

# Reaction of Grafted Cashew Genotypes to Powdery Mildew Disease in the Western Province of Zambia

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### ABSTRACT

Cashew powdery mildew disease (CPMD), caused by the fungus *Pseudoidium anarcadii*, has become an economically significant disease of cashew trees in the Western Province of Zambia and many tropical parts of the world. The disease causes significant yield losses of 70 to 100 %. The most effective, sustainable, and environmentally friendly approach to the management of CPMD elsewhere has been through the use of resistant genotypes. Nevertheless, in Zambia, information on the reaction of existing cashew genotypes to CPMD still needs to be discovered. Therefore, this study aimed to evaluate the reaction of cashew genotypes to CPMD in Western Zambia. 90-day-old seedlings from seven cashew genotypes from the ZARI collections were planted and artificially inoculated with cashew powdery mildew spore suspension in a completely randomized design with 15 plant replicates. The experiment was conducted in a screenhouse at Simulumbe Agricultural research station in the Mongu district of Zambia from 20<sup>th</sup> June to 30<sup>th</sup> July 2020. Disease incidence and severity were evaluated weekly. Results indicated high variability in the response of the genotypes to powdery mildew disease (P < 0.05). Genotype Mab/J/T01 was highly tolerant, while genotype Mab/T117 and Itufa/T01 were moderately tolerant, and genotypes KK/T139 and KK/T65 had low tolerance to CPMD. The rest were highly susceptible. The identified tolerant cashew genotypes are recommended for further direct utilization or incorporation into breeding programs to develop resistant varieties against CPMD in Zambia.

Keywords: Powdery Mildew disease, resistance, genotypes, Anarcadium occidentale, Dwarf, giant cashew type

# INTRODUCTION

The Cashew industry has become the primary source of employment for more than 60,000 smallholder farmers in Zambia's Western Province (Chisengele *et al.*, 2022). It is a significant source of household income, nutrition, and employment for the local communities. Cashew is the only tree crop other than Mango that has successfully adapted to the conditions of Western Province since its introduction in the 1950s (Isimwaa, 1993). Despite its acceptability and economic advantages, significant crop damage has been observed worldwide in most cashew plantations and farmer's fields, with qualitative and quantitative losses caused mainly by fungi (Cardoso *et al.*, 2017; Freire *et al.*, 2002).

Cashew powdery mildew caused by *Pseudoidium anarcadii* first reported in Zambia in 1979 (Uaciquete *et al.*, 2013), has recently become a vital cashew disease in Zambia (Chisengele *et al.*, 2022). Powdery mildew in Tanzania has been associated with crop losses ranging from 70 to 100% (Sijaona *et al.*, 2001; Wonni, 2017). The disease affects all young parts of the shoot, including leaves, inflorescences, apples, and nuts, reducing the quality and yield of cashew apples and nuts (Lima *et al.*, 2019; Pinto *et al.*, 2018). The disease is effectively controlled with sulfur powder applications (Martin *et al.*, 1997; Nathaniels *et al.*, 2003; Swart, 2004). However, chemical control is associated with legal, practical, and environmental problems, including the need for legislative approval by environmental agencies, application equipment acquisition, and labour costs (Pinto *et al.*, 2018). Therefore, genetic resistance is a perfect option for long-term economic and environmental strategies to control powdery mildew disease (Pinto *et al.*, 2018). Early studies have suggested the potential use of resistance genotypes for integration into disease management (Nathaniels *et al.*, 1996; Waller *et al.*, 2008). The main



constraints of disease evaluation methods include the irregularity in budding time among different genotypes and differences in environmental conditions at the plant's vulnerability to infection, which makes comparisons among cashew genotypes subject to errors (Freire *et al.*, 2002; Pinto *et al.*, 2018). In addition, studies by Sijaona *et al.* (2001) revealed that powdery mildew disease developed on inoculated young leaves 48 h after inoculation; however, mature leaves were immune to infection. Despite these limitations, several methods for assessing the reaction of cashew genotypes to CPMD under laboratory and field conditions have been studied (Nathaniels, 1996; Sijaona *et al.*, 2001). These include pathogen inoculation on detached leaves, disease development on plants and flower comparison, and the infection of seedlings also proved successful under field conditions (Sijaona *et al.*, 2001). Currently, more than 80 cashew genotypes are available for growers in Zambia, but no scientific report is available on their reaction to CPMD. As a result, hardly any commercial cultivars have been identified that are either tolerant or resistant to the disease. Therefore, this study's objective was to evaluate seven cashew nut genotypes for resistance against powdery mildew disease in the Western Province of Zambia, thereby selecting promising genotypes for inclusion in crop improvement programs for disease control.

# MATERIALS AND METHODS

#### **Preparation of Plant Materials**

Cashew Seedlings raised to 50 cm in height were softwood grafted using scions from seven cashew genotypes pre-selected by Zambia Agricultural Research Institute (ZARI) in 2018 following the Nursery Operators' guidelines manual (Chisengele *et al.*, 2022) at Simulumbe Research Station, ZARI in Western province. The seedlings were planted in the greenhouse and cared for routinely, and fifteen (15) seedlings per genotype with similar height and girth and without visible attacks of any disease and pest were selected when they had grown to 50 cm tall.

#### **Preparation of Inoculum**

Diseased plant leaves were collected from farmers' fields in Nalolo, Mongu districts, and Simulumbe Research Station. First, the conidia were dislodged from infected leaves by brushing fungal materials into water containing 20% tween. Then, using a haemocytometer, the conidial suspension was adjusted to a concentration of  $1 \times 10^5$  conidia/ml (Rizhsky *et al.*, 2002).

#### **Inoculation and Disease Evaluation**

Inoculation was done by spraying the spore suspension onto 90-day-old seedlings arranged in a completely randomized design with fifteen (15) replications in a greenhouse. The control consisted of uninoculated cashew seedlings to monitor the eventual development of the disease from natural infection and was replicated 15 times. After inoculation, the greenhouse was maintained at  $27 \pm 2^{0}$ C and a relative humidity of 80 to 90 %. Disease severity was done according to Nathaniels (1990). Briefly, the number and size of lesions on the leaves were rated on a five-scale rapid disease screening technique, with a score of one implying powdery mildew fungus covering less than 5% of the leaf and a score of five standing for powdery mildew fungus covering more than 35% of the leaf. The lesions were scaled based on their size.

Infection scale	Infection Index
0	No lesions visually detectable
1	Powdery mildew fungus covering less than 5% of the leaf
2	Powdery mildew fungus covering 5- 15% of the leaf
3	Powdery mildew fungus covering 15 - 25% of the leaf

Table 1. A five-scale rapid disease screening technique by (Nathaniels & Kennedy, 1996) Nathaniels (1990)



4	Powdery mildew fungus covering 25 - 35% of the leaf
5	Powdery mildew fungus covering more than 35% of the leaf

The Infection index was calculated using the formula

DSI (%) = [sum (class frequency × score of rating class)]/[(total number of plants) × (maximal disease index)] × 100

Where DSI =Infection index; xi = Infection scale; ni = number of leaves suffering corresponding infection scale; I = Different scales of infection; N = Total number of leaves under observation and Severity values $\sum(yi+yi+1)/2$  x dti, where yi+yi+1 are the values observed in two consecutive evaluations and the dti is the interval between the evaluations. Nathaniel's scale is associated with genotype degree susceptibility as follows:

Infection scale	Tolerance Scale (%)
1	Not tolerant (infection scale $\geq$ 35)
3	Low tolerant ( $25 \le$ infection scale < $35$ )
5	Moderately tolerant ( $15 \le$ infection scale $< 25$ )
7	Highly tolerant ( $5 \le$ infection index < 15)
9	Very highly tolerant (infection index < 5)

#### Data Analysis

Data were processed using the GenStat 18th Edition statistical package. The ANOVA procedure was used to compare the genotype reaction to the disease, and means were compared by Fischer's Least Significant Difference (LSD<sub>0.05</sub>) (Gomez & Gomez, 1984).

### RESULTS

#### The reaction of cashew genotypes to powdery mildew disease

Un-inoculated plants showed no symptoms of disease development. Therefore, the assessed disease development resulted from the inoculation effected in this trial. There were significant differences (P < 0.001) in the response of the seven cashew genotypes (Table 3) studied to powdery mildew disease. One genotype was highly tolerant, two (2) were moderately tolerant, two had low tolerance, and the other two were highly susceptible to powdery mildew disease (Table 4.0). The most tolerant genotype was **Mab/ptn/ Jumbo** (9.78%), while **KK/Kas/T136**, with a PMD severity of (42.47%) (Table 4.0), was the most susceptible.

Table 3. Analysis of the variation table showing the reaction of the seven cashew genotypes seedlings to powdery mildew disease

Source of variation	d.f	S.S	m.s	v.r	F pr
Genotype	6	12619.15	2103.19***	120.79	<.001
Error	98	1706.39	17.41		
Total	104	14325.54			



\*\*\*Data significant at P=0.05, PMD-Powdery mildew incidence severity; sum of squares (ss); mean square (ms).

S/N	Genotype	Tree type	DSI	Resistance Criteria
1	Mab/ptn/ Jumbo	Dwarf	9.78 <sup>a</sup>	Highly Tolerant
2	Mab/ptn/ T117	Dwarf	19.19 <sup>b</sup>	Moderately Tolerant
3	Itufa - 01	Giant	20.74 <sup>b</sup>	Moderately Tolerant
4	MS/K/KK/T139	Dwarf	32.83 <sup>c</sup>	Low Tolerance
5	MS/K/KK/T65	Dwarf	33.47 <sup>c</sup>	Low Tolerance
6	Itufa-T05	Giant	38.80 <sup>d</sup>	Susceptible
7	KK/Kas/T136	Giant	42.47 <sup>e</sup>	Very Susceptible

Table 4. Mean CPMD Severity among cashew genotype

Means with the same letter (s) in the same column are not significantly different following Boniferroni Test ( $P \le 0.05$ ). DSI-Disease Severity Index Means.

### DISCUSSION

In this study, seven (7) selected cashew genotypes planted at the study sites between 1985 and 2014 (Chisengele *et al.*, 2022; Eijnatten *et al.*, 1984; Isimwaa, 1993) were assessed for CPMD incidence and severity. The results showed that none of the genotypes exhibited complete resistance to CPMD, but the tolerance levels differed. This agrees with early publications involving different cashew genotypes (Sijaona *et al.*, 2001; Sijaona & Mansfield, 2001). Thus, one of the genotypes was very tolerant, two moderately tolerant, two low tolerance to CPMD, and the other very susceptible. Genotypic variations in cashew tree reactions to CPMD were previously reported by Faenza *et al.* (1982) as cited by Majune *et al* (2018) on cashew trees in Tanzania. Similar findings were also reported by (Martin *et al.* 1997; Masawe, 2016; Agboton *et al.*, 2013). The ability of the cashew genotypes to tolerate infection by the CPMD-causing fungi has been attributed to their inherent genetic tolerance (Sijaona & Mansfield, 2001). All the genotypes evaluated are of foreign origin and were introduced in Zambia between 1988 and 1990 for the common giants and 1990 to 1992 for the dwarf genotypes and were introduced for experimental purposes and multiplication (Chisengele *et al.*, 2022; Eijnatten *et al.*, 1984; Isimwaa, 1993). The dwarf genotypes were imported from Brazil, while the common giants were imported from Mozambique and Tanzania (Chisengele *et al.*, 2022).

In the Zambian collection of elite mother trees, dwarf genotypes were more tolerant to the disease than the common giant trees. Furthermore, these dwarf genotypes are said to have been sourced from Brazil (Chisengele *et al.*, 2022), which agrees with the studies carried out by (Pinto *et al.*, 2018), who evaluated the response of commercial cashew nut cultivars to powdery mildew by monitoring the disease during three disease epidemic cycles. The authors' findings showed partial resistance of cashew cultivars to powdery mildew that was Dwarf (Pinto *et al.*, 2018). According to these authors, some of the imported planting materials had genetic resistance to the disease, and the environment may not have affected their reaction to the disease. Other related studies in Tanzania (Masawe, 1994; Nathaniels, 1990, 1996; Sijaona *et al.*, 2001), Mozambique (Uaciquete, 2013) and Brazil (Lima *et al.*, 2019) reported similar findings. For all these authors, resistant cashew genotypes could be integrated into the disease management programmes of cashews, in screening disease susceptibility trials as check genotypes, and in breeding resistant varieties.

Efforts to address the CPMD by initiating cashew breeding programmes are in preparation in Zambia. The starting step is identifying elite mother trees as planting material and genetic resources for breeding at ZARI



Simulumbe (unpublished). Therefore, the presence of tolerant cashew genotypes in Zambia is an excellent opportunity for a young cashew industry already struggling with powdery mildew disease to immediately commence breeding of even more tolerant varieties and quickly integrate these genotypes into disease control programmes. Currently, CPMD in Zambia is controlled by chemical spraying using hexaconazole (Chisengele *et al.*, 2022). It is not only environmentally unfriendly to natural pollinators but also raises public health concerns, and it is not sustainable for resource-limited cashew farmers (Pinto *et al.*, 2018). As suggested, tolerant genotypes are the most natural and sustainable way of managing CPMD in Zambia (Pinto *et al.*, 2018). In the Western province of Zambia, the cashew industry represents a significant proportion of employment and income for more than 60,000 smallholder farmers (Chisengele *et al.*, 2022). Therefore, proper disease management will safeguard the farmers' livelihoods.

Furthermore, using resistant cashew genotypes could be the most feasible and sustainable approach to managing CPMD and avoiding long-term breeding programmes, as Pinto *et al.* (2018) suggested. Unfortunately, such genotypes were not found in our experimental lot. The Dwarf genotypes and Common Giants are the cashew trees that are in existence in Zambia, but to date, no commercial varieties have been bred. Currently, genetic and phenotypic characterization is being conducted by the Zambia Agricultural Institute (ZARI) to study the genetic diversity of cashew germplasm in Zambia.

This is the first scientific study on the response of cashew genotypes to powdery mildew disease in Zambia. The results presented here provide valuable information for cashew growers, policymakers, and researchers involved in breeding cashew plants and studying the response to the most severe cashew disease nationwide. Given the widespread CPMD epidemic in Zambia, this investigation contributes to further studies that will elucidate the genetic basis of resistance. The results also indicate a highly diverse cashew population in response to the CPMD, indicating the polygenic nature of resistance (Ponomareva *et al.*, 2022), which is an excellent source of genes for a successful breeding programme.

# CONCLUSION AND RECOMMENDATIONS

The present study has identified five out of seven cashew genotypes exhibiting varying levels of tolerance to the disease, while the rest were very susceptible. Of the five (5) tolerant genotypes, *Mab/ptn/Jumbo*, *Mab/ptn/T117*, *Itufa-01*, *MS/K/KK/T139* and *MS/K/KK/T65* are recommended for immediate integration into national breeding programmes and distribution to the cashew farmers in the province. In the Zambian collection of genotypes context, the number of dwarfs tolerant to CPMD was generally higher than that of the common giants. Therefore, it can be stated that the likelihood of finding tolerant genotypes is higher among dwarfs compared to common cashew populations. The presence of tolerant cashew genotypes in Zambia is an excellent opportunity for a young cashew industry already struggling with powdery mildew disease to immediately commence breeding of even more tolerant varieties and integrate these genotypes into disease control programmes. In the current study, none of the genotypes showed complete resistance, which is known to be fragile under high disease pressure due to its single gene dependence.

It is recommended that a study should investigate the reaction of other cashew populations under Zambian conditions.

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