Sweetpotato: Germplasm Evaluation for Wound Healing Efficiency

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ABSTRACT

The short shelf-life of sweetpotato storage roots under tropical marketing conditions is a major limitation in many developing countries. The shelf-life under sub-optimal environments typical of marketing conditions, is related to the ability of roots to heal their wounds. A rapid method was developed to assess the wound healing efficiency by staining for lignin, which forms an important barrier above the new wound periderm that grows underneath a wound. A wide range of cultivars (44), sourced from various regions of the world, were screened and tested for their wound healing efficiency, dry matter content, sugar content, and the rate of water loss after wounding. Wound healing efficiency was related to the origin of cultivars and African cultivars were poor wound healers. Dry matter and sugar content were also found to be related to wound healing efficiency, although other factors are clearly more important. The actual rate of wound-healing under optimal conditions was not related to the ability to wound heal under sub-optimal market conditions. Blocks of tissue show wound-healing capacity and will be used for further investigations. Desiccation of tissue blocks inhibits wound-healing in a cultivar specific manner, although no differences in rates of water loss among cultivars could be found. Studies on the water activity within the tissue, changes with water loss and the relationship to activity of key enzymes during lignification are underway.

INTRODUCTION

Surveys conducted in East Africa have highlighted the short shelf-life of sweetpotato and its role in limiting potential both as a food security crop and for marketing (Fowler and Stabrawa, 1993; Kapinga *et al.* 1995). Thus, the marketing system in Tanzania is constrained by the assumption that roots can remain in the market for a maximum of three days. Traders reduce prices to clear stocks, and yet still report high levels of physical loss (Bancroft *pers. comm.*). Recent work conducted in Tanzania has confirmed that during marketing roots rarely keep for longer than two to three weeks (Rees *et al*., 2001). Recommendations have therefore been made that less perishable sweetpotato cultivars should be developed and that improved handling techniques be devised.

The potential for selecting cultivars with better keeping qualities has been demonstrated by the finding that under simulated marketing conditions in East Africa a large range in the storability of sweetpotato exists among the available germplasm (van Oirschot 2000, Rees *et al.*, 1998) . Under these conditions deterioration is dominated by water loss through unhealed or incompletely healed wounds. An important factor in the variation between cultivars is their ability to heal wounds under non-ideal curing conditions, specifically at sub-optimal humidities (van Oirschot 2000).

Optimal conditions for wound-healing in sweetpotato are high humidities (>85%) and temperatures between 26 and 28ºC (Kushman and Wright, 1969). In the USA sweetpotatoes are cured under such conditions prior to storage. During wound-healing four to five layers of cells beneath the wound become lignified to form an initial barrier against infection and desiccation. A wound periderm is then formed below this, even if

the roots have been removed from curing conditions (Walter and Schadel, 1982; 1983). Lignification is considered to be a crucial step in the wound-healing process, and the thickness of the lignified layer, as indicated by phloroglucinol staining, has been used by several scientists as a measure of the progress of the wound healing process (Walter and Schadel, 1982; 1983). However, van Oirschot (2000) found that under moderate relative humidities sweetpotatoes were not always able to complete the wound healing process. An inability to heal was often accompanied by a discontinuous lignin layer that formed deep below the wound, underneath a thick desiccated layer. Successfully healed wounds had a continuous lignin layer, but this could be very thin. It was thus concluded that continuity of the layer was more important than the thickness. A protocol was therefore developed to assess wound-healing at moderate humidities by staining for lignin with phloroglucinol and scoring for the continuity of the lignified layer. This method was used to demonstrate that wound healing efficiency at moderate humidities is related to storability and shelf-life of roots (van Oirchot 2000). It was documented that lignification prevents water loss and reduces the chance of rotting. Further for the cultivars tested it was found that high dry matter (DM) cultivars tended to be less efficient at wound-healing at moderate humidities (Van Oirschot *et al*., in press). This was in agreement with Rees *et al.* (1998) who reported that high dry matter is often related to short shelf-life.

The finding that cultivars with higher dry matter content have less efficient wound healing is unwelcome. High dry matter is associated with sensory characteristics (*flouriness*) important for consumer acceptability in east Africa (Kapinga *et al*., 1997) and is a key attribute for processing. World-wide the characteristic is considered so important that the International Potato Center (CIP) has a specific initiative to breed for higher dry

matter cultivars. It thus becomes very important to determine whether it is possible to breed for cultivars with high dry matter content and good wound-healing characteristics.

This report summarises results obtained from a study in which a wide range of sweetpotato germplasm was screened for wound-healing efficiency at moderate humidity to gain a better understanding of why sweetpotato cultivars differ in wound healing at moderate humidity, and to determine whether cultivars with both high dry matter and good wound healing efficiency exist.

MATERIALS AND METHODS

Plant material

The results presented here include screening results for three sets of cultivars, each of which was assessed twice. Roots were screened between 1 and 4 weeks after harvest unless otherwise stated.

Set A: 16 Cultivars were grown in Nairobi by CIP as part of a world-wide trial on germplasm by environment (GxE) interactions. The cultivars were: Blesbok, Brondal, Mugande, Mafutha, Cemsa 74-228, Kemb 37, Jayalo, Naveto, Zapallo, Santo Amaro, Yan Shu 1, NC 1560, Xu Shu 18, Tainung No.64, Mogamba, Kemb 10. (Note Jayalo was not included in the experiments following the first harvest.) Five additional check cultivars (Yan Shu 1, Kemb 10, KSP 20, Zapallo, SPK 004) were planted in a separate field trial. Trials consisting of the check cultivars were planted in January 2000 and were harvested in May and July 2000.

Set B: 18 cultivars were grown by Janice Bohac of the U.S. Vegetable Laboratory (USDA-ARS): Beau Regard, PI 538354, PI 595856, PI 595873, Picadito, Regal, SC 1149-19, Sumor, Tanzanian, Tinian, W287 Ruddy, W-308, W-317, W-325, W-341, W-345, W364 97k-11, White Regal. The first season trials were planted in May and harvested October 1999, then cured and stored for 2 months before being assessed in January 2000. The second season trials were planted May 2000 and harvested in November 2000.

Set C: Cultivars were grown in Nairobi, Kenya by CIP. These included 8 Tanzanian cultivars (Bilagala, Budagala, Iboja, Kagole, Mwanamonde, Polista, Sinia B, SPN/0), 5 check cultivars (Kemb 10, KSP 20, SPK 004, Yanshu 1, Zapallo) and four cultivars from North and South America (Beauregard, Jewel, Hernandez, L-86-33). First season trials were planted in May and harvested in September 2000; second season trials were planted on November 2000 and harvested in February 2001.

Experiments

The postharvest experiments were conducted at the Natural Resources Institute (NRI), Chatham, UK. Assessment of wound healing efficiency at moderate humidity was conducted with the roots placed in three controlled environment chambers maintained at 65% RH and 26ºC. For assessment at high humidity the roots were placed in an enclosed bin with a layer of water in the base. RH was greater than 95% throughout the assessment. The humidity and temperature of the storage environment were recorded using Tinytalk miniature dataloggers (Gemini, Chichester, UK). A minimum of 12 roots

per cultivar were assessed at moderate humidity and a minimum of 4 roots per cultivar at high humidity.

One day after roots were placed in the chambers a shallow wound was cut with a potato peeler. The size of the wounds was approximately 2 x 5 cm and 1.7 mm deep. After 5 days, wounds were cut out of the roots, and four thin transverse sections (approximately 0.5 mm thick) per wound were cut with a razorblade. The sections were stained with phloroglucinol (1% in 95% ethanol) for 2 min and transferred to concentrated HCl for 30 s, then rinsed in water for 30 s. Each wound was given a score between 0 and 1 based on the continuity of lignification seen in 3-4 sections across the wound (Table 1). The average lignification score for 3-4 sections was termed the lignification index (L.I.).

Dry matter contents were assessed using approximately 20 g of diced or sliced tissue which was then dried in an oven at 80°C for 48 hours.

Root soluble sugar content was measured by high performance liquid chromatography (HPLC). Freeze-dried samples were ground and extracted in water (1 g sample in 20 ml water) by shaking for one hour at room temperature. The extract was filtered through muslin and filter paper, diluted with acetonitrile to 80% acetonitrile and further filtered through a 0.45 um PTFE syringe filter. 10 µl samples were injected onto an amino-bonded HPLC column (Hypersil APS-2, 20 cm) maintained at 30°C, using 80% acetonitrile running at 0.6 ml/min as the mobile phase. Sugars were detected using a refractive index detector (Hewlett Packard), and peak heights were calculated using a Perkin Elmer LCI-100 Integrator.

Statistical analysis

Statistical analyses were carried out using the statistical package Genstat (Rothamsted, UK). Analysis of variance was used to determine differences among the cultivars. The relationships between rate of water loss and storage time, and between the lignin index and DM content were assessed using linear regression, for which the mean values of each cultivar were taken.

RESULTS AND DISCUSSION

The range in wound-healing efficiency among cultivars

For all sets of cultivars a large range in lignification index (L.I.) (wound-healing efficiency at moderate humidity) was found. However, a comparison of the results obtained for the two harvests of Set A, and for the two seasons for Sets B and C indicated that cultivar behaviour was consistent (Fig 1a-c). Correlation coefficients (r) of 0.80 ($p< 0.001$), 0.47 ($p<0.001$) and 0.82 ($p<0.05$) were calculated for sets A, B and C, respectively. The lower correlation seen for Set B may be due to the fact that roots were stored prior to screening in the first season.

Table 2 presents the mean L.I. for each cultivar measured both at moderate and high humidity. In some cases cultivars were included in more than one set of trials. As cultivar results were again found to be consistent between trials, data has been combined in this table. As previously suggested (van Oirschot *et al.,* 2000) almost all cultivars have high L.I. at high humidity, although there were a few exceptions, for example, Beauregard, Hernandez and L86-33.

The effect of DM content on wound-healing efficiency

One of the objectives of these trials was to determine to what extent DM content affects wound-healing efficiency at moderate humidity. When the overall results are considered, a highly significant correlation is found between L.I. and DM content (Figure 2), indicating that high DM is associated with a lower ability to heal wounds. This was consistent with previous observations (Van Oirschot *et al.*, 2000, in press)

A key question is whether dry matter content is directly affecting wound-healing efficiency, or whether the fact that we often see that low DM cultivars have high efficiency is an indirect effect, and actually dependent on another related factor. Two approaches were used to gain a better understanding of this relationship.

Firstly, the origin of the cultivar was considered. Figure 3 classifies the cultivars according to their origin. It is apparent that the cultivars cluster by origin, for both DM and L.I. For example, cultivars originating from East Africa had higher DM contents and lower L.I. than cultivars from the USA or Central /South America. Correlation analysis carried out separately for cultivars of each origin did not reveal any significant relationships between DM and L.I. .

Secondly a set of five experiments was conducted for a selection of cultivars in which DM content and L.I. at moderate humidity was measured for each individual root. In this way it was possible to use multivariate linear regression analysis to model L.I. in terms of cultivar and DM content. For the five experiments conducted, the linear regression models obtained with their significance levels are presented in Table 3. In all cases

cultivar was the most important factor. However, in each case root DM content made an extra contribution, albeit a small one, to the strength of the model. The general conclusion from this data is that DM content does affect wound-healing ability at moderate humidity, but that there are other cultivar factors that are much more important.

In sweetpotatoes DM content is affected primarily by levels of starch. We have no hypothesis as to how starch levels can affect wound healing efficiency directly. However, there may be an indirect effect related to root sugar levels. Sugar content of selected roots were measured prior to screening of the first harvest for the cultivars of Set A. Table 4 shows correlations between sugar levels, DM content and L.I. at moderate humidity by cultivar. A consistent positive correlation between levels of fructose, glucose and total sugars was found with L.I. measured at moderate humidity. A strong negative correlation was noted between these sugar levels and DM. It is possible that wound-healing efficiency at moderate humidity is affected directly by sugar levels, rather than DM content *per se*. Experiments are under way to distinguish the effects of DM and sugars, and also to test hypotheses as to how sugar levels might control lignification at moderate humidities.

Hypotheses

Given that almost all cultivars tested are able to wound-heal at high humidity, but have a wide range of abilities to wound-heal at moderate humidity, the following broad hypotheses on how cultivars differ are being considered.

1. Sweetpotato cultivars differ in rate of wound-healing at all humidities. Slow healing cultivars are unable to complete wound-healing as desiccation reaches a critical level before they complete the process.

2. Sweetpotato cultivars differ in drying characteristics at moderate humidity. Those that desiccate more rapidly reach a state where wound-healing is inhibited earlier.

3. Sweetpotato cultivars differ in the effect of desiccation on wound-healing efficiency.

These hypotheses are not mutually exclusive.

In order to test hypothesis 1, experiments were conducted to measure the rates of lignification for a range of cultivars at high humidity. Figure 4 illustrates the results of one such experiment, where it was found that although rates of lignification did differ significantly among cultivars, the ranking of cultivars bore no relation to their efficiency at moderate humidity. A second experiment on a different set of cultivars also found significant differences in lignification rate at high humidity that did not relate to L.I. at moderate humidity. Assuming lignification is a valid indication of wound-healing against desiccation, this provides evidence against hypothesis 1.

We have found that small tissue blocks cut from either the parenchyma, or cortex are able to lignify all surfaces whether or not they include portions of the periderm. This has allowed us to develop a model system for experiments to investigate further the physiological basis of cultivar differences.

So far we have found that controlled desiccation of tissue blocks inhibits wound-healing in a cultivar specific manner, although no differences in rates of water loss among cultivars could be found. However, if there are differences in the pattern of desiccation near to the surface of the blocks, our methodology may not be sufficiently accurate to detect differences. Studies on the water activity within the tissue, changes with water loss and the relationship to activity of key enzymes during lignification are planned.

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Figures

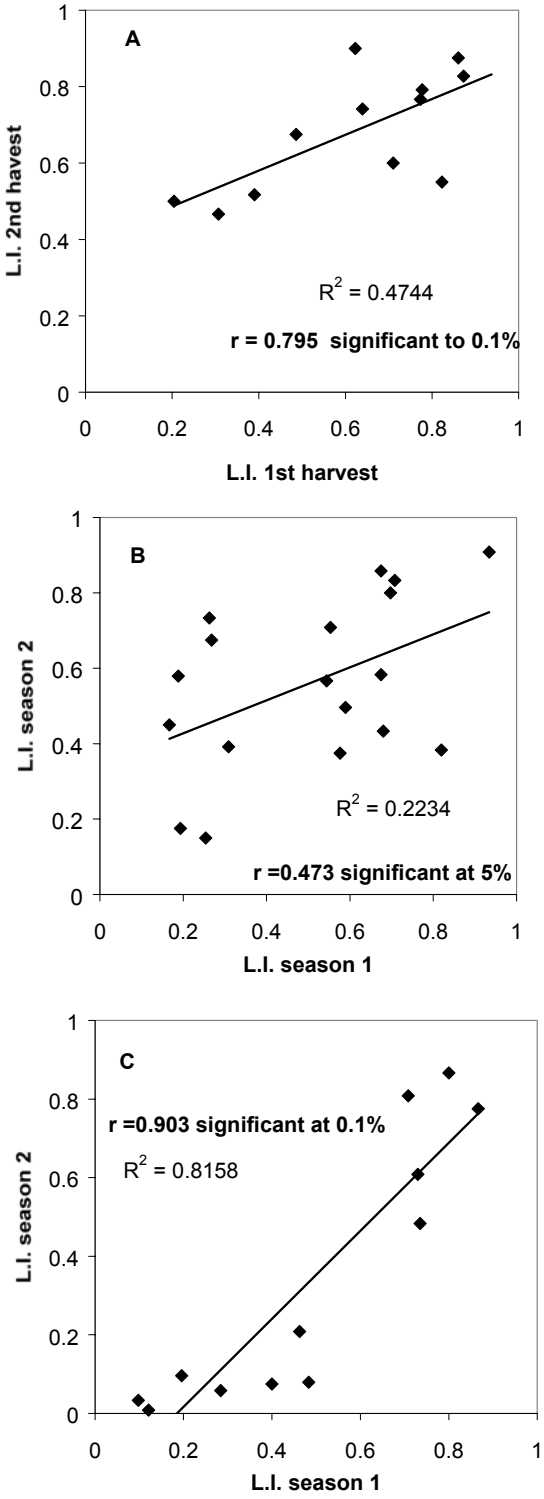


Figure 1: Comparison of lignification index measured at moderate humidity by cultivar a) Set A, first and second harvest b) Set B, first and second season c) Set C, first and second season

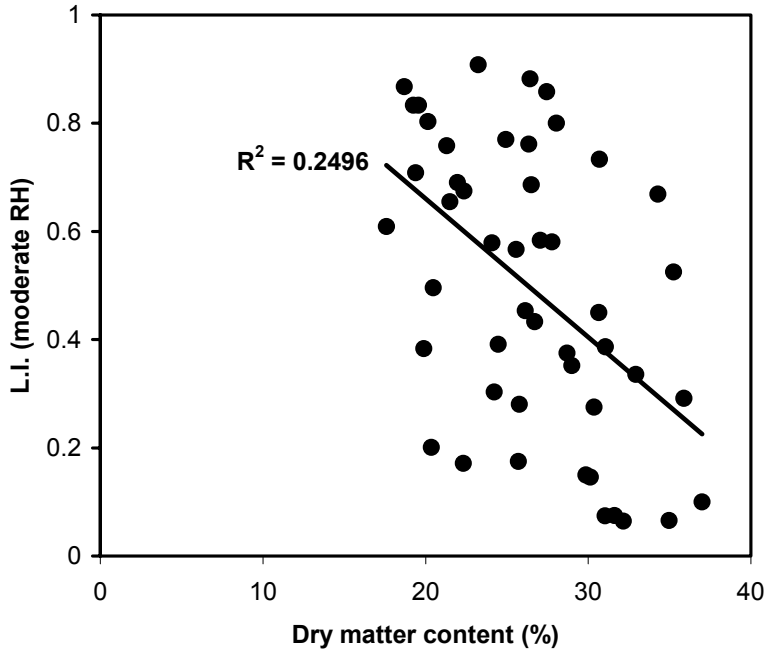


Figure 2: Relationship between L.I. (at moderate humidity) and DM (%) by cultivar for all cultivars tested.

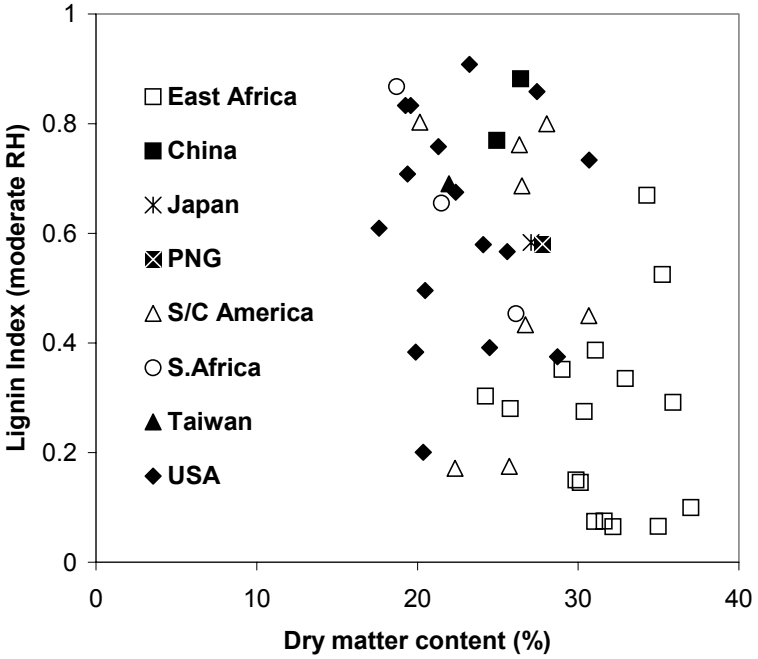


Figure 3: Relationship between lignification index (at moderate RH) and dry matter content for all cultivars tested, classifed by location.

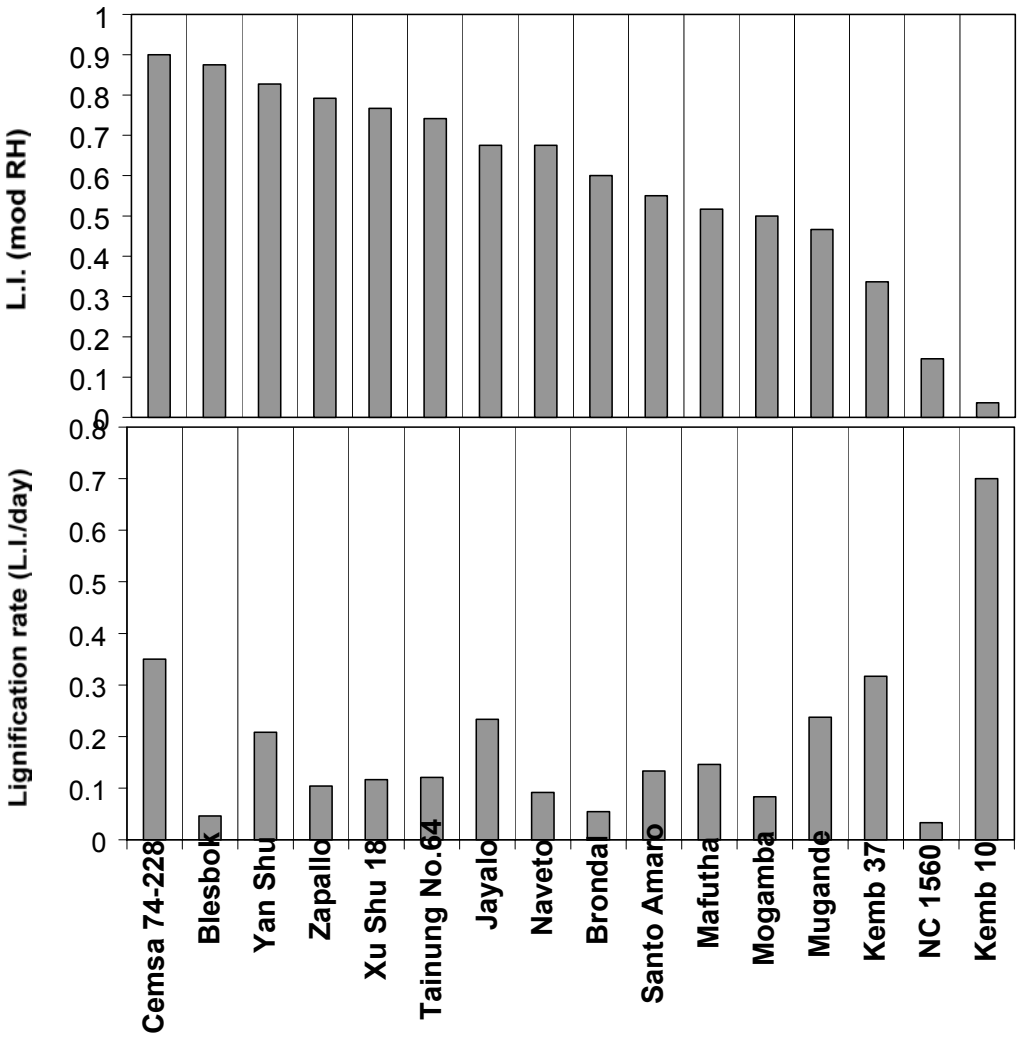


Figure 4: L.I. after 5 days at moderate humidity and the rate of lignification under optimal curing conditions (RH>95%, 22ºC) for 16 cultivars.

Tables

Table 1: Scores for lignification of sweetpotato wound sections

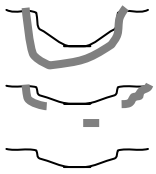
	Score	Completeness of the lignin layer
	Lignification score	Distribution of lignin in wound = lignin
Complete lignification	1	
Patchy lignification	0.5	
No lignification at all	0	

Table 2: Mean lignification indices measured at high and moderate relative humidity, for all cultivars screened.

cultivar	LI high RH	LI mod RH
W-308	0.99	0.93
Blesbok	0.83	0.87
Yan Shu 1	0.98	0.86
Zapallo	0.91	0.80
Jewel	0.78	0.78
Cemsa 74-228	0.90	0.77
Xu Shu 18	0.83	0.77
PI 595856	0.96	0.75
W-287	0.78	0.74
Sumor	0.93	0.71
Tainung No 64	0.78	0.71
Beauregard	0.64	0.68
Brondal	0.90	0.68
Jayalo	-	0.68
Naveto	0.90	0.68
W-325	0.91	0.67
Tinian	0.95	0.63
Sinia	0.95	0.60
W-317	0.94	0.57
L-86-33	0.64	0.57
PI 595873	0.97	0.56
Santo Amaro	0.90	0.55
W-341	1.00	0.55
W-345	0.94	0.48
Mafutha	0.93	0.45
Mugande	0.98	0.39
97K-11	1.00	0.38
Mogamba	0.90	0.37
Regal	0.98	0.35
SC 1149-19	0.95	0.33
Kemb 37	0.85	0.31
SPN/0	0.84	0.29
Bilagala	0.73	0.29
White Regal	0.96	0.27
Picadito	0.94	0.26
KSP 20	0.96	0.23
Kemb 10	0.94	0.22
Tanzania	0.98	0.20
NC 1560	0.95	0.20
PI 538354	0.96	0.18
Hernandez	0.61	0.13
Budagala	0.94	0.13
Polista	1.00	0.10
Kagole	0.96	0.08
SPK 004	0.94	0.05
Iboja	1.00	0.05
Mwanamonde	1.00	0.05

Table 3: Multivariate Linear Regression Models for L.I. at moderate humidity

Expt	Model (cultivar)	Model (DM and cultivar)
1	L.I. = 0.303 + constant*cultivar P<0.001 34.3% variance accounted for.	L.I. = 1.182 – 0.030 DMCi +constant*cultivar P<0.001 36.0% variance accounted for.
2	L.I. = 0.625 + constant*cultivar P<0.001 23.1% variance accounted for.	L.I. = 1.276 – 0.036 DMCi +constant*cultivar P<0.001 28.2% variance accounted for.
3	L.I. = 0.272 + constant*cultivar P<0.001 40.0% variance accounted for.	L.I. = 0.902 – 0.021 DMCi +constant*cultivar P<0.001 41.7 % variance accounted for.
4	L.I. = 0.224 + constant*cultivar P<0.001 43.1% variance accounted for.	L.I. = 1.075 – 0.027 DMCi +constant*cultivar P<0.001 45.4% variance accounted for.
5	L.I. = 0.37 + constant*cultivar P<0.045 7.2% variance accounted for.	L.I. = 1.59 – 0.058 DMCi +constant*cultivar P<0.001 18.3% variance accounted for.

DMCi initial DM content. For experiments 1-4 this was estimated using final DM content and weight loss during the experiment. For experiment 5 the root was cut in two halves at the start of the experiment, and one half used to measure DMC.
Expt 1: 18 roots each of 10 cultivars; Expt 2: 13-18 roots each of 13 cultivars; Expt 3: 182 roots each of 12 cultivars; Expt 4: 17-18 roots each of 11 cultivars; Expt 5: 12 roots each of 9 cultivars.

Table 4: Relationship between lignification index measured at moderate humidity, dry matter content and sugar levels by cultivar for the first harvest of the GxE trial and the five check cultivars (20 cultivars).

	Correlation coefficient (r)	
	Lignification index	%Dry matter content
% Dry matter content	-0.535*	
Fructose [mg/g dry wt]	0.447*	-0.670**
Glucose [mg/g dry wt]	0.516*	-0.685***
Fructose+glucose [mg/g dry wt]	0.480*	-0.681**
Total sugars [mg/g dry wt]	0.592**	-0.882***

***, **, *** indicates significance to 5, 1 and 0.1% respectively.**