

Preliminary experiments on potential use of blue monochromatic light for identification of cashew diseases

Ensaio preliminar sobre o potencial uso da luz azul monocromática para a identificação das doenças do cajueiro

Ensayos preliminares sobre el uso potencial de la luz azul monocromática para la identificación de las enfermedades en plantas del marañón

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Abstract

Cashew is a valuable crop for the world due to its nutritional, environmental, and economic qualities. However, it is challenged by abiotic and biotic constraints such as diseases that cause significant yield losses. Various disease diagnostic and management strategies have been developed over time. But their adequacy is bound to a complex of advantages and disadvantages depending on host/pathogen relationships. Thus, the objective of this article was to describe a new approach that distinguishes cashew diseases by light at 405 nm. Symptomatic tissues were, under asepsis, prepared for pathogen culturing and morpho-cultural identification of the pathogen(s) associated. By inoculation and incubation of pathogens, disease symptoms were reproduced on cashew seedlings. Later, symptomatic tissues were then cut macerated in a 1/1 (weight/water) extraction and allowed to precipitate at 4 °C. The supernatant was submitted to absorbance reading in a monochromatic beam of 405 nm. Sole extraction water and sole healthy tissue extracts were also considered. Three experiments with different treatments were conducted. Absorbance data were then processed statistically following a completely randomized design in which treatments consisted of individual plant/pathogen(s) extracts. The biotrophic pathogen *O. anacardii* resulted in the highest absorbance. However, it reduced in association with the necrotrophic pathogen *Cryptosporopsis* spp. No significant differences were discovered between the two necrotrophic pathogens *Cryptosporopsis* spp. and *Septoria* spp. The method demonstrated high potential for diagnosis of cashew diseases. Future research must focus on adjustable wavelength spectrophotometry and standardization. The method can be recommended for direct identification of field disease samples.

Keywords: Cashew diseases; Spectrophotometry; Absorbance diagnosis; Anacardium.

Resumo

O caju é uma cultura internacionalmente valiosa. No entanto tem sido desafiada por fatores abióticos e bióticos tal como as doenças que limitam os seus rendimentos. Para as doenças, diversas estratégias de diagnóstico e controlo vêm sendo desenvolvidas. Todavia, a sua adoção está ligada a um complexo de vantagens e desvantagens interconectada à complexidade da relação patógeno/hospedeiro. Assim, este artigo teve como objetivo descrever uma nova abordagem para distinguir doenças do cajueiro através do uso da luz monocromática de 405 nm. Tecidos sintomáticos foram preparados sob assepsia para o cultivo e identificação morfo-cultural do(s) patógeno(s) associado(s). Por inoculação e incubação dos patógenos, os sintomas das doenças foram reproduzidos em mudas de cajueiro. Destas, tecidos sintomáticos foram cortados e triturados em extrato de 1/1 (peso/água) e deixados a precipitar a 4°C. Ao sobrenadante fez-se leitura da absorvência à 405 nm em um espectrofotômetro. Extratos de plantas sãs e de água de extração foram igualmente consideradas. Conduziu-se três ensaios com diferentes tratamentos. valores da absorvência foram estatisticamente processados seguindo o desenho experimental com tratamentos completamente casualizados. Tratamentos consistiram em extratos de cada associação planta/patógeno(s).

O *O. anacardia* gerou a mais alta absorvância, porém reduzida pela associação de *Cryptosporipsis* spp. na muda. Não foram detectadas diferenças significativas na absorvância de *Cryptosporipsis* spp. e *Septoria* spp. Portanto, o método da absorvância demonstrou alto potencial para o diagnóstico de doenças do cajueiro. Investigação futura de incluir espectrometria de leitura contínua e padronizada. O método é recomendado para uso direto em amostras de campo.

Palavras-chave: Doenças do cajueiro; Espectrofotometria; Absorvância; Diagnóstico; Anacardium.

Resumen

El marañón es un cultivo de interés internacional. Sin embargo, los factores abióticos y bióticos como las enfermedades afectan los rendimientos. Para las enfermedades, se han desarrollado estrategias de diagnóstico y control, sin embargo y su adopción tiene ventajas y desventajas interconectadas a la relación patógeno/hospedero. Por lo tanto, este artículo tenía el objetivo de describir un nuevo método para distinguir las enfermedades del marañón utilizando luz monocromática de 405 nm. Tejidos sintomáticos se prepararon bajo asepsia para cultivo e identificación morfo-cultural de patógeno(s) asociado(s). Mediante la inoculación e incubación de patógenos, los síntomas de la enfermedad se reprodujeron en plántulas del marañón. En estas, tejidos sintomáticos se cortaron y trituraron en extracto 1/1 (peso/agua) y se dejaron precipitar a 4 °C. La absorbancia del sobrenadante se leyó a 405 nm en un espectrofotómetro. También se consideraron extractos de plantas sanas y agua de extracción. Se realizaron tres ensayos con diferentes tratamientos. Los valores de los tratamientos de absorbancia se procesaron estadísticamente utilizando el diseño completamente al azar. Los tratamientos consistieron en extractos de cada asociación de planta/patógeno(s). El *O. anacardia* generó la mayor absorbancia, pero ésta se redujo por la asociación de *Cryptosporipsis* spp., en la plántula. No se detectaron diferencias significativas en la absorbancia de *Cryptosporipsis* spp. y *Septoria* spp. Por lo tanto, el método de absorbancia demostró un alto potencial para diagnosticar enfermedades en plantas del marañón. Las investigaciones futuras deberían incluir espectrofotometría de lectura adecuada y estandarizada para uso directo en muestras del campo.

Palabras clave: Enfermedades del marañón; Espectrofotometria; Absorbância; Diagnóstico; Anacardium.

1. Introduction

Cashew (*Anacardium occidentale* L.) is a member of the Anacardiaceae botanical family and known to have dispersed from Brazil (Khatoon et al., 2017). Today, it is cultivated in 32 countries worldwide (Wonni et al., 2017) as result of its economic, environmental, and nutritional value (Manjusha et al., 2023). Cashews are rich in phenols, healthy oil and sugars required in food industry and its apple contains juice that can be transformed into wine, syrup, or beer (Patrice et al., 2021). The crop is challenged by many biotic and abiotic constraints that cause significant yield losses (Freire et al., 2002; Wonni et al., 2017) among the prevalent disease such as cashew powdery mildew (*Oidium anacardii* Noack), anthracnose (*Colletotrichum gloeosporioides* Penz.), dieback (*Phomopsis anacardii* Punithalingam), pestalotia leaf spot (*Pestalotia* spp.), damping off disease (*Fasarium* spp.; *Pythium* spp.; *Phytophthora palmivora* Butler), cashew leaf and nut blight disease (*Cryptosporipsis* spp) and wilt disease (*Fusarium oxysporium*, complex) are the most common in the Southern Africa region (Majune et al., 2018; Patrice et al., 2021; Udayanga et al., 2011).

Disease diagnoses requires both host symptoms, pathogens' signs, morpho cultural and more recently molecular studies (Monteiro et al., 2022). This becomes more complicated when, in nature and more frequently, the exhibited cashew symptoms reflect more than a single pathogen infection. For instance, dieback has been found associated with *Colletotrichum* spp. and *Lasiodiplodia* spp.; from leaf blight symptoms, *Pestalotia heterocornis* and *C. gloeosporioides* can be isolated, in drying buds, *C. gloeosporioides*, *Phomopsis anacardii* and *Curvularia lunata*, have been found together and many other complexities (Khatoon et al., 2017; Majune et al., 2018; Dooh et al., 2021; Udayanga et al., 2011).

With the above reality in mind, a quick, reliable, and un-expensive diagnoses system that leads to appropriate control strategy is required on cashew. On the other hand, fungi have been found to perceive near-ultraviolet, blue, green, red and far-red light using specific photoreceptors and signaling mechanisms to control a large proportion of its morphogenetic pathways and thereby photobiologically adapt to environmental conditions (Yu & Fischer, 2018). The objective of this article was to describe a new approach that distinguishes cashew diseases through the use of light at 405 nm as key to disease diagnoses in cashew patho-systems.

2. Methodology

From symptomatic samples, cashew pathogens were isolated and inoculated on cashew seedlings. By cutting the symptomatic sections and macerate them with double distilled water in 1/1 (w/v) ration, liquid extracts were obtained and later used for absorbance readings. Finally, the absorbance readings were used to distinguish cashew/patho systems as per the experimental treatments and statistical analysis.

Trial site

The experiment took place at Northeast Zonal Center of the Agriculture National Research Institute of Mozambique, IAM-NE, in Nampula province. The climate of area is tropical humid with two seasons: hot and rainy between November and April and old and dry between May and October (MAE, 2012). Maximum temperature is 33.9 °C and mean annual rainfall is 1.045 mm (FAO, 2005). Nampula is main cashew region of Mozambique and contributes 60 to 70% to the national commercial cashew production.

Isolation of pathogens

From cashew leaf, mummified fruits, flowers and twigs, the following pathogens were isolated under aseptic and morho-culturally identified (CAB International, 1996) in Petri dishes plated SDA (Sabouraud Dextrose Agar) or PDA (Potato Dextrose Agar).

The isolates were further confirmed by reproductive structures observation under the microscope at 10x; 40x e 100x magnifications as necessary and visually compared previously published pictorial keys (Menge & Shomari, 2016; Udayanga et al., 2011; CABI, 1983; Nakapalo et al., 2017; Wonni et al., 2017). For the *O. anacardii*, being obligate parasite (Muntala et al., 2021) no cultures were made. Disease signs and symptoms (Majune et al., 2018, Nene et al., 2020; Freire et al., 2002) were satisfactory for identification purpose. The following necrotrophic pathogens were identified: *Cryptosporiopsis* spp.; *Colletotrichum gloeosporioides*; *Septoria* spp. and *Pestalotia* spp. (CAB International., 1996; Rodrigues et al., 2019; Sijaona et al., 2006).

Production of seedlings.

Pots were sowed with single seed from cultivar 11.8PA obtained from Nassuruma cashew research center. A total of 150 seedlings were produced and cared as described by Abanum et al., (2022) and Masawe et al., (2020). Seedlings were considered mature for inoculation at 3-4 reddish leaves stage.

Experimental design

Three consecutive experiments following a completely randomized experimental design with 4 to 5 treatments in 4 replicates each were conducted (Gomez & Gomez, 1984). Treatments varied according to individual experiments as indicated in Table 1. A total of six seedlings were considered for each treatment.

Before laying out the experiment, seedlings were inoculated by placing 3 discs of the culture on individual apical leaves and 2 on the upper stem terminal for the necrotrophic pathogens (*Colletotrichum*, *Cryptosporiopsis*, *Septoria* e *Pestalotia*). A uniform spore suspension was sprayed on seedlings for the biotrophic *Oidium anacardii*. Twenty-nine to 30 days post inoculation and incubation, symptomatic edges of the inoculation sites, were cut from various tissues infected with the same pathogen and weighed up to 5 grams for each patho-system. This material was then mortar grinded with addition of 5 ml of double distilled water. Disease free tissues was also extracted to compose the negative control. Both extracts were

separately released into plastic tubes and vertically preserved in a freezer for 24 hours at 4 °C for sedimentation. The liquid supernatant portion was sucked through a lab aseptic syringe into Nunc vials.

Table 1 - Treatment codes and respective description per experiment.

Treatment Code	Experiment		
	First	Second	Third
	Description	Description	Description
1	Distilled water	Distilled water	Healthy leaves
2	Healthy leaves	Healthy leaves	<i>Oidium anacardii</i>
3	<i>Oidium anacardii</i>	<i>Oidium anacardii</i>	<i>Septoria</i> spp.
4	<i>Cryptosporiopsis</i> spp	<i>Cryptosporiopsis</i> spp.	<i>Cryptosporiopsis</i> spp.
5		<i>Oidium anacardii</i> * <i>Cryptosporiopsis</i>	

* = Simultaneous infection. Source: Authors (2024).

Absorbance reads

The reads of absorbance were taken in a spectrophotometer as described by Soares, (2008) using a monochromatic beam of blue light of 405 nm through the Nunc plates containing the supernatant extract from each patho-system.

Statistical data analysis

An excel table was built with individual treatment reads of absorbance per trial and replication. Definite disease associated reads were obtained by subtracting the reads of sole double distilled water (negative control), from individual sample reads. Definite absorbance reads were further processed in SAS package for ANOVA and Tukey's test at 5% significance for treatment means separation (Gomez & Gomez, 1984).

3. Results

Three experiments in which pathogen/host systems were created through inoculation and incubation. On symptoms developed from each pathogen/host system, samples were taken for trituration and water based supernatant extraction. Supernatant blue light absorbance means values, with deduction of water extraction values, were used to explore pathogen/host specificity and the results are presented in Table 2.

Table 2 - Mean absorbance comparison of cashew extract from different patho-system symptoms in three experiments conducted at IIAM-NE zonal center, Nampula, Mozambique.

Experiment	Treatment	CV	ANOVA Pr>f	(Abs-wa) Mean
First		10.6327	<0.0001	
	<i>Oidium anacardia</i>			2.26100 ^d
	<i>Cryptosporiopsis</i> spp.			0.86750 ^c
	Healthy leaves			0.3675 ^a
	Distilled water			0.000 ^b
Second		2.56778	<0.0001	
	Distilled water			0.000 ^d
	Healthy leaves			0.92125 ^c
	<i>Oidium anacardia</i>			2.7000 ^a
	<i>Cryptosporiopsis</i> spp.			1.90400 ^b
	<i>O. anacardii</i> * <i>Cryptosporiopsis</i>			1.08050 ^b
Third		8.2183	<0.0001	
	Healthy leaves			1.92650 ^b
	<i>Oidium anacardia</i>			2.25025 ^a
	<i>Septoria</i> spp.			1.44725 ^c
	<i>Cryptosporiopsis</i> spp.			1.52600 ^c

* = Simultaneous infection, CV=Coefficient of variance, Abs= Absorbance, wa=water absorbance. Means followed by the same letter in each experiment, were not statistically different by Tukey's test at 5% significance. Source: Authors (2024).

Analysis of variance (ANOVA) from the three trials confirmed the existence highly significant differences in water extract absorbance (Pr>f, 0.0001) means as per the symptoms induced by single or multiple pathogen infection (Table 2). The coefficient of variance varied according to each experiment, from 2.6 to 8.2 and 10.6% respectively in second, third and first experiment.

In the first experiment, the extract's absorbance of healthy leaves was significantly lower than that from infected tissues. The *O. anacardii* infected tissue extract had significantly higher absorbance compared to *Cryptosporiopsis* spp. (Table 2).

In the second experiment, extract from healthy leaves, again had the lowest absorbance in relation to that from infected tissues (Table 2). This is contrary to *O. anacardii* that induced the highest significant extract absorbance. But the effect of *O. anacardii*, was reduced by interaction with *Cryptosporiopsis* spp. which induced relatively lower absorption of light at 405 nm and thus no significant differences detected between this later and its interactions effect with *O. anacardii* (Table 2).

From the third experiment, *O. anacardii* infection has shown consistence in inducing the highest extract absorbance in relation to other patho-systems (Table 2). The absorbance induced by *Cryptosporiopsis* spp. and that of *Septoria* spp., was not statistically significant. Contrary to experiments 1 and 2, where the effect of *Cryptosporiopsis* spp. was significantly higher than that of healthy tissues. In experiment 3, the absorbance from healthy tissues extract was higher (Table 2). This reflects relative inconsistency comparison of healthy tissues versus *Cryptosporiopsis* spp. infected tissues. *Septoria* spp. and *Cryptosporiopsis* spp. infected tissue extract absorbance could not be statistically distinguished in this experiment (Table 2).

4. Discussion and Conclusions

Significant discrepancy concerning the causal agents of cashew diseases is common due to lack of proper species identification through morpho-cultural and molecular approaches (Monteiro et al., 2022). In this set of experiments a beam of blue light at 405 nm was successfully used to distinguish some cashew diseases. Absorption of visible blue light by microbes is well known. Its molecular mechanism consists of photoexcitation of either endogenous or exogenous photosensitizers such as porphyrins and flavins in the biological medium or microbe's vicinity (Haridas & Atreya, 2022). Likewise, un-infected healthy cashew tissues supernatant extracts were also found absorbing light, confirming on one hand, the basic knowledge about photosynthetic harvest of light by plants in general (Beattie et al., 2018).

Un-infected, healthy tissues' absorbance was statistically different from the infected ones. Either higher in two experiments or lower than disease symptomatic tissues. This suggests an interference of the pathogen on the tissue extract absorbance. Indeed, this finding is in line with the previous studies indicating that pathogens possess specific array of photoreceptors that modulate their gene expression and resulting in different plant/pathogen systems' responses to light (Assefa & Gobena, 2019).

Sensitivity of certain species toward specific wavelengths seems different (Yu and Fischer, 2018). This could explain the observed differences absorbance between different plant/pathogen systems and the lack of distinction among the investigated necrotrophic diseases. This observation suggests that changes in wavelength range of visible blue light (400-470 nm) could give better resolution into distinguishing cashew pathogens. Therefore, oncoming experiments must address wavelength adjustments for different patho-system to ensure case specific resolution. The hypothesis is soundly and further supported by the fact that patho-systems are known to express differences in integrated plant phytochromes and fungi LOV blue light sensing proteins (Beattie et al., 2018).

The biotrophic fungus (*O. anacardii*) (Muntala et al., 2021) plant/pathosystem was found to induce significantly higher absorption of blue light than the necrotrophic fungi (Dominic et al., 2014; Martins et al., 2018) *Septoria* spp. and *Cryptosporiopsis* spp, in all three experiments. This supports the observations that light-sensitive proteins seem to harmonize infectivity and virulence with the defense mechanism of the plant host (Losi & Gartner, 2021).

In addition, the current study demonstrated that absorbance of the biotrophic fungus plant/pathosystem (*O. anacardii*) was reduced upon interaction with the necrotrophic *Cryptosporiopsis* spp. This suggests the pathogen/host stability (Losi & Gartner, 2021) can be disturbed by the presence of a necrotic pathogen causing a disruption of photo-responsive systems.

Numerous methods for plant disease detection have been reported. The adoption of one or another depends on various factors including cost, specificity, purpose, spatial coverage, simplicity for use, etc. (Martinelli et al., 2015 and Balodi et al., 2017). At the current experimental wavelength however, the necrotrophic pathogens, *Septoria* spp. and *Cryptosporiopsis* spp. could not be differentiable from one another (Table 2, third experiment). Therefore, monochromatic blue light diagnosis is proposed as an option. Further research with changes in the light wavelength through a multiple range spectrophotometer is recommended. This becomes a need because in cashew/pathogens systems, more than 12 diseases (Wonni et al., 2017; Patrice et al., 2021) are involved and most of which are necrotrophic, producing closely related symptoms on fruits, twigs, and leaves.

The potential success, suitability, and accuracy of diagnosis in cashew diseases will depend also on wavelength, cultivar, host tissue involved and probably environmental factors as well. Thus, future research must focus on standardization of wavelength for different patho-system specificity, looking at potential development of portable and on-field direct absorbance readers.

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