

Infection in Tuber Head, Middle and Tail by *Rhizopus Stolonifer* (Ehrenb.) Lind in Relation to Calcium Content in *Dioscorea Rotundata* Poir.

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Abstract

Calcium content in tuber head, middle, tail region in four white guinea yam varieties (*Dioscorea rotundata*) was investigated in this study in relation to infection by *Rhizopus stolonifer*. This is informed by the observed higher sprouting/germination of “head” yam setts than the middle or tail. The experimental design was 4 by 3 factorial in randomized complete block arrangement of head, middle, tail per block with three replications, and variety as main plot treatment. After 4 weeks incubation, varietal mean infection of 1.32%, 1.45% and 2.74%, for varieties Oniyere, Iseosi, Efuru respectively was significantly lower than the 11.1% in variety Dakaa. There were no significant differences in infection of head (4.24%), middle (2.9%) or tail (5.33%) region, but middle region infection of 0.92% was lower in variety Oniyere and Efuru. Mean tuber region calcium content in mg/100g dry matter were not significantly different in variety Oniyere (23.33), Iseosi (21.67) and Efuru (25), but all three were higher than Dakaa (5.51). Head and middle region calcium of 6 and 6.08 mg/100g dm was lower than the 3.72 mg/100g dm of the tail in variety Dakaa. Calcium role in the plant cell wall confers structural integrity which resists pathogenic enzyme deterioration but analysis of total phenolic compounds which may confer structural integrity as well as toxic metabolites which destroy the pathogen may render explanation to the “hardy” nature of yam tuber head region compared to the middle and tail regions.

Introduction

Yams, *Dioscorea species* especially the white guinea yam varieties, *Dioscorea rotundata* Poir are probably more popular in South West Nigeria as food cultivars than the water yam, *Dioscorea alata* L. The water yam in South West Nigeria is popular among the Ijebu sect in Ogun state who prepare local dish referred to as “Ikokore” from the grated cooked and spiced meal. Water yam may also be peeled, grated, spiced, made into balls and fried into a meal snack called “Ojojo”. Apart from these two, preparations, the Yoruba in the South West relish various preparations from White guinea yams which outnumber those for which water yam is put. Also many whiter guinea yam varieties are available in a

yam (South West Nigeria) market relative to water yam varieties. The South East Nigerian region however has numerous dishes/preparations from water yam and relatively more water yam varieties. Yoruba farmers in South West Nigeria plant sections usually cut or prepared from whole tubers, which are referred to as yam “setts”. The setts are preferred for the smallholder farmer because ware yams are more costly for planting.

The alternative is the smaller-sized yams referred to as “seed yams” which are formed after the milk harvest (at six months after planting) when big-sized tubers are severed with sharp knives from the shoots in a yam mound. The severing is done such that a part of the proximal (head) region is left, still attached to the shoot. The latter are then buried again for the last 3 months (month 6 to 9) of the growth cycle when the severed portion bulks again to produce the small-sized yams at physiological maturity which are referred to as “seed yams”. The latter are usually expensive especially towards the onset of rains which is generally the time to plant yams. A small holder farmer buys as many seed yams as can be afforded financially and supplements with yam “setts” cut from the ware yams which normally may be as big as 2 to 6 kilogram weight. Ware yams are harvested at physiological maturity when yam leaves become senescent, generally 9 months for white guinea yam.

It is usual to have faster emergence from setts prepared from the proximal region/head region as farmers are familiar with rots affecting more setts from the distal portion of the tuber in the field. Differences in infection by *Aspergillus niger* from different geographical locations (Nigeria, Asia and Europe) on cut regions of the yam (*Dioscorea species*) tuber were not consistent probably because of a mix up of varieties from their sources (provenances) (Otusanya and Jeger, 1994).

Calcium fertilization in two improved varieties of *Dioscorea species* namely *D. rotundata* TDr 131 and *D. alata* TDa 92-2 reduced infection by *Aspergillus niger* and *Botryodiplodia theobromae* after long-term storage, especially in yam sections/yam setts (Otusanya et al., 2016).

This study investigated infection in different regions namely head, middle and tail of tubers of four varieties of white guinea yam from South West Nigeria, by *Rhizopus stolonifer*, an important yam tuber rot pathogen. Calcium content of the head, middle and tail of tubers of the four varieties were also analysed to ascertain relationship between it and infection.

Materials and Methods

Yam, *Dioscorea* species tubers

Tubers of four varieties of white guinea yam *Dioscorea rotundata* were sourced from the yam farmers' market in Gbonogun main market, Abeokuta, Ogun State in South West Nigeria. They are varieties Efur, Oniyere, Iseosi and Dakaa.

Analysis of Tuber Calcium

Three tubers per variety were selected for the tuber calcium analysis. The selected tubers were free of abrasions or injuries or disease symptoms. They were washed in running tap water without bruising them and left to dry on top of a laboratory bench under a low-speed fan. Each tuber was then cut into sections of head, middle and tail. Each section was sliced thereafter into thin chips inside labelled plastic trays. Drying of the chips was for three days on yam storage structures inside the COLPLANT (College of Plant Science and Crop Production), Federal University of Agriculture, Abeokuta (FUNAAB), Screen house. The dried chips were ground to powder with a high powered mill at the Central Workshop of COLENG (College of Agricultural Engineering), FUNAAB. They were then poured into new labelled polyethylene bags for calcium content analysis. Analysis of calcium content was carried out with the routine methods of mineral (Calcium) analysis of the Association of Official Analytical Chemists [1].

Infection and weight loss experimental design and procedure

Tubers which had no bruises, lacerations or disease rot symptoms (soft, dry or wet) of the four varieties were selected. They were cut into fairly equal regions of the head, middle, and tail perpendicular to the tuber length, after the head and tail tips had been cut off, with a sharp surface-sterilized steel knife [3]. They were left to dry for 24 hours on top of surface-sterilized Laboratory benches in the Crop Protection Laboratory of the Department of Crop Protection, COLPLANT, FUNAAB, Abeokuta, Ogun State in the South West area of Nigeria. Each of these cut regions of head, middle and tail from each tuber had two cut surfaces and served as the experimental units [4]. Each yam section was labeled and weighed with an electronic balance at the beginning of the experiment. Cut sections of the four varieties were then set up, in a 4 x 3 factorial experiment, arranged in randomized complete block, each of head, middle and tail, and three replications.

Each experimental unit was inoculated with a 7-day old pure culture of *Rhizopus stolonifer* which had earlier been isolated from a partially rotted tuber of variety Efur. Inoculation of experimental units was according to the method of Otusanya and Jeger (1994). Surface sterilized 4 mm and 3 mm cork borers,

scalpel and forceps were used to bore 10 to 12 mm hole into each unit and to place a 3 mm agar (potato dextrose agar) disc of the pathogen into the hole. After which the upper incision on the periderm was sealed with Vaseline (petroleum jelly). The inoculated sections were transferred in trays into a raised wooden netted/ventilated yam storage structure inside the COLPLANT screen house. The yam storage structures are protected from rain, rodents/reptiles and allow free air flow which disallows increase of humidity. Incubation period was 4 weeks. Each section was weighed again with an electronic balance after the 4 week incubation. Then, each yam section was cut open with a knife, after the Vaseline had been removed with spatula and cotton wool. Infected tissue was cut into a pre-weighed petri-dish and its weight measured with an electronic balance. Percent infection was determined with the formula:

$$\% \text{ Infection} = \left(\frac{C}{A} \right) 100$$
 Where C and A are corrected weight of infected tissue and weight of the yam section at the beginning of the experiment respectively. C was calculated with the formula: $C = \frac{100X}{100 - Y}$; where X and Y are the weight of infected tissue and percentage weight loss respectively. Percent weight loss was calculated with the formula:

$$\% \text{ Weight loss}(Y) = \left(\frac{A - B}{A} \right) 100$$
 where A and B are weight of the section at the beginning and at the end of the experiment respectively.

Data Analysis

Percent data was transformed appropriately before Analysis of variance. Means were separated with Tukey's (HSD) test.

Results

Infection in tuber head, middle and tail and calcium content

Percent infection in the varieties was significantly different (Table 1). Variety Dakaa had higher infection of 11.1% than the three other varieties which were not significantly different from one another, with the values 1.32% Oniyere/1.45% Iseosi/2.74% Efur. Tuber regions were comparable/similar in infection, and the head region had 4.24%, the middle region 2.90% and the tail region 5.31%. Interaction effects showed significantly lower infection of 0.92% in the middle region of variety Oniyere, whereas the head and tail which were comparable or similar had 1.22% and 1.81% respectively. The middle of variety Efur with 0.92% was also significantly lower than the head and tail which had comparable/similar infection of 4.02% and 3.27% respectively. However, variety Iseosi had comparable infection in all three regions of 1.3% (head), 1.41% middle and 1.63% (tail). Higher overall mean infection than in the three other varieties occurred in variety Dakaa which had 11.1% compared to the other three varieties which had overall mean infection of over 1% each. The head middle and tail regions of variety Dakaa had comparable/similar infection of 10.43% (head), 8.33% (middle) and 14.53% (tail). Interaction effects indicated comparable calcium content in the three regions in three of the four varieties namely Oniyere, Iseosi and Efur. Each of these varieties had overall mean calcium content of over 21 mg/100 gdm in the three

Table 1: Infection by *Rhizopus stolonifer* in 4 weeks and calcium content in the head middle and tail region of the tuber of four varieties of white guinea yam, *Dioscorea rotundata* Poir.

Variety		% infection	Calcium mg/100gdm	Content
Oniyere		1.32 ^b	23.33 ^a	
Iseosi		1.45 ^b	21.67 ^a	
Dakaa		11.10 ^a	5.51 ^b	
Efuru		2.74 ^b	25.00 ^a	
TUBER REGION				
	Head	4.24 ^a	18.38 ^a	
	Middle	2.90 ^a	20.45 ^a	
	Tail	5.31 ^a	17.81 ^a	
INTERACTION				
White variety	guinea	yam	Tuber region	
Oniyere	Head	1.22 ^b	22.50 ^a	
	Middle	0.92 ^c	22.50 ^a	
	Tail	1.81 ^b	25.00 ^a	
Iseosi	Head	1.30 ^b	17.50 ^a	
	Middle	1.41 ^b	25.00 ^a	
	Tail	1.63 ^b	22.50 ^a	
Dakaa	Head	10.43 ^a	6.00 ^b	
	Middle	8.33 ^a	6.80 ^b	
	Tail	14.53 ^a	3.72 ^c	
Efuru	Head	4.02 ^b	27.50 ^a	
	Middle	0.92 ^c	27.50 ^a	
	Tail	3.27 ^b	20.00 ^a	

Means in a column followed by the same letter are not significantly different at P = 0.05

regions. Variety Dakaa however had significantly lower overall mean calcium content of 5.51 mg/100 gdm and also significantly lower calcium in the tail of 3.72 mg/100 gdm compared to that of the head (6 mg/100 gdm) and middle (6.8 mg/100 gdm) regions which had comparable/similar calcium content.

Discussion

Overall mean infection of head, middle and tail regions of the tuber in varieties Oniyere, Iseosi and Efuru in the 4 weeks of incubation with *Rhizopus stolonifer*, was in the range 1.32% to 2.74%. This mean was lower than in variety Dakaa which had overall mean of 11.1%. However, there were no significant differences in infection of the tuber regions. But interaction effects in two of the four varieties namely Oniyere and Efuru had significantly lower infection in the middle region than the head or tail regions. The two other varieties, Iseosi and Dakaa had comparable/similar infection in the head, middle and tail regions. Head portions of yam (*Dioscorea*) tubers especially in white guinea yam are known to be "hardy" than the middle or tail regions and are naturally preferred as setts for planting as they sprout easily and are less likely to rot after planting in the field compared to the middle and tail portions. Farmers in Nigeria

are therefore more careful with setts prepared from the distal portion of the tuber as emergence is usually known to be lower with them except cultural management is high and supported by management pesticides. Calcium fertilization (CaCO₃) and NPK reduced infection by *Aspergillus niger* and *Botryodiplodia theobromae* especially in cut regions in two improved varieties of *Dioscorea* species namely *D. rotundata* TDr 131 and *D. alata* TDa 92-2, after long-term storage [5]. This was the basis for calcium content analysis in this study. But calcium content analysis in 3 of the 4 varieties in this study had no significant differences in either head, middle or tail. Only variety Dakaa had significantly different calcium contents in the tuber regions. In this variety, calcium content of 6mg/100gdm and 6.8 mg/100 gdm of the head and middle region, were comparable/similar and higher than that of the tail region which was 3.72 mg/100 gdm. Probably analysis of total phenolic compounds of tuber regions of head, middle and tail in white guinea yam (*Dioscorea rotundata*) may establish which region is potentially higher in resistance to the tuber rot pathogen *Rhizopus stolonifer*. Phenolics or phenolic compounds have a wide variety of structure and potential which provides basis for their specificity as antimicrobial agents [2].

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