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Research Application Summary

Screening of selected cassava genotypes for resistance to Cassava Brown Streak Disease by mechanical grafting in Southern Mozambique

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Abstract

Cassava brown streak disease (CBSD) is a major biotic stress of cassava in Southern, Eastern and Central Africa. It is caused by two virus species; the Ugandan cassava brown streak virus (UCBSV) and the cassava brown streak virus (CBSV). The CBSV is the most aggressive virus of the two. The disease can effectively be managed by exploiting host plant resistance through identification of tolerant and/or resistant genotypes. The aim of this study was to screen and identify genotypes resistant to CBSD among selected cassava lines as well as to determine the effect of CBSD on cooking time and dry matter content. Ten CBSV graft-inoculated genotypes were grown in a randomized complete design with four replicates. Data were collected from two weeks after inoculation up to nine months. Results indicated that there was no immune genotype. Genotypes exhibited different forms of response ranging from dilatory, tolerance, discriminatory and true resistance. Foliar and root severity varied significantly among genotypes ($P<0.05$). A weak positive correlation ($r=0.0593$ $P>0.05$) between foliar and root severity was observed. The CBSD did not have a significant effect on Dry matter content (DMC) but significantly affected cooking time with a point increment in root severity increasing cooking time by 6.4 minutes. Genotypes Amarelinha, Cucci, Munhaca, Mukalane, Timbilu and Fpo were classified under resistant category thus should be considered as breeding stock for CBSD resistance given that they displayed different levels of resistance to CBSV.

Key words: Cassava brown streak disease, cooking time, disease resistance, dry matter content, Mozambique

Résumé

La maladie de la striure brune du manioc est un stress biotique majeur du manioc en Afrique australe, orientale et centrale. Elle est causée par deux virus; le virus de la striure brune du manioc ougandais et le virus de la striure brune du manioc. Le dernier est le virus le plus agressif. La pathologie peut être efficacement gérée en exploitant la résistance de la plante hôte à travers l'identification de génotypes tolérants et/ou résistants. Le but de cette étude

était d'explorer et d'identifier des génotypes résistants à la pathologie à partir des lignées sélectionnées de manioc ainsi que de déterminer l'effet de la pathologie sur le temps de cuisson et la teneur en matière sèche. Dix génotypes de virus de la striure brune du manioc inoculés par greffe ont été cultivés suivant un dispositif aléatoire complet avec quatre répétitions. Les données ont été collectées deux semaines après inoculation pendant neuf mois. Les résultats ont indiqué qu'il n'y avait pas de génotype immunitaire. Les génotypes ont montré différentes formes de réponse. La sévérité foliaire et racinaire variaient significativement entre les génotypes ($P < 0,05$). Il y avait une faible corrélation positive ($r = 0,0593$ $P > 0,05$) entre sévérité foliaire et racinaire. Le virus de la striure brune du manioc n'a pas eu d'effet significatif sur la teneur en matière sèche, mais a significativement influencé le temps de cuisson avec une augmentation de la sévérité racinaire augmentant le temps de cuisson de 6,4 minutes. Les génotypes Amarelinha, Cucci, Munhaca, Mukalane, Timbilu et Fpo ont été classés dans la catégorie résistante et devraient donc être considérés pour la résistance au virus de la striure brune du manioc.

Mots clés: Pathologie de la striure brune du manioc, temps de cuisson, résistance aux maladies, teneur en matière sèche, Mozambique

Introduction

Cassava (*Manihot esculenta*) is a perennial shrub that belongs to the family *Euphorbiaceae* and genus *Manihot*. The crop is a staple food crop for most developing countries including but not limited to; Nigeria, Democratic Republic of Congo, Angola, Ghana, Mozambique, Uganda and Rwanda (FAOSTAT, 2014). In Mozambique, cassava is the largest produced food crop in the country with an estimated annual production of seven million tonnes (FAOSTAT, 2014). It is also the number one food security crop of the country. However, cassava production has been affected by Cassava brown streak disease (CBSD) leading to tremendous yield gap (IIAM, 2006). On average farmers harvest 9.5MT (MIC, 2007), as opposed potential yield (18.1-22.7 MT ha^{-1}) that is achieved for the same variety under research stations (IIAM, 2006). The disease has been endemic in Northern part of the country but its recent identification in the South possesses a great threat to the cassava industry in the whole country. Despite the challenges, cassava production in Mozambique needs to be increased to meet the food needs of the growing population. In order to further increase cassava production in Mozambique, tolerant genotypes to the new disease need to be identified and improved for yield and market attributes.

Literature review

The CBSD is a viral disease caused by two virus species namely the cassava brown streak virus (CBSV) and the Ugandan cassava brown streak disease (UCBSV). It is transmitted mainly through infected cuttings and vectors specifically *Bemisia tabaci* of the family Aleyrodidae (Maruthi *et al.,* 2005). The CBSD is regarded as the most devastating disease of cassava (Donald, 2010). This is due to necrosis that forms on the roots which renders them unsalable or for food use. Worse still, tubers can be affected as early as five months after planting (Alicai, 2007). This leaves farmers with no choice other than harvesting them

early before symptom accumulation. This is a threat to food security in countries like Mozambique where cassava is the main food security crop (Donovan and Tostao, 2010).

The disease has been endemic in the Northern part of Mozambique, but recently in 2015, the national virology program reported the disease in Inhambane province (Southern Mozambique). There is also growing fear that due to the monocyclic spread nature of the disease, it will not take long before the disease spreads to other parts of the country if management practices are not put in place. Currently, identification of tolerant genotypes is the main recommended strategy for managing the disease (Legg and Hillocks, 2002). Such genotypes are identified at early stages of the disease epidemic and disseminated to affected communities. Indeed early identification of tolerant genotypes in the Northern endemic area has reduced disease incidence in Nampula although the situation is still alarming in Zambezia and Cabo Delgado. In Zambezia farmers do not accept total loss of such infected tubers; they cut out the necrotic part and consume the non-necrotic part (Zacarias, personal communication). There are claims that farmers using such tubers may take more time to cook them, in comparison to cooking healthy ones. In this case this would be a waste of fuel and time to already constrained farmer. This claim was investigated in this study under two specific objectives; (i) identify genotypes that may be tolerant to CBSD in Southern Mozambique, and (ii) determine effect of CBSD on cooking time and dry matter content.

Study description

Ten selected genotypes were inoculated with CBSV through mechanical grafting. One well known susceptible-infected variety (Tomo) was used as a scion against all the 15 healthy genotypes that acted as scions. The grafted plants were laid out in a Randomised Complete Design with in a screen house. The treatments for the experiment were (genotype + disease). Each of this treatment was replicated four times within experimental units (Planting pots). For each genotype four healthy plants (grafted) were included as negative controls and also four plants of an infected Tomo (non-grafted) genotype were planted as positive controls. Thus eight plants per genotype were established to give a plant population of 84 (10 genotypes × 8 replicates +4 plants of non -grafted infected Tomo). A Reverse transcription polymerase chain reaction (RT-PCR) was used to confirm successful inoculation and presence of immune genotypes. Data on disease severity were then collected at two months interval starting from 1, 3, 5, 7 and 9 months after planting/inoculation. At nine months, plants were harvested, harvest parameters recorded and cooking tests carried out.

Results and discussion

Results of RT-PCR indicated that no genotype was immune (Fig. 1). This meant that the genotypes were good host receptors for the pathogen to express its virulence. Foliar and root severity varied significantly among genotypes (Table 1). A few genotypes kept mild symptoms both in leaves and roots (Amarelinha, Cucci and Munhaca). Pathogen-host interaction involves host recognition, translation, replication, cell to cell and long distance movements. This suggests that these genotypes poses genes that additively work together to obstruct one or more of these steps involved in virus accumulation. Such genes involved should be identified and used in breeding for resistance.

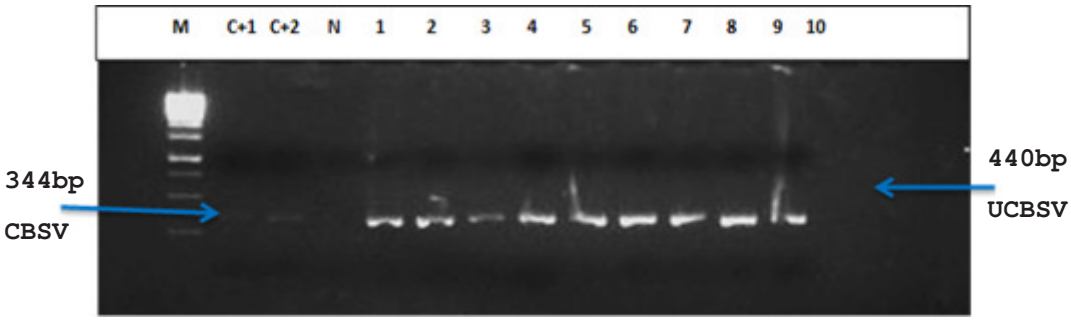

Figure 1. Molecular screening of genotypes by gel electrophoresis at three months after planting. Agarose gel (2 percent), visualized under Gel Doc M = Molecular Marker 1kb; N = negative control; C + 1, C + 2 = positive control. 1, 2, 3,…. 10- are samples genotypes

Table 1. Root severity and harvest parameters assessed at nine months after planting

Genotype	Foliar severity	Root severity	Harvest index	Root weight (kg)	Storage roots	Commercial roots
Diocese	2.88±0.11^{bd}	4.41±0.22^{c}	0.46±0.24	0.64±0.26	22	5
Mukalane	4.42±0.13^{c}	2.06±0.32^{a}	0.13±0.04	0.15±0.08	13	3
Branco	3.33±0.16^{d}	3.67±0.38^{abc}	0.30±0.08	0.23±0.07	9	4
Timbilu	4.75±0.11^{c}	2.33±0.33^{a}	0.39±0.11	0.48±0.09	13	6
Fpo	4.46±0.15^{c}	2.83±0.17^{abc}	0.38±0.05	0.23±0.08	3	3
Chinembwe	1.29±0.09^{a}	2.50±0.29^{ab}	0.20±0.07	0.08±0.02	4	0
Amarelinha	1.71±0.15^{a}	2.07±0.47^{a}	0.27±0.06	0.25±0.06	9	3
T11	2.58±0.15^{b}	3.22±0.40^{abc}	0.24±0.10	0.15±0.08	6	1
Munhaca	2.63±0.15^{b}	2.07±0.64^{a}	0.55±0.08	0.52±0.23	6	4
Cucci	1.38±0.08^{a}	2.17±0.17^{a}	0.28±0.08	0.28±0.04	11	3
P-value	0.0001	0.0003	0.2284	0.0945		
CV	47.5	35.45	60.67	97.32		

CV = Coefficient of variation, Pvalue = Probability values at 95 percent confidence

Genotypes Timbilu and Fpo accumulated the highest foliar disease symptoms throughout the evaluation period but interestingly the two accumulated mild to moderate root symptoms and bulked more commercial roots. This means that plants of these genotypes allow disease accumulation but restrict the effect of the disease on the roots. Genotype Mukalane expressed a unique reaction to infection. It showed early symptom accumulation in all plants but later disease symptoms phased off. According to Politowski and Browning (1978), Mukalane possess dilatory resistance since it reduces the rate of development of initial pathogen population. Mukalane should therefore be used in CBSV breeding program, since it has special attributes which need to be incorporated in the quest for breeding resistant genotypes that can be accepted by farmers.

Cooking time varied significantly between genotypes in infected and healthy state ($P<0.05$). The disease had an effect on time taken to cook a cassava tuber in infected state. Time taken to cook an infected tuber ranged from 6-15 minutes while it took between 4-11 minutes to cook a healthy tuber. This is possibly due to accumulated lignin that reduced starch content in diseased tubers. As earlier reported by Alicai *et al.* (2007), there is high lignin content in diseased tubers, which would mean a low starch content (Nuwamanya, 2014).

Conclusion

Genotypes Amarelinha, Cucci, Munhaca, Mukalane, Timbilu and Fpo were classified as tolerant and thus should be considered as breeding stock for CBSD resistance given that they appeared to possess different genes involved in resisting CBSV and can be distributed to affected communities. Basing on the results it is concluded that resistance in the screened genotypes is controlled by many genes some which limit symptom expression, accumulation and development. Such genes need to be identified as they will be important for breeding resistant varieties. The study results also indicate that households consuming CBSD infected tubers spend more time on cooking than for healthy tubers. The CBSD affected cooking time significantly with a point increment in root severity increasing cooking time by 6.4 minutes. Thus farmers need to be sensitized to use tolerant/resistant cultivars to save the time and fuel consumed in cooking infected tubers.

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