a major weapon used by plant breeders to control fungal pathogens such as wheat powdery mildew (*Blumeria* (*Erysiphe/ graminis* f.sp. *tritici/*, the generally race-specific and hence non-durable nature of this resistance has shifted the focus to race-non-specific partial resistance. Unfortunately, the fact that partial resistance is the resultant effect of many genes, each with a small individual effect, has made investigation of partial resistance genes (a vital study, if breeding for this relatively low heritability trait is to be made more efficient) a difficult process. Partial disease resistance is expressed as a reduction in the rate of development of the disease in the host. It can be caused by alterations in one or more of the following components: increased incubation period (time from inoculation to appearance of symptoms), increased latent period (time from inoculation to sporulation on the resulting colony), reduced infection frequency (number of colonies per unit leaf area), reduced infectious period (length of time the colony produces viable spores), reduced infection size (colony size), and reduced spore production (number of spores produced per infection or per unit leaf area over a particular length of time). Assessment of partial resistance in the field can be subject to large experimental error, because of the effects of environmental factors such as field heterogeneity and other pathogens or pests. On the other hand, measurement of single components of resistance under a controlled environment involves much smaller experimental error values. If a single component were highly correlated with field partial resistance, then it would be possible to assess this component only, and hence select indirectly (and more efficiently) for increased resistance. Significant correlations between one component of resistance and partial resistance in the field have been reported, e.g., adult plant latent period with partial resistance to leaf rust (*Puccinia hordei*) on barley (Parlevliet *et al.,* 1980) and lesion size with partial resistance to bacterial blight in rice (Koch *et al.,* 1991). In these examples, it was necessary to measure only the specific component in order to obtain an accurate estimate of partial field resistance.

Cashew (*Anacardium occidentale Linn)* a native to the coastal parts of North eastern Brazil belongs to Anacardiaceae family (Chipojola *et al.,* 2009). Tanzania is the Africa's third largest producer of cashew nut producing around 120,000 metric tonnes during the year 2011 according to Cashew Nut Board of Tanzania. Cashew nut production in Tanzania is a major export earner. The main growing areas in Tanzania include Mtwara, Lindi, Coast, Ruvuma and Tanga regions. Cashew trees are well adapted to harsh conditions and can grow successfully in areas with annual rainfall as low as 50cm. Cashew grows best in well drained soils. Cashew blight caused by *Cryptosporiopsis* spp causes upto 48.4% nut yield loss in Tanzania (ACRR, 2006). Disease symptoms are

characterized by spot lesions with brown margins formed on young tender leaves, apples and nuts. Infected nuts display a dark tan color while undergoing shrinking and defoliated eventually. Cashew blight damage include reduction in shoots hence low number of flower panicles, reduction in nuts and apples produced. High susceptibilty and inadequate knowledge on management of cashew have been shown to be among the constraints facing cashew nut production in eastern and southern Africa (Shomari, 2002). Cashew resistance to blight is not well understood; therefore host resistance studied will be a practical management. In cashew, there is no report of resistance to *Cryptosporiopsis* spp causing blight. Fungicide application is a wide spread practice of controlling the disease. However, the increassing production costs and pollution of the environment by the fungicides have demeed it necessary to find an alternative. Chemical control measures are too expensive and therefore host resisntce is given priority in cashew blight control strategy.

There are two types of cashew tree each defined by size and denominated as the common type and the dwarf type (Bezerra *et al.,* 2008). Common cashew tree varies from 8 to 15 m in height and 20 cm crown span. Common type of cashew was selected because it is popularly grown in the southern region of Tanzania. There is need to select desirable genotypes from the existing gene pool with improved resistance to blight pathogen. We examined methods for assessing the resistance of cashew genotypes under various environmental conditions using isolates of *Cryptosporiopsis* spp causing blight.The aim of this study was to characterize the partial resistance of four cashew cultivars with different levels of *Cryptosporiopsis* spp resistance through an analysis of the components of resistance in the seedling.

2.0 Materials and Methods

Two experimental fields were used over two periods of study. Both fields were located in Mtwara region of Tanzania. The data was obtained at Naliendele Research Station, Mtwara (040° 09' 15.61'' E 10° 22' 40.98'' S, 141 M a s l) from Jan 2011 until March 2012. The rainy season of November/December to April/May is single peaked, the peak being reached in January but occasionally in February or March. Mtwara district rains vary from 935 mm to 116 mm in the hills and the plateau. Likewise temperatures vary from 27°C as the highest monthly mean at Mtwara on the coast in December to 23°C in July. Relative humidity goes from 87% in March to 79% in October in Mtwara. Temperatures and humidity are lower inland. Four parents of promising cash clones were selected basing on yield results of over 15 years (Anonymous 1990). The clones were AC4, AC10, AZA2 and AZA17. In addition, AC4 parent clone known to be susceptible to leaf and nut blight (Shomari personal communication) was also selected.

2.1 Pathogen and Genotypes (Clones)

A collection of the fungus (*Cryptosporiopsis* spp) was established with 10 isolates (AA1, AA2, AA3, AA4, AA5, AA6, AA7, AA8, AA9 and AA10) collected in different geographic regions from cashew leaves and proved to be pathogenic to cashew were used. *Cryptosporiopsis* spp isolates from the diseased leaves were carried out on Potato Dextrose Agar (PDA) slants according to (Sandeep *et al.,* 2005) and grown routinely at 27ºC on malt extract agar in Petri dishes. For the pathogenicity tests, detached young leaves were inoculated on the abaxial side with mycelial disks of agar or with conidia suspensions 10^6/ml. The leaves were then put in petri dishes lined on the bottom with filter papers and then covered and put in an incubator at 27ºC for 8 days. Cashew genotypes reffered in this paper were produced from grafting. The 4 genotypes were originally selected on the basis of overall yield.

2.2 *In vivo* determination of Infective Inoculum Density and Resistant/Tolerant Cultivars

Cashew seedlings of the four cultivars widely grown in the country were sprayed with four inocula densities (10^4, 10^5, 10^6, 10^7 10^8 10^9ml/L), ascertained using a neubar counter for spore counting. A batch of seedlings of each cultivar was arranged in a completely randomized design (CRD) in the green house and inoculated with each of the densities using a hand sprayer. The experimental layout was a 2x4 factorial replicated 3 times in a CRD. The factors under study were genotypes resistance/susceptibility to blight disease and conidia densities infectivity. The variables recorded were number of spots/lesions per plant and number of leaves.

2.3 Leaf – Disk Assay

The relaibility of a leaf – disk assay to assess partial resistance of cashew clones (AC4, AC10, AZA2 and AZA17) to *Cryptosporiopsis* spp, the causal agent of cashew blight was evaluated. 2nd leaf surface characteristics of the four cashew clones used in the leaf disk assay were studied. Preparation of leaf disks for inoculation was carried out according to the methodology of Santos *et al.,* 2011. 20mm diameter leaf discs from four cashew clones leaves were obtained using a 20mm diameter cork borer. Leaf disks were obtained from young fully expanded 2nd and 3rd leaves of each cashew clone. The discs were maintaned in 9cm petri dishes lined up with moistened filter papers with distilled water. Conidial suspension of *Cryptosporiopsis* spp was prepared and adjusted to 10^6/ml and sprayed using a hand sprayer immediately to maintain a uniform inoculum. The covered plates were incubated at 28ºC for 6 days. Percentage leaf damaged was calculated in all of the four clones. The results were subjected to analysis of variance and t test comparison of means, at $\alpha = 0.05$. The experiment was replicated twice.

2.4 *In vitro* Screening of 4 Cashew Cultivars (AZA 2, AZA 17, AC 4 And AC 10) for Resistance to *Cryptosporiopsis* Spp Causing Blight Disease

Cashew cultivars plant materials of the four cultivars collected from the field were washed in sterile distilled water. The materials were then cut into small pieces and 50 g of each then autoclaved at 121°C and 15psi for 20 minutes with sterile 50 ml distilled water and 3.9 g of PDA powder to infuse the plant extracts in the plant extracts in the PDA media. Water was dispensed to 3 petri dishes separately to act as a negative control. The petri dishes (3 per treatment) were then centrally inoculated with 9mm mycelial plugs from 6 day old culture and incubated at 28°C. The fungus colony radial diameter was measured after 8 days of inoculation and the conidia sporulation determined at the 8th day. 8 days after inoculation, conidial suspensions were prepared by washing spores off the plates with 10-15ml of deionized water. Suspensions were filtered through three layers of muslin cloth to remove mycelial fragments. Growth rate of the colony was determined by dividing the diameter of the colony with the total number of days. Number of conidia per 5ml in each of the treatments was measured. The results were subjected to analysis of variance and t test comparison of means, at $\alpha = 0.05$.

2.5 Mechanism of Slow Blighting

A field experiment was conducted to study the slow blighting mechanism at Naliendele Agricultural Research Station, Mtwara. Four genotypes were evaluated for slow blighting mechanism in randomised block design with four replications. Cashew seeds were sown in a 1.6 m x 5.2 m plot. When approximately 13cm high with 4-5 fully expanded leaves were grafted using the scions of five susceptible cashew genotypes. Softwood grafting technique using two to three month old cashew seedlings with scions of 12 to 15 cm length was adopted (Sawke, 1992). This ensured uniformity in the genotypes required for the experiment. Grafted seedlings were cut after developing to obtain new uniform shoots. Thereafter they were allowed one month to harden before being inoculated. The test clones were inoculated with *Cryptosporiopsis* spp at 10^6ml. Inoculation was done once followed by water spray so as to maintain humidity for infection. Following observations were recorded:

(i) Per cent disease index: Observations on blight severity was recorded at 3, 5 and 7 days after inoculation. On the basis of PDI, the cashew cultivars were categorized as resistant, R (PDI = 0-5%); moderately resistant, MR (PDI = 6-10%); moderately susceptible, M.S (PDI = 11-20%); susceptible, S (PDI = 21-50%) and highly susceptible, HS (PDI > 50%). Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were conducted using SAS statistical software.

$$\text{PDI} = \frac{\text{Sum of individual disease ratings}}{\text{No. of observations assessed}} \text{ x } \frac{100}{\text{Maximum disease rating}}$$

(ii) Infection frequency (IF) was measured by counting the number of pustules per unit area (cm^2) and it was calculated on the same leaves where latent period was measured.

(iii) Latent period: Latent period was recorded as the period between inoculation and appearance of symptoms on leaves. Latent period was decided when 50% of visible lesions developed on leaves (Patil *et al.,* 2006)

(iv) Lesion number: Lesion number per plant was taken from five randomly selected plants from each cashew clone.

(v) Lesion size: Observations on lesion size at 3, 5 and 7 days were recorded in cm^2 based on length and width of lesion using a ruler from five tagged plants in each clone and rate of increase in lesion size per day was calculated.

(vi) Rate of infection (r): The per cent disease indices calculated at 2 days interval for each genotype was used to calculate 'r' value by adapting formula given by Vander Plank (1963). The rate of disease increase over time was estimated following the logistic model (van der Plank, 1963) as the regression coefficient of the logit x on time in days

$$r = \frac{2.3}{t_{2-}\ t_1}\left[\text{Log}\frac{x_2}{1-x_2} - \text{Log}\frac{x_1}{1-x_1}\right]$$

Where, r = apparent rate of infection as spread

x_1 and x_2 = Percent disease index at time t_1 and t_2

$t_2 - t_1$ = Time interval in days between two consecutive observations

Intact seedling inoculation experiment was performed by 1x4 factorial experiments in completely randomized design (CRD) with three replications. Treatments consisted of one isolate of *Cryptosporiopsis* spp and four clones of cashew. Three successive experiments were performed. Area under disease progress curves (AUDPC): The per cent disease indices obtained at 2 days interval for each genotype were used for AUDPC calculation. The AUDPC values for each genotype was measured using trapezoidal method, as described by Madden *et al.,* 2007.

$$\text{AUDPC} = \sum_{i=1}^{N_i - 1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

Where AUDPC= Area under disease progress curve

y_i = Disease severity at the end of time i

t_i = Number of successive evaluation of blight severity

N = interval between two observations

3.0 Results

3.1 *In vivo* Determination of Infective Inoculum Density and Resistant/Tolerant Genotypes

Differences in virulence among isolates were examined by comparing the number of clones on which isolates induced symptoms. The absence of isolates with differential virulences was observed with the 10 isolates. However, it may be too early to rule out this possibility until more isolates have been studied. Therefore screening was done on a mixed inoculum given that some isolates may be aggressive than others. The concentration of arriving spores determines the disease incidence on the leaves (Table 1). There was a significant host genotype/fungal isolates interaction for each of the three cashew genotypes examined (P<0.0001). Disease incidence increased with inoculum concentration. AC10 genotype was severely diseased regardless of the amount of inoculum. AZA2 had lower disease incidence in all concentration. Low concentration caused no disease infection in AZA2 genotype suggesting it will require large influx of inoculum to cause an infection. AZA2 and AZA17 genotypes had lower disease incidence in all spore concentrations suggesting they could slow the infection rate and can only be infected only at high spore loads. Rapid screening aimed at selecting only the most resistant genotypes using spores application methods have proved effective.

There was significant differences between the amount of inoculum and the number of the lesions it produces (P<0.0001). 10^7/ml produced the highest mean number of lesions (70) followed by 10^6/ml (43), 10^5/ml (37), 10^8/ml (24), 10^4/ml (15) and 10^9/ml(9) respectively. The disease/inoculum curve is no longer straight, as in table 1, but curves to the right. The more the spores in the inoculum, the ratio of lesions to spores decreases. Cashew clones were inoculated with suspensions of spores, and after 24 h the number of lesions were counted. With the increasing *Cryptosporiopsis* spp inoculum the number of lesions increases to a maximum of about 70 lesions per 4 leaves of each cashew seedlings. Thereafter, with still further increases of inoculum, the trend reversed and the number of lesions decreased to about 24 and 9 lesions for 8000 and 9000 spores/ml respectively. The germination of the spores in abundance of inoculum may be attributed to self-inhibition of germination of *Cryptosporiopsis* spp

fungi. Spores are shown to compete with one another for the susceptible sites on the leaves. Inhibitors of germination have shown to occur in other fungi as well**.**

3.2 Leaf – Disk Assay

Results from the two leaf disk assays were not significantly different (P>0.05), so the data were pooled for final statistical analyses (Fig 1). Cashew clone AC10 had the highest disease severity (68%) followed by AC4 (69%), AZA17 (14%) and AZA2 (7%) respectively. AC10 and AC4 appeared to be susceptible while AZA2 and AZA17 were partially resistant to *Cryptosporiopsis* spp causing blight. There were no significant differences between cashew clones AC4 and AC10 (t=-.028, df=3, P=0.979). However, in there were significant differences in cashew clones AC4 and AZA17 (t=5.435, df=3, P=0.012). There were significant differences in between cashew clones AZA17 and AZA2 (t=3.11, df=4, P=0.036). There were significant differences in between cashew clones AC4 and AZA2 (t=6.756, df=3, P=0.007). There were significant differences in between cashew clones AC10 and AZA17 (t=11.965, df=4, P<0.05). There were significant differences in between cashew clones AC10 and AZA2 (t=6.756, df=3, P=0.001).

3.3 *In vitro* Screening of 4 Cashew Cultivars (AZA 2, AZA 17, AC 4 And AC 10) for Resistance to *Cryptosporiopsis* Spp Causing Blight Disease

There were significant differences (P<0.05) in the colony radial growth of *Cryptosporiopsis* spp inoculated on different cashew clone leaf extracts infused on Agar (Table 1, Fig 2). The fungal colony radial diameter over the experimental period was highest in AC4 (67mm), followed by AC10 (65mm), AZA2 (58mm) and AZA17 (55mm) respectively. The control had the lowest colony radial diameter (13mm). There was no significant differences (t=0.685, df=5, P=0.524) between AC4 and AC10 clones in colony radial diameter. In the *Cryptosporiopsis* spp sporulation in different cashew clones extracts (AC4, AC10, AZA17 and AZA2) and the control was significantly different (P<0.05). AC4 leaf extract had the highest sporulation (Table 3) followed by AC10. The total number of conidia per colony was highest in AC4 (2.83×10^8) followed by AC10 (2.33×10^8), AZA17 (1.36×10^8) and AZA2 (1.18×10^8) respectively. AZA2 had a reduction in sporulation as compared to other cashew genotypes. AC4 recorded the highest number of spores indicating maximum sporulation.

3.4 Mechanism of Slow Blighting

The various slow blighting components such as spore density, percent disease index, latent period, lesion size, lesion density, rate of infection and area under disease progressive curve were evaluated against cashew blight in the field. The study used whole plants to prevent the senescence associated with detached leaves thereby clearly observing later forming leaves which showed greater resistance. Slow blighting checks the rate of spread of the disease and also detects the possible occurrence of epidemics without causing any adverse effect on yield. This phenomenon has been described as horizontal resistance (Van der plank, 1963). The components assessed in this study showed variation. Resistance can be inherited as a qualitative or quantitative trait (Mehta *et al.* 2005; Mohan *et al.* 2010) and extensive variation in disease response has been observed in populations developed to determine the genetics of resistance (Erpelding 2007); therefore, the diversity in disease response and overall lower disease severity would suggest genetic variation for host resistance.

3.4.1 Spore Density

There were differences in the number of spores per unit area in cashew genotypes studied (P<0.0001). AC10 (2.27×10^7) had the highest number of spores per unit area followed by AC4 (1.61×10^7), AZA17 (7.25×10^6) and AZA2 (6.67×10^6) respectively (Table 3). AZA2 revealed a long time that infectious propagules appears this could slow the progress of *Cryptosporiopsis* spp. AZA2 showed reduction in the total number of infectious units over time. There was a significant difference in colony radial growth diameter whereby AC4 (67mm) revealed the highest followed by AC10 (65mm), AZA2 (58mm) and AZA17 (55mm) respectively (Table 3).

3.4.2 Latent Period

Latent period is defined as the mean number of days taken for appearance of cashew blight lesion from the day of inoculation varied from genotype to genotype. It was in the range of 20 h in AC4 to 26 h in AZA2. The results (Table 4) revealed that, latent period varied from 20 to 26 h for all cashew genotypes. AZA2 recorded significantly higher

latent period (26 h) followed by AZA17 (23 h), AC10 (22 h) and AC4 (20 h). AZA17 and AZA2 recorded longer latent period (23-26 h) while AC4 and AC10 had shorter latent periods (20-22 h).

3.4.3 Lesion Size

The results presented in Table 4 reveal that, among the cashew genotypes lesion size (mm) and rate of increase in lesion size (mm/day) were maximum in AC4. There was significant differences in lesion size 3 DAI (P<.0001). AC10 had the highest lesion size 3 DAI followed by AZA17, AC4 and AZA2 respectively. There were significant differences in lesion size 5 DAI whereby AC10 had the maximum lesion size followed by AC4, AZA17 and AZA2. There was significant differences in lesion size 7 DAI (P=0.014) whereby AC10, AC4, AZA17 and AZA2.

3.4.4 Lesion Density

Lesions are easily counted and their absence could be used as a simple way of separating resistant genotypes from less resistant ones. There were significant differences in the average number of lesions per day in the cashew genotypes studied (Table 4). The number of lesions per plant ranged from minimum of 27 in AZA2 to maximum of 59 in AC4. AC10, AC4 and AZA17 genotypes had the highest number of lesions increase per day (2) while AZA2 revealed a low increase of lesions per day. The rate of increase in size of lesions per day was evaluated and AC10 (0.32) recorded the maximum followed by AC4 (0.26), AZA17 (0.25) and AZA2 (0.06) respectively. The low rate of lesion size increase observed in AZA2 suggested that the genotype is resistant. The smaller lesions in AZA2 have the potential of slowing lesion growth over time. There were significant differences in the total number of lesions in the four cashew genotypes (P<0.0001). AC10 (59) revealed the highest total number of infections followed by AC4 (48), AZA17 (29) and AZA2 (27) respectively (Table 4).

3.4.5 Infection Frequency

Infection frequency is a component of resistance which expresses the efficiency by which the fungus is able to complete the whole infection cycle. The more resistant the cultivar, the smaller the lesion and the slower its growth. Expression of resistance increased as plants aged in all clones. AC4 had the highest infection frequency followed by AC10, AZA2 and AZA17 respectively (Table 4).

3.4.6 Percent Disease Index (PDI)

There were significant differences in PDI among the genotypes. AZA17 and AZA2 showed significantly lower disease severity. It shows that cashew clones AZA2 and AZA17 are more tolerant to cashew pathogen (Table 4).

3.4.7 Apparent Rate Of Infection (r)

Rate of infection in the field varied from 0.10 to 0.45 per day. Lowest mean rate of infection (r) was observed in AC4 wchich ranged between 0.10 and 0.29 whereas AC10 recorded higher rate of infections which ranged between 0.45 and 0.48. There were no consistency in any genotypes and no particular trend observed (Table 4).

3.4.8 Area under Disease Progress Curve (AUDPC)

The estimated AUDPC was selected to be the descriptor for each epidemic to compare the reaction of cashew clones for blight resistance. The AUDPC incoporates variations in time of disease onset and finish. AUDPC quantifies the disease repression on infected plants. There was a high variation among cashew genotypes in AUDPC values (Table 4). The AUDPC values differed significantly in the genotypes. The highest AUDPC value was observed in AC10 (403) followed by AC4 (386), AZA17 (217) and AZA2 (113). AC10 (403.83) and AC4 (386.83) genotypes revealed high AUDPC values while AZA17 (217.33) and AZA2 (113.5) recorded low AUDPC values. AC4 and AC10 recorded higher AUDPC values and were considered as fast leaf blighters, while, AZA17 and AZA2 exhibited low AUDPC values and were inditified as slow leaf blighters (Table 4). Among the genotypes, AC10 exhibited higher AUDPC and AZA17 showed medium AUDPC and was considered as moderate blighter while AZA2 showed least AUDPC and was considered as slow leaf blighter. The higher AUDPC value in AC10 revealed fast leaf blighter.

4.0 Discussion

AC4 and AC10 leaves had waxy surface characteristics that might have an effect on spore deposition or catch. AZA2 and AZA17 had smooth leaf surfaces. Waxy leaf surfaces exhibited by AC4 and AC10 genotypes may contribute to increased disease incidence because of more spore catch as compared to AZA2 and AZA17. AZA2 and AZA17 might be possessing reduced catching capabilities. The availability of young tender cashew leaves showed to trigger an infection in all cashew genotypes. Mature leaves appeared to be resistant from *Cryptosporiopsis* spp infection causing cashew blight in the four genotypes. Therefore the control measures of *Cryptosporiopsis* spp causing cashew blight can be scheduled to coincide with specific host phenological observations like the availability of young tender leaves. The seasonal development of young tender cashew shoots was studied. The establishment of the seasonal changes in cashew is important especially to tome the fungicidal sprays to control *Cryptosporiopsis* spp causing blight. 2nd and 3rd leaves of each cashew clone were used in leaf disk assay to reduce discrimination of clones for disease resistance. This selection corresponds with Browne *et al.,* 2006 findings that the selection of leaves for use in a detached leaf assay is important with regard to discrimination of cultivars for disease resistance. There was no evidence of contamination in leaf disk assay employed here. *In vitro* detached leaf assays have been used as a tool for investigating Partial disease resistance in the whole plant for a wide range of diseases including Septoria tritici (Arraiano *et al.,* 2001; Chartrain *et al.,* 2004) rust resistance in Salix to Melampsora larici-epitea (Pei *et al.,* 2004) and against Fusarium head blight (Diamond and Cooke, 1999; Browne and Cooke, 2004b; Browne *et al.,* 2005). Cashew genotypes AZA17 had the least colony radial growth; this could be attributed to the partial resistance to *Cryptosporiopsis* spp causing blight. AC4 recorded the highest colony radial growth revealing susceptiblity of the cashew clone to *Cryptosporiopsis* spp causing blight disease.

Quantitative resistance to cashew leaf and nut blight reduces the rate of disease development in the field by hampering the development of the fungus at different stages during the infection cycle. The extent to which the fungus is hampered in its development can be assessed through a study of components of resistance in monocyclic experiments. In the experiments described here, spore density, latency period, infection frequency, lesion size and density, disease severity and colony radial growth were assessed on cultivars. Partial disease resistance has characterized by a reduced rate of epidemic development in a host population attributed to various components of Partial disease resistance including lower infection frequency and a longer latent period (Browne *et al.,* 2006). Overall observations revealed that, AZA2 genotype had longer latent period as compared to AZA17, AC4 and AC10 genotypes. The latent period of the *Cryptosporiopsis* spp pathogen was extended in AZA2 genotype; this could have resulted in fewer cycles of reproduction of the pathogen. Cashew clones with shorter latent periods probably had more number of blight cycles than clones with long latent periods. This implies reduced intial inoculum load on the genotypes with longer latent period which suggests partial resistance ability. Susceptible genotypes reacted faster than the resistant ones after pathogen inoculation. For a pathogen of high multiplication rate, latent period is major determinant of epidemic speed (Campbell and Madden, 1990). Shorter latent periods produced in AC4 and AC10 were directly proportional to more number of lesions per plant and high AUDPC values. Reduced rate of infection (r) was observed in AC4 which ranged between 0.10 and 0.29 whereas AC10 recorded higher rate of infections which ranged between 0.45 and 0.48.

Similar results were obtained with seedlings of British winter wheat cultivars with durable (quantitative) resistance to yellow rust which showed a small reduction of the infection type and a lower pustule formation rate (Mares & Cousen, 1977). The data presented here show that these cultivars carry different levels of quantitative resistance. Breeders who use above mentioned classification to discard susceptible cultivars or lines in the seedling stage take the risk that valuable source of quantitative resistance are eliminated. Quantitative resistance measured as infection type is better expressed at high than at low temperatures (Broers, 1997). The temperatures used in this study were much appropriate which may explain the association between infection type and level of resistance.

Infection frequency is a component of resistance which expresses the efficiency by which the fungus is able to complete the whole infection cycle. The infection cycle consists of many steps and can be obstructed at any time from spore germination until sporulation (Mares & Cousen, 1977). Reduced infection frequency in AZA17 and AZA2 cultivars revealed low levels of quantitative resistance. The method used to assess infection frequency assumes

that each spot equals one infection. AC4 had the highest infection frequency followed by AC10, AZA2 and AZA17 respectively. Lesion size and lesion density are both components that express the growth of established lesions in the leaf as does latency period. This study shows that both Lesion size and lesion density were affected by the level of resistance in the tested cultivars. In general, maximum lesion size and rate of increase in lesion size were observed AC10 while AZA2 showed smaller lesion size and the least rate of increase in lesion size. AZA2 indicated to have limited the lesion size forming fewer spores while AC4 had larger lesion surface available for sporulation.

Fewer infections on AZA2 reflect partial resistance ability while AC10 shows susceptibility to *Cryptosporiopsis* spp infection under the same conditions. Partial resistance was greater in late expanding leaves against *Cryptosporiopsis* spp in cashew. The infection rate of *Cryptosporiopsis* spp can be used to predict the future epidemic development and spray timing schedules after initial disease occurrence. The components assessed in this study showed associated variation. Increasing levels of quantitative resistance were characterized by longer latency period, lower infection frequency and incidence, smaller and slower growing lesions resulting in less leaf area affected. The study revealed that different components may be affected by different genes. Individual components of quantitative resistance reduce the epidemic. Although all individual components could explain to a large extent the observed variation for quantitative resistance in the field, not all components are epidemiologically of equal importance (Broers, 1997).

The AZA2 genotype has more quantitative resistance against *Cryptosporiopsis* spp as compared to AC4, AC10, and AZA17. AUDPC has been shown to evaluate the resistance of plant species to the pathogens (Contreras-Medina *et al.,* 2009). Slow leaf blighting AZA2 genotype is important in reducing the rate of spread of the disease. The source of cashew resistance found in this study may be included into the overall approach for integrated cashew management. AZA17 and AZA2 showed delayed onset of the disease and recorded significantly the lowest disease severity whereas AC4 and AC10 showed very high disease severity and shorter latent periods. The lesion number was high in susceptible genotypes (AC4 and AC10) while resistant genotypes recorded the lowest number of lesions. The highest AUDPC values were observed in AC4 and AC10. Cashew clones with small lesion size and lower number of lesions per day recorded very low AUDPC values and vice versa.

5.0 Conclusion

Cashew clones AZA2 and AZA17 are more tolerant to cashew blight pathogen. It is concluded that, the plant breeders could utilize the slow blighting genotypes identified, for improving the cashew varieties especially AC4 and AC10, which are highly susceptible to blight pathogen, but having good export potential.

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Table 1: Disease incidence in different cashew clones

Spore Concentration	Cashew clones			
	AC10	AC4	AZA17	AZA2
10^4	75	50	50	25
10^5	50	62.5	75	62.5
10^6	62.5	87.5	50	25
10^7	87.5	62.5	25	25
10^8	25	37.5	50	25
10^9	62.5	25	50	25
Mean	60.42	50	50	31.25
CV (%)	20.69	28.87	0	23.10
LSD	30.58	35.32	0	17.66

Table 2: linear and quadratic relationship between number of lesions per 4 leaves of each clone of A. Occidentale and number of spores of Cryptosporiopsis spp

Equation	**Model Summary**					**Parameter Estimates**		
	R^2	**F**	**df1**	**df2**	**Sig.**	**Constant**	**b1**	**b2**
Linear	.270	3.707	1	10	.083	38.467	-2.682E-8	
Quadratic	.311	2.030	2	9	.187	40.683	-1.446E-7	1.164E-16

Table 3: Influence of different cashew extracts on sporulation and growth of Cryptosporiopsis spp causing blight

Cashew clones	**Total no. of conidia per colony**	**No. of spores per unit area (cm^2)**	**Growth rate per day (mm)**	**Colony radial growth diameter (mm)**
AC10	$2.33 \times 10^{8}_{b}$	$2.27 \times 10^{7}_{a}$	8.17_{a}	65.33_{a}
AC4	$2.83 \times 10^{8}_{a}$	$1.61 \times 10^{7}_{b}$	8.39_{a}	67.17_{a}
AZA17	$1.36 \times 10^{8}_{c}$	$7.25 \times 10^{6}_{c}$	6.87_{b}	55.00_{b}
AZA2	$1.18 \times 10^{8}_{c}$	$6.67 \times 10^{6}_{c}$	7.27_{b}	58.17_{b}
Control	$0.23 \times 10^{8}_{d}$	$1.25 \times 10^{6}_{d}$	1.625_{c}	13.00_{c}
Grand mean	1.70×10^{8}	1.16×10^{7}	6.81	54.48
CV (%)	16.72	20.74	4.99	4.99
LSD	3.63×10^{7}	3.08×10^{6}	0.435	3.4798

Table 4: Slow leaf blighting in cashew clones against Cryptosporiopsis spp causing cashew blight disease

Name of clone	Total no. of lesions on four leaves	Average no. of lesions per leaf	Size of lesions (sq.cm^2)			Average no. of lesions per day	Rate of increase in size of lesions/day	Latent period (h)	Infection frequency (IF)	AUDPC	Rate of infection (r)		Percent Disease Index (PDI)
			3 DAI	5 DAI	7 DAI						r1	r2	
AC10	*59[a]	17.25	2.1[a]	2.33[a]	2.33[a]	2.26	0.32	22	4.67a	403.83[a]	0.48[a]	0.45[a]	61.89[a]
AC4	48[a]	14.08	1.4[b]	1.83[a]	2.17[a]	1.8	0.26	20	5.00a	386.83[a]	0.29[a]	0.10[a]	62.33[a]
AZA17	29[b]	6.50	1.67[b]	1.73[a]	1.77[a]	1.72	0.25	23	1.67c	217.33[b]	0.47[a]	0.38[a]	34.11[b]
AZA2	27[b]	7.92	0.17[c]	0.23[b]	0.8[b]	0.4	0.06	26	4.00b	113.5[c]	0.33[a]	0.26[a]	22.67[c]
Mean	41	11.44	1.34	1.53	1.66	1.77	0.22	23	3.83	280.38	0.396	0.30	45.25
CV	15.3	18.5	16.63	15.4	25.93				19.29	8.83	17.896	18.23	5.03
LSD	13.83	3.99	0.4175	1.2041	0.8628				4.2798	46.617	0.2828	0.4281	4.29
P value	<0.0001	0.0007	<0.0001	0.0077	0.0140				0.0061	<.0001	0.269	0.328	<0.0001

*values followed by different letters within a column are significantly different according to the LSD test (p=0.05)

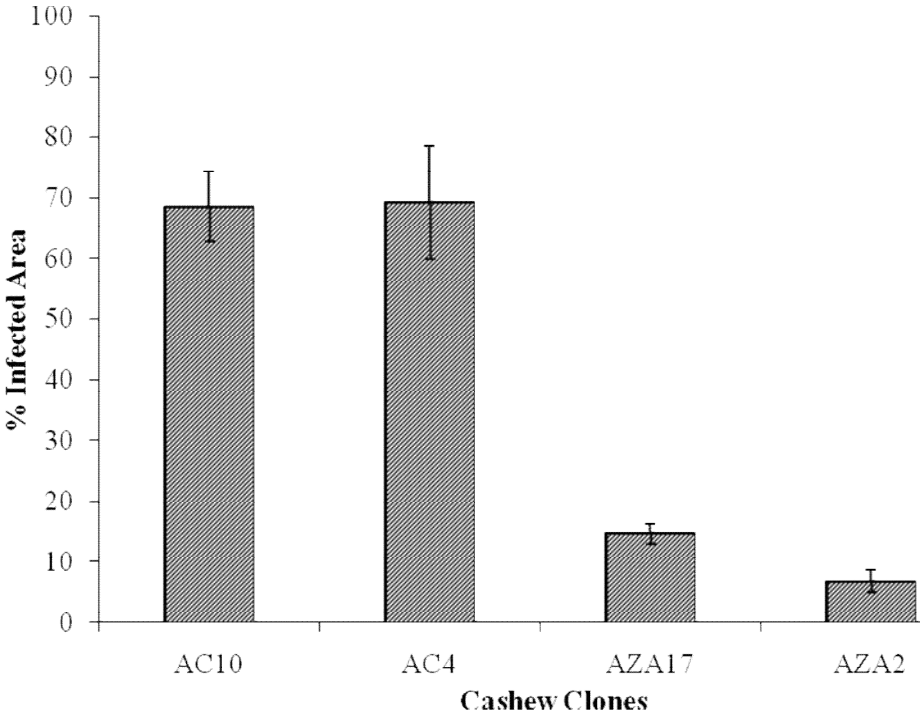

Figure 1: Percentage necrotic area

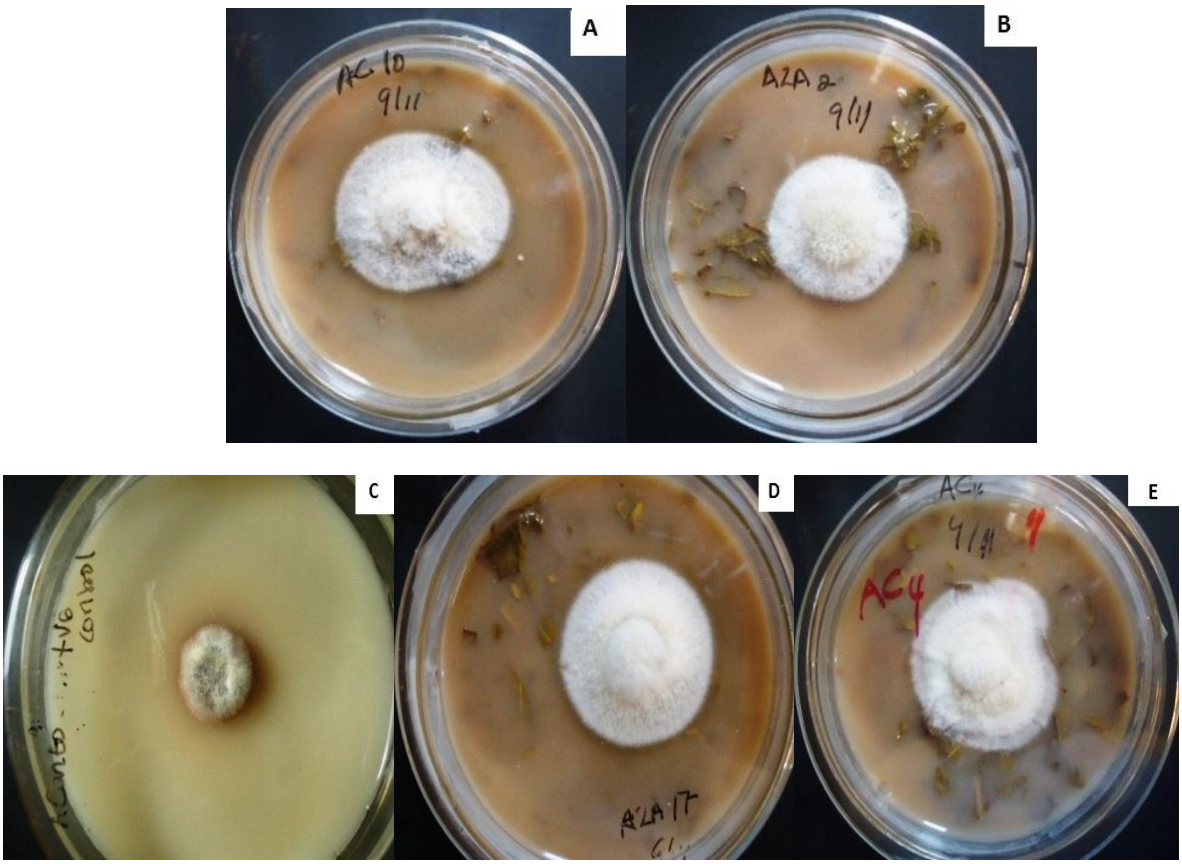

Figure 2: Different levels of resistance exhibited by cashew clones against blight