Investigating the relationship between carbohydrate metabolism and wound-healing at sub-optimal humidities.

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Introduction

We have shown previously that although sweetpotato cultivars are generally all able to heal wounds efficiently when placed at high humidity, they differ significantly in their ability to heal wounds when kept at lower humidities (Van Oirschot *et al.*, 2002, 2003, Rees *et al*., 2003). We have often observed a relationship between wound-healing efficiency and dry matter content, with low dry matter cultivars having more efficient wound-healing. We have no physiological explanation as to how such a relationship can exist. However, there is a close relationship between dry matter content and sugar levels, and therefore, we have hypothesised that the results we have seen are due to a relationship between sugar levels and wound healing efficiency. In support of this hypothesis, we have previously observed a strong relationship between shelf-life and root monosaccharide levels (Rees *et al*. 2003a)

Conversion of starch into soluble sugars is one mechanism by which plant tissues can increase their osmotic potential and thereby slow down water loss. For this reason, in leaves it has been observed that water deficit leads to the accumulation of soluble sugars. This appears to be brought about by several processes: stimulation of sucrose synthesis, inhibition of starch synthesis and stimulation of starch breakdown. Specifically, there is evidence that activation of Sucrose Phosphate Synthase (SPS) by reversible protein phosphorylation is involved. It appears that the same mechanism occurs to protect potato tubers from the effects of water deficit. The water content of potato tubers can fluctuate between 10 and 20% during the day (Geigenberger *et al.* 1997 and references therein).

In this report we present data which provides more information on the relationship between sugar content and wound-healing in sweetpotato roots. Specifically we report expeirments designed to enable us to look at the sugar levels and their changes near to tissue wounds, during the process of healing at sub-optimal humidities. To conduct part of this study, we exploited our previous findings that small sections of tissue cut from roots are able to lignify. Our main objective is to determine whether the ability of sweetpotato cultivars to lignify wounds at sub-optimal humidities is related to their ability to increase sugar levels in tissues close to wounds in order to slow down desiccation by the increased osmotic potential.

Methods

Root supply
Field trials were grown by CIP, Nairobi, Kenya and by USDA, South Carolina, USA. Harvested roots were air-freighted to the NRI, UK in May 2000, November 2000, May 2002 and September 2002 for experiments 1-4 respectively.

The cultivars used for experiments 1 and 2 were set A and set C, as described in Rees *et al.* (2003b).

The cultivars for experiments 3 and 4 were chosen to cover a range of dry matter content and wound healing ability as follows, as determined in previous experiments (Rees *et al.* 2003b):

Low Dry matter, good healers: Zapallo, Yanshu, Beau Regard
High Dry matter, good healers: Sinia, Naveto, Cemsa 74-228
Low Dry matter, bad healers: Hernandez, KSP20, Kemb37
High Dry matter, bad healers: Bilagala, Kemb10, Polista

Beau Regard and Naveto were not included in experiment 3 due to low yields.

Experiment 1
Comparison of root composition and wound-healing efficiency by cultivar
Six roots of each of 17 cultivars were sampled on arrival in the UK. Three were assessed for DM content and three analysed for sugar content. Further roots were then used to screen for LI at moderate humidity (as described in Rees *et al.* 2003b).

Experiment 2
Comparison of root composition and wound-healing efficiency by individual root
12 roots of each of 9 cultivars were cut into two longitudinally. One half was assessed for DM content and sugars. The other half was assessed for LI at moderate RH (as described in Rees *et al.* 2003b).

Experiment 3
Assessment of carbohydrate changes under wounds during healing, and the relationship with LI.
12 roots of each cultivar were placed in CE chambers maintained 65% RH, 25°C for one day, after which they were wounded by removing a thin section from one side of the root. After removal of most of the periderm, this section was freeze dried for subsequent sugar analysis. Wounded roots were maintained under the same conditions and left to heal for 5 days.
The roots were weighed at the start of the experiment, before and after wounding and after 1, 4 and 5 days of healing.
After 5 days, the lignification index (LI) was measured at the wound, and a tissue slice (approximately 4mm thick) cut from under the wound for freeze drying and subsequent sugar analysis.

Experiment 4
Assessment of carbohydrate changes in tissue cuboids during healing, and the relationship with LI.
Three cuboids (5 x 15 x 50 mm) were cut from the central tissue (parenchyma) of each of 9 roots of each cultivar. All three cuboids were weighed. One was immediately frozen for subsequent freeze drying and sugar analysis, while the other two were placed in CE chambers maintained at 73% RH 25°C. After one day both remaining cuboids were weighed, one was frozen for freeze drying and sugar analysis and the other returned to the CE chamber. This final cuboid was weighed after 4 and 5 days, at which time it was cut into two. One half was weighed and frozen for freeze drying and sugar analysis, while the other was assessed for LI and scored for discolouration.
The wound healing efficiency (LI) of whole roots of the cultivars used in this experiment was tested prior to the main experiment as previously (Rees *et al.* 2003b).

Dry matter (DM) content
DM content was calculated using the sample weights before and after freeze drying.

Sugar analyses
Freeze-dried samples were ground and extracted in water (1 g sample in 20 ml water (expt 1,2), or 200 µg in 4 ml water(expt 3,4)) by shaking for one hour at room temperature. The extract was centrifuged (filtered for experiments 1 and 2), the supernatant diluted with acetonitrile to 80% acetonitrile and filtered through a 0.45 µm PTFE syringe filter. 20 µl

samples were injected onto an amino-bonded HPLC column (Hypersil APS-2, 20 cm) maintained at 30°C, using 80% acetonitrile running at 0.8 ml/min as the mobile phase. Sugars were detected using a refractive index detector (Hewlett Packard), and peak sizes were calculated using a Perkin Elmer LCI-100 Integrator.

Assessment of Lignification index (LI)
The wounds on whole roots were cut from the root to a depth of approximately 10mm. Three thin cross sections approximately 1 mm thick were cut from the wound, or the cuboid using a razorblade. The sections were stained with phloroglucinol (1% in 95% ethanol) for 1 min, transferred to concentrated HCl for 1 min, then rinsed in water for 1 min. Each wound was given a score between 0 and 1 based on the continuity of lignification across the wound, and the average score was calculated.
The method used for cuboids was very similar, using sections cut across the whole cuboid.

Experimental design and statistical analysis
For all experiments the roots or cuboids were arranged on shelves in 3 CE chambers in a randomised complete block design, with one root per replicate.
Statistical analyses were carried out using Genstat (Rothamsted, UK.)

Results and Discussion

Experiment 1

The root composition and LI for the cultivars of experiment 1 are given in Table 1. As reported previously there was a wide range in LI, which correlated negatively with DM content. There was a stronger positive correlation between LI and total sugar content, and there was also a very strong negative correlation between DM content and sugar content (Table 2). This data supports, but does not prove our hypothesis that sugar levels are more directly linked to wound-healing efficiency than is DM content.

Experiment 2

In experiment 2, in order to be able to sample roots for initial sugar levels and DM content, as well as assessing lignification, we assessed healing in roots that had been cut into two longitudinally. Figure 1 summarises the data obtained for LI and DM content and compares it with data obtained during screening of cultivars using the same consignment of roots. Although the DM contents are very consistent, the LI results indicate that healing across cut roots is not as efficient as healing of the surface wounds used for screening. Correlation of the cultivar LI measured in the two cases was not significant.

We attempted to create linear regression models of LI in terms of cultivar, DM content and sugar content. The best models are summarised in Table 3, but none of them were very strong, accounting for less than 20% variance in all cases. As we have observed previously (Rees *et al.* 2003b), a model using cultivar can be improved by including DM content. The same was true of total sugar content, but it contributed less than DM content. This data does not support our hypothesis of sugar involvement in wound-healing efficiency. However, we believe that in this experiment the data was weak due to cultivars differing in their response to cutting.

Experiments 3 and 4

Experiments 3 and 4 were designed to enable us to determine the sugar levels near to healing wounds, and their changes during the healing process. In the case of experiment 3, we assessed tissue composition at a wound made on an intact root at wounding, and after healing was complete. In experiment 4, by considering the healing of tissue cuboids, and using several cuboids cut from adjacent tissue from one root we were able to assess sugar levels at wounding and after one and five days of healing

The main parameters measured are summarised in Table 4 for experiment 3 (root experiment) and Table 5 for experiment 4 (cuboid experiment). In both cases, the cultivars are ordered by decreasing LI.

As previously observed there is a significant difference among cultivars in ability to wound heal (LI) for both experiments reported here, and in an initial assessment of the roots supplied for experiment 4 (Tables 4, 5 and 6). However, for the cuboid experiment the extent of lignification was generally lower than for the root experiment and also lower than we have found in previous experiments on whole roots. Nevertheless, comparison of wound-healing efficiency in these and earlier experiments indicates that the relative performance of cultivars was reasonably consistent (Table 7).

Changes in sugar levels during healing

The cultivar sugar levels and the changes during the experiments are illustrated in Figures 2 and 3. Initial sugar levels differed significantly among cultivars for both experiments. As predicted from our hypothesis there was a general trend for the tissues close to wounds to accumulate sugars, so that in both cases the sugars after five days tended to be higher than the initial levels. In the case of the cuboids (Figure 3), it can also be seen that there was a greater accumulation of sugars over the first day from the time the cuboids were cut, and in most cases a subsequent decline from day 1 to day 5. The day 0 and day 5 data is very consistent between the experiments (Correlation of total sugar levels $r = 0.846$ (significant to 1%), 0.933 (significant to 0.1%)). We assume, although we could not do the measurements, that there was an initial accumulation beneath the wounds of the intact roots as well, so that the maximum sugar levels would have been higher than those measured on day 5.

Factors associated with wound-healing efficiency

In order to examine which characteristics might be associated with wound-healing efficiency (LI), the correlation between various characteristics was examined for individual roots for each experiment. The most notable relationships are summarised in Tables 8 and 9.

As observed previously there is a very significant negative correlation between LI and initial DM for both experiments. However, in both experiments there are also very significant positive relationships between LI and total sugar levels after healing. For the root experiment, although the relationship for LI with total sugar levels is not as strong as that with DM, the correlation with sugar increase during healing is stronger. For the cuboids the correlation with total sugar content one day after the cuboids were cut, is stronger than with initial DM (see Figure 4).

Linear regression models of root and cuboid LI in terms of various parameters are given in Tables 10 and 11.

The strong relationship between DM content and sugar levels, means that the two cannot be included in the same models. As observed previously LI can be modelled in terms of DM content. However, better models can be obtained by considering sugar increase for the root experiment, and sugar levels after day 1 for the cuboid experiment. Notably a model using sugar levels after one day accounted for 33% variance between cuboids. For all models, the addition of cultivar as a factor increased the % variance accounted for, suggesting that other cultivar factors are important. However, comparison of the levels of variance accounted for by the models indicate that LI is strongly cultivar dependent, and most of the cultivar effect is due to cultivar differences in sugar levels. Differences in sugar levels among roots/cuboids of each cultivar also have an effect

The similarity of the models for experiments 3 and 4, give us confidence in the validity of using the cuboids to investigate wound healing of whole roots.

In summary we believe that the data from experiment 4 supports the view that sweetpotatoes which are better able to heal wounds at low humidities are those which have efficient conversion of starch to sugar. This effect can be best observed with a system that allows us to follow sugar levels during the healing process, as in the cuboid experiment. We chose for convenience to measure sugar levels after one day of healing. It is probable that maximum levels would have occurred either earlier or later, and that peak sugar levels would provide an even better model of wound-healing efficiency. We might therefore have underestimated the contribution of this mechanism to wound-healing efficiency.

Water loss

We have hypothesised that the increased sugar levels protect the tissue against desiccation by increasing the osmotic potential. If our hypothesis is correct, we would expect to see some relationship between sugar levels and water loss at the start of the healing process.

Although in most of our experiment it has been very difficult to compare initial rates of water loss between cultivars due to differences in root size and shape, and non-uniformity of wound size, the use of cuboids should remove these problems. However, in experiment 4, (and also experiment3) there is no significant difference among cultivars in rate of weight loss for day 1 (Table 5).

Note:There is no correlation between weight loss and LI throughout the experiment (Table 6). Moreover, there is a very strong relationship between Initial DM and weight loss at day 4 and day 5, with low DM roots losing more weight. In practice it appears that by the end of the experiment the cuboids had lost most of their water, so that those with high initial water content (low DM) lost more weight (see Figure 4). It would appear that lignification provided little or no protection against desiccation in this experiment. This may be because effective healing was only possible on the upper surface of the cuboids due to limited access of oxygen to the lower surface.

Implications for selection of cultivars

One of the long-term objectives of this work is to facilitate the selection of cultivars with high DM content and high wound-healing efficiency. At the start of this work we identified specific cultivars with both characteristics, notably Cemsa, Naveto and Sinia. Although in experiment 4, Sinia did not show high LI, Cemsa in particular continues to show very good characteristics. From our data, the sugar accumulation in Cemsa is not as high as we would expect from the LI. It may therefore be that there is another mechanism that makes this cultivar such a good healer.

Conclusions

By using a model system consisting of tissue cuboids cut from adjacent positions within the sweetpotato we have been able to follow changes in sugar levels during the process of wound-healing at moderate humidity.

We have demonstrated that there is an initial accumulation of sugars close to the wound.

We have shown that the efficiency of wound-healing at moderate humidity is cultivar dependent, and that the level of accumulated sugars is a major cultivar factor in controlling efficiency.

We have hypothesised that this is due to protection against desiccation by high osmotic potential, but cannot demonstrate a difference in rates of initial weight loss as might be predicted. It is possible that accumulation of sugars is protecting specific cellular compartments against water loss, and that this would not be reflected in the water loss of the bulk tissue.

It has been previously observed that physiological deterioration of cassava is reduced in cultivars with a high sugar:starch ratio. We therefore assume that the mechanism involved in the two cases is similar. We believe that manipulation of carbohydrate metabolism (by biotechnology) could be a relatively easy route to improve shelf-life of sweetpotatoes and likewise of cassava.

Our previous observations of a relationship between DM content and wound-healing efficiency is likely to be a result of the link with ability to mobilise starch. Cultivars with rapid starch mobilisation would tend to have lower DM content.

References

Regulation of sucrose and starch metablism in potato tubers in response to short-term water deficit.
Geigenberger, P., Reimholz, R., Geiger, M., Merlo, L., Canale, V., and Stitt, M. (1997)
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Table 1: root composition and lignification index by cultivar for experiment 1

Cultivar name	DM content	Mean L.I.	Fructose [mg/g dry wt]	Glucose [mg/g dry wt]	Sucrose [mg/g dry wt]	Maltose [mg/g dry wt]	Fructose +Glucose [mg/g dry wt]	Total sugars [mg/g dry wt]	Fructose +Glucose [mg/g fresh wt]	Total/ sugars [mg/g fresh wt]
Yan Shu 1 (2)	24.01	0.94	66.49	58.52	57.66	1.66	125.01	184.33	30.01	44.25
Yan Shu 1	25.07	0.87	65.40	55.46	68.25	13.84	120.87	202.96	30.30	50.88
Blesbok	17.89	0.86	81.34	67.61	69.90	3.27	148.95	222.11	26.64	39.73
Santo Amaro	23.47	0.82	31.71	31.63	88.40	21.12	63.34	172.86	14.86	40.56
Zapallo (2)	18.71	0.80	71.24	53.33	98.32	3.43	124.57	226.32	23.31	42.34
Zapallo	19.81	0.78	36.43	35.09	164.86	8.21	71.52	244.59	14.17	48.46
Xu Shu 18	23.37	0.77	16.22	17.34	105.63	6.81	33.57	146.00	7.84	34.12
Brondal	18.60	0.71	32.14	27.93	151.76	3.70	60.07	215.53	11.17	40.08
Tainung No 64	21.72	0.64	53.48	49.03	93.91	1.71	102.51	198.13	22.27	43.03
Cemsa 74-228	25.46	0.62	20.30	21.54	127.77	4.35	41.83	173.96	10.65	44.28
Naveto	26.94	0.49	60.26	46.76	40.00	2.40	107.02	149.43	28.83	40.26
Kemb 10 (2)	30.59	0.46	0.00	2.74	99.38	4.63	2.74	106.76	0.84	32.66
Mafutha	25.91	0.39	11.45	11.62	132.93	16.23	23.07	172.23	5.98	44.62
Mugande	31.22	0.31	0.00	1.10	103.49	12.13	1.10	116.72	0.34	36.44
KSP 20 (2)	23.66	0.28	65.63	57.78	44.99	7.90	123.41	176.30	29.20	41.72
Kemb 37	23.25	0.27	67.33	36.28	30.39	6.99	103.61	141.00	24.09	32.79
NC 1560	20.15	0.26	61.47	51.41	90.54	8.45	112.88	211.87	22.74	42.68
Kemb 10	26.29	0.22	0.74	4.39	125.56	8.98	5.14	139.68	1.35	36.72
Mogamba	27.23	0.20	0.28	8.11	146.67	7.36	8.39	162.42	2.29	44.23
SPK 004 (2)	29.56	0.14	5.41	9.75	91.90	5.94	15.16	113.00	4.48	33.41

2: roots grown in a separate field trial. Although some of the cultivars wer duplicated they were considered separately.

Table 2: Relationship between lignification index measured at moderate humidity, dry matter content and sugar levels by cultivar for experiment 1

	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***
Fructose +glucose [mg/g fresh wt]	0.438+	-0.542*
Total sugars [mg/g fresh wt]	0.427+	n.s.

n.s. not significant

+, *, **, *** indicates significance to 10, 5, 1 and 0.1% respectively.

Table 3: Models of root LI in terms of individual root characteristics determined in experiment 2

Factors considered	% variance accounted for	Model
Cultivar	7.2%	L.I. = 0.37 + cultivar F = 0.045
Cultivar and DM content	18.3%	L.I. = 1.59 – 0.058%DMC + cultivar F < 0.001
Cultivar and Total sugars	9.2%	L.I. = -0.177 + 0.0024(Total sugars) + cultivar F = 0.026
Cultivar and (Fructose + glucose)	6.9%	L.I. = 0.105 + 0.0015 (F+G) + cultivar F = 0.057

Correlations (considering 108 roots)
%DMC v F+G -0.626 ***
%DMC v total sugars -0.8 ***
No significant correlations between L.I. and sugar levels

Table 4: Wound healing ability, weight loss and sugar levels adjacent to wounds for roots from 10 sweetpotato cultivars (experiment 3)

Cultivar no.	Cultivar name	Initial sugar level [mg/g dry wt]	Final sugar level under wound [mg/g dry wt]	Sugar increase [mg/g dry wt]	% sugar increase	DM 1	DM 2	LI	DM of tissue under wound after healing	% wounded wt loss*	% healed wt loss**	% healed wt loss / % wounded wt loss	Calculated water loss from under wound (% of initial tissue wt)
3	Cemsa-74-228	134.63	146.65	12.03	10.68	23.77	23.48	0.88	34.86	3.56	1.42	0.40	32.47
12	Zapallo	173.96	239.90	65.94	40.53	20.72	19.62	0.65	33.60	4.25	1.78	0.43	37.67
10	Sinia	121.79	129.36	7.57	12.98	29.74	28.66	0.53	52.37	10.74	1.66	0.42	42.03
11	Yanshu	152.16	155.48	3.32	2.66	21.21	20.20	0.48	40.82	5.84	2.76	0.48	45.47
7	KSP20	132.86	142.54	9.68	8.38	25.54	25.26	0.44	49.36	3.42	1.33	0.39	48.27
6	Kemb37	133.50	160.08	26.58	20.46	24.91	24.59	0.32	47.08	2.68	1.05	0.40	46.95
5	Kemb10	112.36	118.78	6.42	8.53	28.39	31.93	0.30	55.95	3.40	1.65	0.48	47.32
9	Polista	98.85	96.86	-1.98	4.97	32.23	32.65	0.23	58.92	4.13	2.03	0.49	44.92
2	Bilagala	107.69	117.97	10.28	11.82	27.32	28.71	0.18	50.30	4.33	2.25	0.49	44.88
4	Hernandez	194.62	193.95	-0.66	1.01	22.17	21.03	0.10	47.26	6.38	3.45	0.54	52.96
	Mean	136.2	150.1	13.9	12.2	25.61		0.41	47.05	4.87	1.94	0.45	44.23
	Cult effect	***	***	***	**	***		***	***	n.s.	***	***	***
	LSD	22.58	25.55	26.48	19.45	2.11		0.24	5.34	6.68	0.70	0.063	6.68

DM 1 is the dry matter content calculated using samples cut from each root which were subsequently freeze dried.
DM 2 is the dry matter content calculated using 3 roots and standard oven drying techniques at the start of the experiment (not sure where excel input of this data is.)
The two methods of calculating DM give similar results. The correlation between the cultivar values is very strong (R = 0.962 significant to 0.1%)
* % wounded weight loss refers to the root weight loss over 24 hours immediately following wounding.
** % healed weight loss refers to the root weight loss from 4 until 5 days after wounding when healing is considered to be essentially complete.

Table 5 : Cuboid parameters measured during healing experiment, averaged by cultivar (experiment 4).

Cultivar no.	Cultivar name	LI	Discoloratn	DM(day 0)	DM(day1)	DM(day5)	% wt loss (day1)	% wt loss (day4)	% wt loss (day5)	% DM loss (day 1)	% DM loss (day5)
12	Zapallo	0.62	0.54	21.98	26.36	62.81	15.38	60.53	67.19	-0.84	7.86
3	Cemsa-74-228	0.29	0.55	28.87	34.31	76.06	15.03	57.2	62.63	-0.35	1.26
6	Kemb37	0.27	0.73	22.52	28.5	75.58	16.4	64.55	70.28	-6.63	0.55
7	KSP20	0.24	0.62	22.62	28.63	76.32	15.9	63.05	68.58	-5.32	-5.99
11	Yanshu	0.22	0.59	31.59	33.09	78.64	15.47	56.94	61.53	12.34	4.31
1	Beau Regard	0.20	0.43	21.56	26.05	71.95	16.41	64.15	70.89	-0.1	3.73
2	Bilagala	0.17	0.49	31.87	37.27	80.99	15.33	57.52	61.35	0.87	1.77
8	Naveto	0.14	0.59	28.43	33.98	79.25	15.21	60.28	64.92	-1.22	1.99
4	Hernandez	0.12	0.56	20.98	25.64	78.09	17.58	67.95	73.41	-0.86	1.00
5	Kemb10	0.12	0.48	31.82	38.26	81.34	16.48	57.66	61.36	-0.25	1.20
9	Polista	0.12	0.41	37.56	44.64	81.5	15.31	51.3	54.38	-0.24	1.04
10	Sinia	0.08	0.28	36.39	42.94	82.61	16.09	53.63	56.38	1.08	0.94
Mean		0.22	0.52	28.01	33.31	77.10	15.88	59.56	64.41	-0.13	1.64
Cult sign. (p value)		<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.	0.05
LSD		0.13	0.17	2.33	4.69	5.07	2.03	3.31	3.16	10.1	6.48

Cultivar	Total sugars (day 0) Mg/gdrywt	Total sugars (day 1) Mg/gdrywt	Total sugars (day 5) Mg/gdrywt	Sugar increase (day1) mg/gdrywt	Sugar increase (day5) Mg/gdrywt	Sucrose increase (day1)	Sucrose increase (day 5)	Monosaccharide increase (day1)	Monosaccharide increase (day5)
12	250.76	275.88	287.38	25.12	36.62	27.07	55.95	-2.11	-24.87
3	142.09	178.87	139.81	52.57	13.51	46.3	15.47	6.73	-5.49
6	170.25	189.59	160.74	19.34	-9.51	25.99	33.85	-7.78	-49.26
7	183.31	195.04	173.09	11.73	-10.22	27.78	42.35	-13.35	-58.29
11	130.81	169.12	130.29	34.05	14.02	30.98	18.51	3.93	-6.33
1	215.13	217.54	220.41	2.41	5.28	22.09	84.65	-18.8	-81.01
2	92.55	119.32	125.88	26.77	33.33	25.45	9.78	1.88	20.31
8	135.07	168.37	137.67	33.31	2.6	33.11	52.8	0.04	-54.45
4	213.69	228.66	227.74	14.98	14.06	33.65	92.88	-16.9	-80.73
5	113.02	152.92	143.5	39.9	30.49	36.04	7.13	2.24	20.14
9	85.05	116.79	102.14	31.74	17.08	29.95	8.29	-0.27	8.43
10	88.41	110.38	108.43	21.96	20.02	23.99	9.64	-0.84	8.24
Mean	151.97	176.95	163.09	26.16	13.94	30.20	35.94	-3.77	-25.28
Cultivar sign. (p value)	<0.001	<0.001	<0.001	n.s.	n.s.	n.s.	<0.001	0.009	<0.001
LSD	38.39	41.61	45.91	37.71	45.84	26.88	32.01	15.02	23.85

Table 6 Consistency of L.I. for sweetpotato cultivars used in whole root and cuboid experiments

	Screening results		May		Sept		
	L.I.	DM	L.I. (expt 3)	DM	L.I.	Cuboid L.I. (expt 4)	DM
Yanshu 1	0.88	26.4	0.48	21.2	0.33	0.22	31.6
Zapallo	0.80	20.1	0.65	20.7	0.79	0.62	22.0
Cemsa-74-228	0.76	26.3	0.88	23.8	0.79	0.29	28.9
Beauregard	0.71	19.4			0.18	0.20	21.6
Sinia	0.67	34.3	0.53	29.7	0.24	0.08	36.4
Naveto	0.58	27.8			0.51	0.14	28.4
Kemb 37	0.30	24.2	0.32	24.9	0.22	0.27	22.5
Bilagala	0.29	35.9	0.18	27.3	0.38	0.17	31.9
KSP 20	0.28	25.8	0.44	25.5	0.18	0.24	22.6
Kemb 10	0.28	30.4	0.3	28.4	0.60	0.12	31.8
Hernandez	0.17	22.3	0.1	22.2	0.23	0.12	21.0
Polista	0.10	37	0.23	32.2	0.43	0.12	37.6
Mean			0.41	25.6	0.41	0.22	28.0
Cult sign			***	***	***	***	***
LSD			0.24	2.1	0.28	0.13	2.3

Table 7: Correlations between cultivar cuboid LI and cultivar root LI measured in previous experiments (12 cultivars)

Roots Sept	0.570+
Roots May	0.542+
Roots (average of May and Sept)	0.623*
Previous screening data	0.509+

Table 8: Relationship (correlation coefficient, r) between wound healing efficiency (LI) and other root factors measured in the whole root experiment (by root)

	LI	DM(day 0)
DM (day0)	-0.282**	
Total sugar content (day 0)	n.s.	-0.629***
Total sugar content (day 5)	0.240*	???
Sugar increase (day 5)	0.291**	-0.165+
% Sugar increase (day 5)	0.289**	
Calculated water loss from under wound	-0.395***	n.s.

Table 9: Relationship (correlationship coefficient, r) between cuboid characteristics

	LI	DM(day 0)
DM (day0)	-0.410***	
Total sugar content (day 0)	0.456***	-0.838***
Total sugar content (day 1)	0.579***	-0.751***
Total sugar content (day 5)	0.399***	-0.695***
Sugar increase (day 1)	0.244*	0.199 *
% wt loss (day 1)	-0.161 n.s.	-0.113 n.s.
% wt loss (day 4)	-0.040 n.s.	-0.756 ***
% wt loss (day 5)	0.156 n.s.	-0.898 ***

Table 10

a) Models of LI in terms of root characteristics (Expt 3)

Parameter included in the model	% variance accounted for	model
Cultivar	29.8%	0.175 + cultivar
DM	6%	0.999 – 0.023 DM
DM, cultivar	34%	1.138 – 0.035 DM + cultivar
% sugar increase	9.6%	0.356 +0.00464 % sugar increase
% sugar increase, cultivar	36.8%	0.1297+0.00383%sugar increase +cultivar
Sugar increase	9.4%	0.368 + 0.00316 sugar incrase
Sugar increase, cultivar	35.8%	0.149 + 0.00256 sugar increase + cultivar
Sugar_day 5	5.3%	0.137 +0.00184 sugar_day 5
Sugar_day 5, cultivar	34.7%	= -0.107 + 0.00239 sugar_day 5 + cultivar

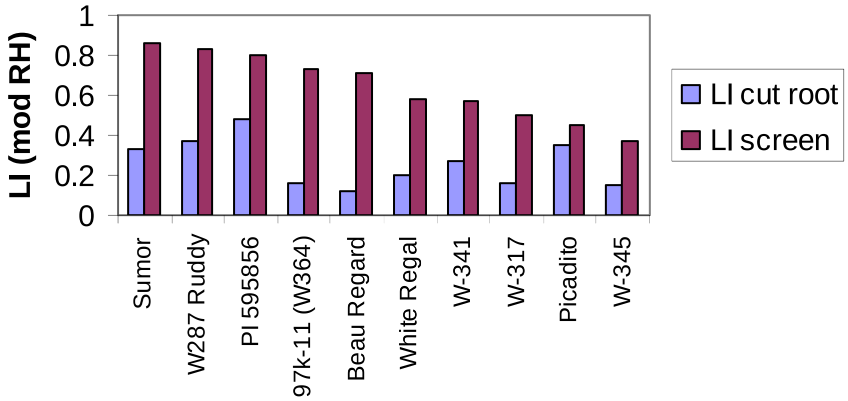
b) Models of Calculated water loss from wound in terms of root characteristics (Expt 3)

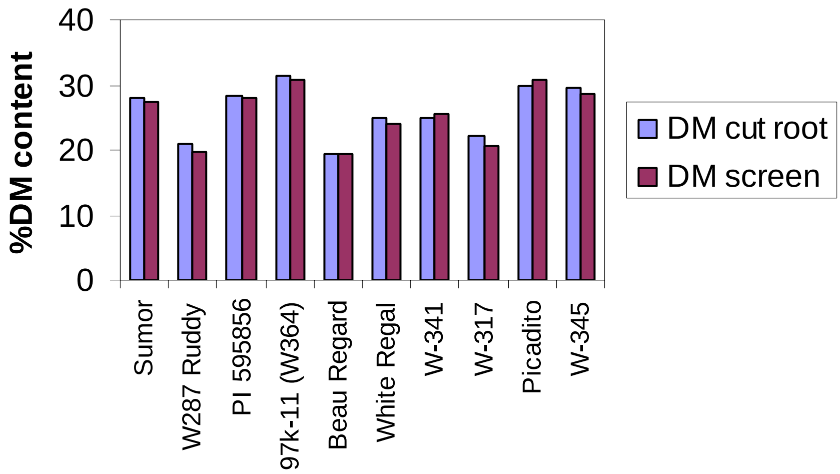
Parameter included in the model	% variance accounted for	model
Cultivar	21.4%	44.88 + cultivar
DM		
DM, cultivar		
% sugar increase	10.8%	46.16 – 0.139 % sugar increase
% sugar increase, cultivar	26.5%	46.21 – 0.1036 %sugar increase +cultivar
Sugar increase	10.3%	45.74 – 0.0921 sugar increase
Sugar increase, cultivar	26.2%	45.68 – 0.072 sugar increase + cultivar
Sugar_day 5	3%	50.54 – 0.04 sugar_day 5
Sugar_day 5, cultivar	25.5%	50.18 – 0.0706sugar_day5 + cultivar

Table 11: Linear regression models of LI in terms of cuboid characteristics for experiment 4

Parameters included in model	% variance accounted for	Model
Cultivar	38%	0.200 + cultivar
Sugars_day 0	20%	-0.0008+0.0014 sugars_day 0
Cultivar, sugar_day 0	40.9%	0.014 +0.0086 sugars_day 0 + cultivar
Cultivar, sugar_day 0, cultivar*sugar_day 0	48.9%	0.071+ 0.0006 sugars_day 0 + cultivar + cultivar*sugar_day 0
Sugars_day 1	32.9%	-0.106 +0.0018 (Sugars _day 1)
Cultivar, Sugars_day 1	50.1%	-0.136 + 0.0015 (TSday1) + cultivar
Cultivar, Sugars_day 1, cultivar*sugars_day1	58.4%	-0.009 + 0.00096(TSday1) + cultivar + TSday1.cultivar
Sugars_day 5	15.4%	0.021+ 0.0012 (sugars_day 5)
Cultivar, Sugars_day 5	37.7%	0.116 + 0.00038 sugars_day 5 + cultivar
Cultivar, sugar_day 5, cultivar*sugar_day 5	39.1%	0.25 – 0.00023 sugar_day 5 +cultivar + cultivar * sugar_day 5
DM	16.1%	0.597 - 0.0136 DM
Cultivar, DM	45.9%	0.719 – 0.024 DM + cultivar
Cultivar, DM, cultivar*DM	54.7%	0.387 – 0.0087 DM + cultivar + DM.cultivar

Figure 1: Comparison of characteristics measured in experiment 2 with those measured in screening experiments on the same consignment of roots.





The LI for cut roots was assessed for the cut surface of 12 roots per cultivar which had been cut into two parts longitudinally. LI screen was assessed for a shallow surface cut of 12 roots per cultivar.

DM cut root was the mean of 12 half roots sampled at the start of the experiment, while DM screen was the mean for 3 roots sampled when the roots arrived in the UK.

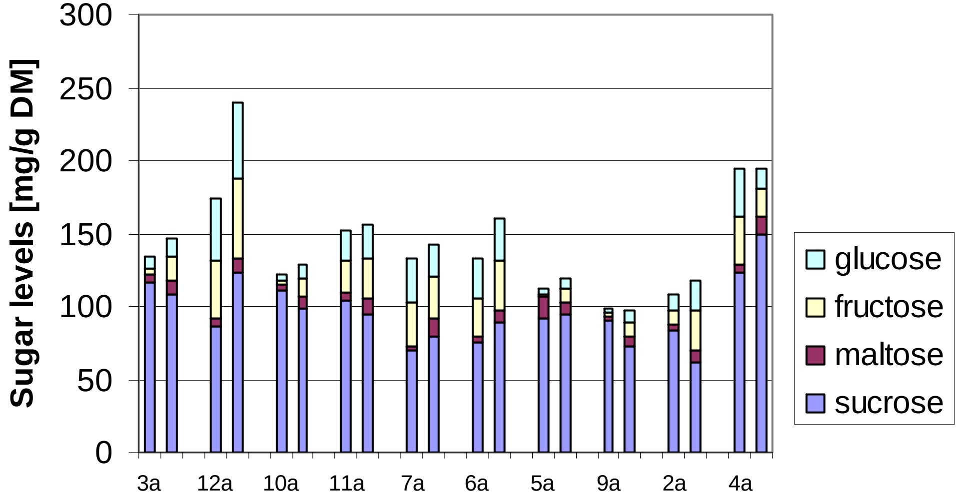


Figure 2 sugar levels near wounds in roots at the time of wounding and after five days of healing.

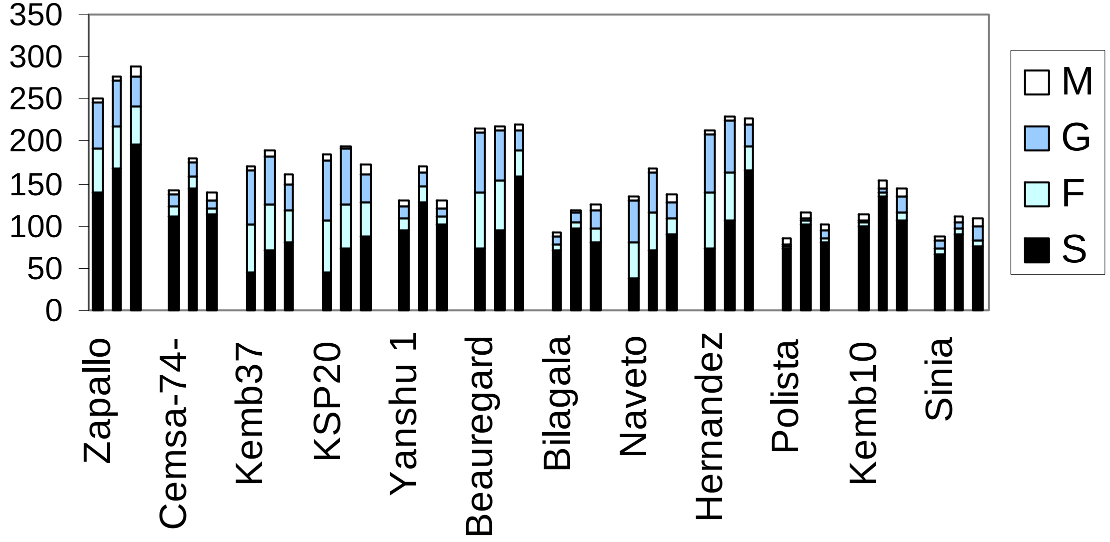


Figure 3 : sugar content of cuboids by cultivar at day 0, 1 and 5 of healing experiment.

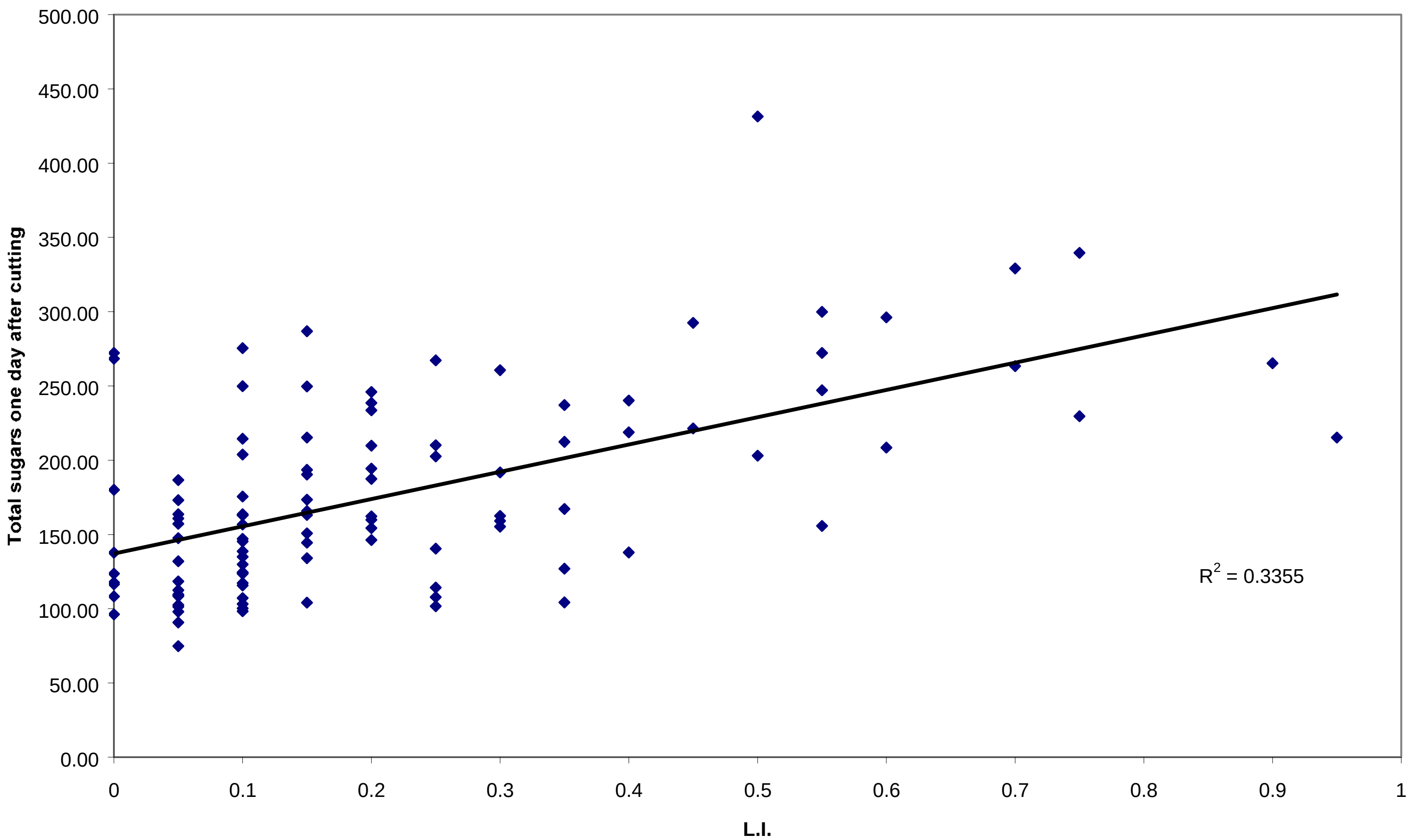


Figure 4 Relationship between LI and total sugar content 1 day after cuboids were cut, for individual cuboids

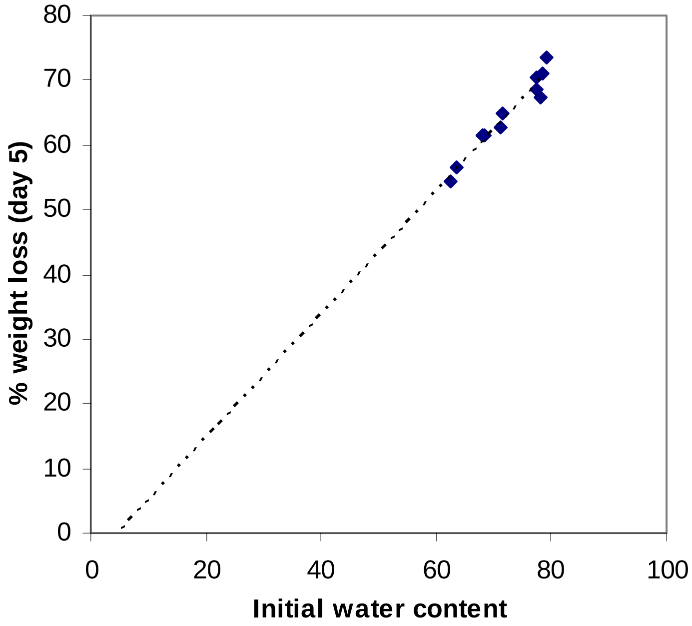


Figure 5 Relationship between cultivar weight loss over 5 days and initial water content