

Studies on osmotic adjustment of tissue discs suspended in mannitol.

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The methods used in these studies were adapted from those used by Geigenberger *et al.* (1997) on potato discs.

Discs of sweetpotato mesophyll tissue were cut and suspended in buffered mannitol solutions of different concentrations. The assumption was that discs placed at low osmotic potential would take up water, thereby increasing in weight. That discs placed in an isotonic solution would maintain approximately the same weight, while discs placed at high osmotic potential would be subjected to water stress and would lose weight. If the tissue discs were able to respond to water stress by osmotic adjustment, weight loss would be less. The hypothesis was that osmotic adjustment might occur to different extents in different cultivars.

Pilot study 1

Discs from 4 cultivars with contrasting wound-healing efficiency were suspended in 0, 100, 300 and 500mM mannitol. (100 mM was supposed to be approximately isotonic)

Figures 1 and 2 show the weight change after 3 and 27 hours respectively

Figure 1

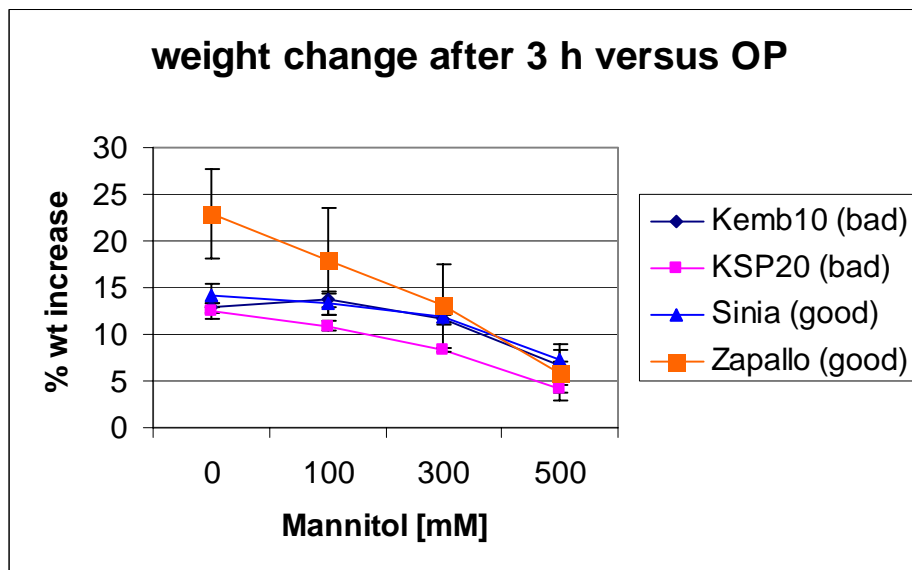
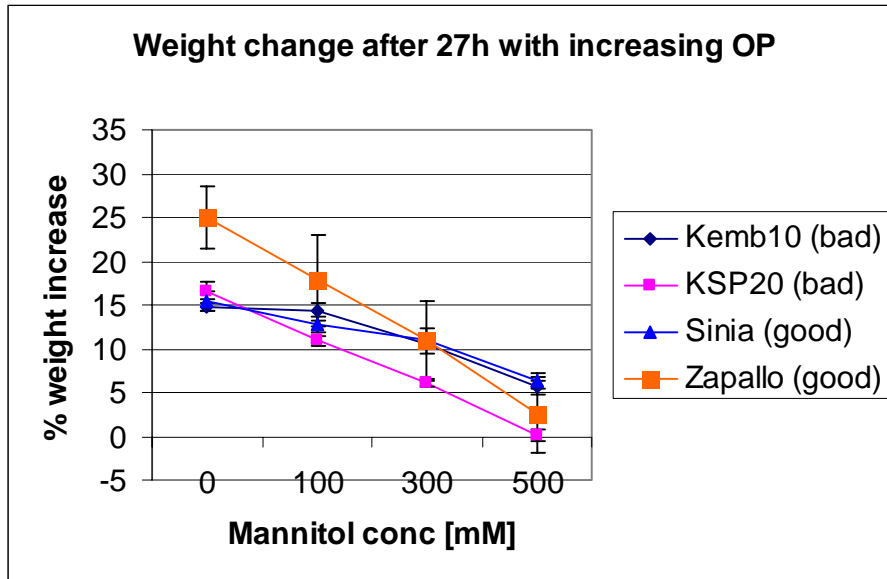


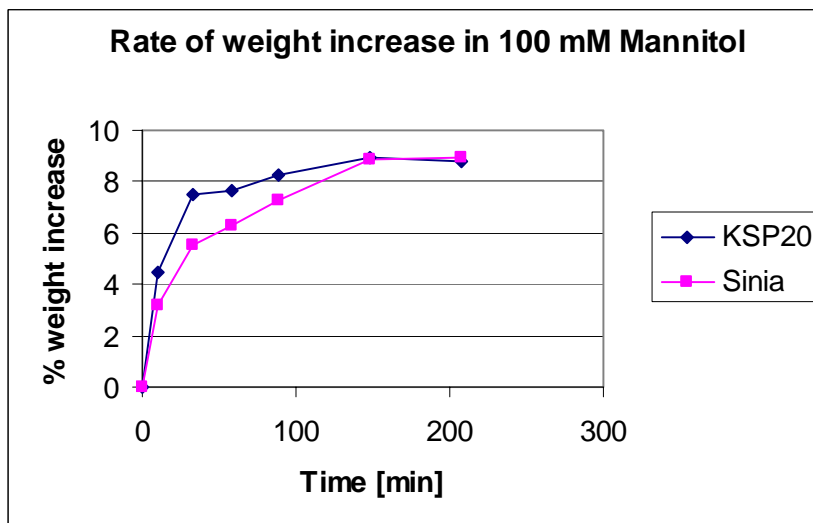
Figure 2.



The fact that there was an increase in weight for all concentrations of mannitol was a surprise. It was proposed that this could be due to air spaces in the tissue that become infiltrated.

Pilot study 2

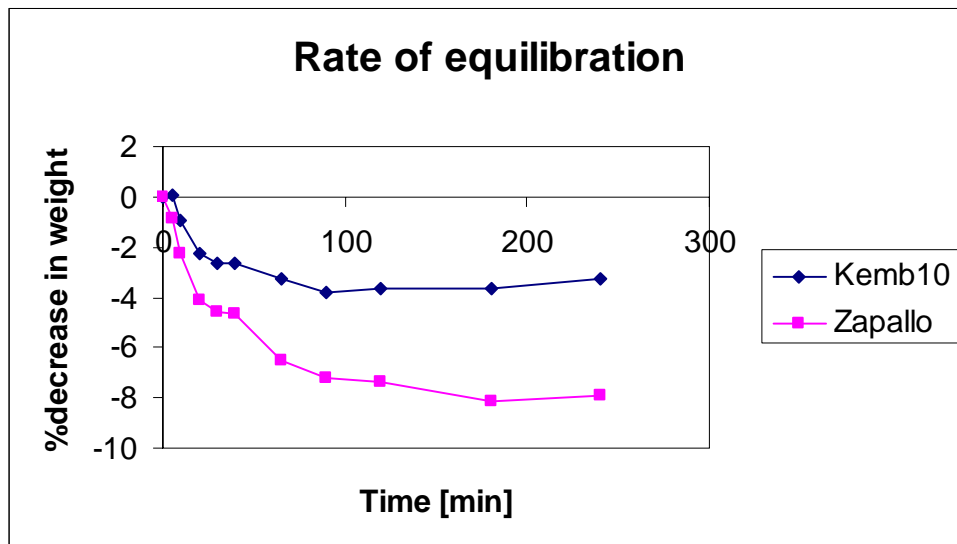
3 disks of each of a single root of KSP20 and Sinia were cut and placed into 100 mM Mannitol. Assuming weight increase is due to uptake into airspaces, this experiment was carried out to determine how long it takes for disks to equilibrate



Equilibration appeared to be complete after 3 hours

Pilot study 3

3 disks from each of Kemb10 and Zapallo were presoaked at 100 mM Mannitol and were then transferred to 500mM Mannitol. This experiment was carried out to determine the rate of equilibration in this case, so that sensible time points could be chosen to assess rates of weight change.



The results of the three pilot studies were used to design an experiment to compare the behaviour of tissue discs from 10 sweetpotato cultivars.

Comparison of 10 cultivars

Disks were cut from 10 cultivars and equilibrated in 100mM Mannitol. They were then transferred to 300 and 500 mM mannitol, and the weight change measured after 50 mins and after 200 minutes.

The results are summarised in the table below

cult	cultivar	DMC*	% loss	% loss	% loss	% loss	L.I.*
			50 mins (300 mM)	200 mins (300 mM)	50 mins (500 mM)	200 mins (500 mM)	
Bilagala	2	0.29	0.60	2.55	5.59	8.17	0.18
cemsa	3	0.23	1.46	3.58	5.54	10.28	0.88
Hernandez	4	0.21	2.27	3.91	9.07	16.54	0.10
kemb10	5	0.32	1.48	3.18	4.46	7.04	0.37
kemb37	6	0.25	1.79	2.71	6.83	11.47	0.30
ksp20	7	0.25	1.57	3.13	7.65	14.47	0.44
Polista	9	0.33	0.69	2.24	3.22	6.17	0.23
Sinia	10	0.29	1.38	2.54	3.45	5.69	0.53
yanshu	11	0.20	1.67	2.98	8.19	15.75	0.48
Zapallo	12	0.20	1.61	3.82	4.12	10.02	0.65

* measured from roots in separate experiment

The following table shows a correlation matrix for the data

DMC	1.000					
li	-0.315	1.000				
%loss50_300	-0.687	0.096	1.000			
%loss50_500	-0.615	-0.265	0.634	1.000		
%loss 200_300	-0.705	0.328	0.710	0.403	1.000	
%loss 200_500	-0.772	-0.100	0.704	0.949	0.544	1.000
	DMC	-li	%loss	%loss	%loss	%loss
			50_300	50_500	200_300	200_500

The most interesting observation was that weight loss was negatively correlated with DMC i.e. high dry matter cultivars showed less weight loss. This is the opposite to what we had predicted. Note L.I. did not correlate significantly with anything including DMC.

Geigenberger, P., Reimhöz, R., Geiger, M., Canale, V. and Stitt, M. (1997). Regulation of sucrose and starch metabolism in potato tuber in response to short-term water deficit. *Planta*, **201**, 502-518. *