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Changes in Carbohydrate Content Following Oxygen Deficiency Stress in Related *Senecio* Species with Different Flooding Tolerances

By

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S u m m a r y

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In the present work we compare responses of the carbohydrate content to hypoxia of related plant species, which naturally grow on sites prone to flooding (*Senecio aquaticus* HILL.-flooding tolerant), with plants from habitats with only a low risk of oxygen shortage (*Senecio jacobaea* L.-flooding sensitive). The sum of water soluble carbohydrates in shoots of *Senecio aquaticus* was nearly tripled following one day of hypoxic treatment. While the amounts of glucose and fructose, changed only slightly, those of sucrose and fructans were two-to fourfold. Following oxygen shortage in the root environment the fructans became the main pool of water soluble carbohydrates in *S. aquaticus*. The shoots of the flooding tolerant species *S. aquaticus* formed more fructans when grown under oxygen deficiency, than their flooding intolerant relative *S. jacobaea*.

The substantial increase in sucrose and fructan content occurred in spite of diminished photosynthetic rates under restricted oxygen supply.

Fructans were found to accumulate as a response to oxygen deficiency in both flooding tolerant and intolerant species but with higher absolute values and ratios between fructan to starch in the flooding tolerant species.

Adding 50 mM sucrose to the nitrogen -flushed nutrient solution did not lead to a further increase of fructan in shoots of *S. aquaticus* but it occurred in *S. jacobaea*, but the CO₂ fixation decreased to nearly 50 % in both species.

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Introduction

Although higher plants require oxygen for growth, they frequently experience low oxygen conditions in natural wetlands and during flooding or irrigation. Without oxygen, mitochondrial respiration ceases for lack of a suitable electron acceptor. The decreased ability to generate ATP under hypoxia is reflected in reduced growth and a general decline in tissue integrity that ultimately leads to death in flooding sensitive species. Therefore substrate consumption associated with glycolysis belong to the most studied aspects of oxygen shortage research (CRAWFORD 1992, PERATA & ALPI 1993). Nonetheless, the question of availability of extensive energy resources and the control of substrate metabolism under oxygen deprivation needs to be clarified. In contrast to accelerated fermentation resulting in starvation of carbohydrates (VANTOAI & al. 1995) many plants accumulate sugars (SETTER & al. 1987) and fructans (ALBRECHT & al. 1993) at low oxygen concentration.

We measured the content of carbohydrates, with emphasis on the analysis of the fructan pool, following growth in aerated, oxygen sufficient (control) and in nitrogen-flushed, oxygen deficient nutrient solution (hypoxia). Fructans are suggested to play a role in tolerance to oxygen deficiency (ALBRECHT & al. 1993) and other plant stresses (HENDRY 1993).

Materials and Methods

Plant growth

Seeds of *Senecio jacobaea* L., which grows preferably in dry habitats (flooding sensitive) and *Senecio aquaticus* HILL which is naturally found on sites prone to flooding (flooding tolerant) were grown on a vermiculite substrate. At the age of 7 weeks they were transferred to an aerated 35% KNOP-solution. After one week the roots were exposed for one or two days to a nitrogen-flushed nutrient solution (H - hypoxia, oxygen concentration $< 0.05 \text{ mg l}^{-1}$) with or without addition of 50 mM sucrose. The control plants were continuously aerated (C- control, oxygen concentration $> 8.3 \text{ mg l}^{-1}$). The shoots were kept in air under light / dark cycles (16 h, 22°C, $310 \mu\text{mol s}^{-1} \text{ m}^{-2}$ / 8h, 17°C).

Assay of carbohydrates

Shoot and root samples (1g fresh weight) were boiled once for 15 min in 10 ml of 20% ethanol and three times for 15 min in 10 ml water. The extracts were pooled together, purified with anion exchange resin (Amberlite IRA-68) and cation exchange resin (Amberlite IRC-50) then by C-18 cartridges (Sep-Pak, Waters-Millipore) and dried under vacuum (ALBRECHT & al. 1993). The carbohydrates were separated using a cation exchange column (Sugar-Pak, Waters-Millipore) and eluted with water at a flow rate of 0.45 ml min^{-1} . Sugars were detected with a differential refractometer (Waters 410) and quantified using fructose, glucose, sucrose and inulin (from Jerusalem Artichokes - Sigma) as standards. Fructan oligomers were quantified individually using HPLC with an rp-column (Spherisorb ODS II, Knauer-Berlin) eluted with a gradient (1-10% methanol in 60 min) at 0.8 ml min^{-1} and detected by Varex ELSD (Evaporative light scattering detector). Fructan standards with DP-3, DP-4, and DP-5 were prepared from kestose syrup (Fa. Bio-Rad, 1m x A2.5 cm ID).

Starch contents were measured on residues from the water-soluble carbohydrate extraction or on replicate tissue samples with the anthrone method.

Gas-exchange measurements

Photosynthetic CO₂ exchange rates from intact attached leaves were measured at 2h from the beginning of the daily light period at a photosynthetic photon flux density of 290- 320 $\mu\text{mol s}^{-1} \text{m}^{-2}$, using an open gas-exchange system (model LCA 4, ADC). Measurements were made at (PO₂, 20kPa; T, 21-23 °C; relative humidity, 65-70 %, and 330-350 ppm CO₂). For these measurements single leaves from five different plants were selected and placed in the cuvette. The measurement was repeated twice one hour before the carbohydrates were extracted.

Results

Hypoxic treatment caused carbohydrate accumulation in shoots and roots of the flooding sensitive *Senecio jacobaea* and in the flooding tolerant *S. aquaticus*, but the absolute levels were higher in the flooding tolerant than in the intolerant species (Fig.1). The sum of water soluble carbohydrates in shoots of *Senecio aquaticus* was nearly tripled following one day of hypoxic treatment. While the amounts of glucose and fructose changed only slightly, those of sucrose and fructans increased two- to fourfold. Following oxygen shortage in the root environment the fructans became the main pool of water soluble carbohydrates (wsC) in *Senecio aquaticus*. The shoots of the flooding tolerant species *S. aquaticus* formed more fructans when grown under oxygen deficiency, than their flooding intolerant relative *S. jacobaea*. The content of wsC increased also in the roots directly suffering from oxygen deficiency, but with a certain delay and to a lower level.

Adding 50mM sucrose to the nitrogen-flushed nutrient solution did not lead to a further increase of fructan in shoots of *S. aquaticus* but it occurred in *S. jacobaea*. Following a restricted oxygen supply the latter species revealed a higher amount of reducing sugars but a lower one of fructans, compared with *S. aquaticus*.

Starch was hardly affected by oxygen deficiency. In contrast to only a slight increase in starch a significant increase in fructan content was induced by restricted oxygen supply. The degree of polymerisation (DP) in *S. jacobaea* increased from DP 6 to 17 and in *S. aquaticus* from DP 6 to more than 24, after the roots were subjected to hypoxia.

A substantial increase in sucrose and fructan content occurred in spite of diminished photosynthetic rates under restricted oxygen supply (Fig. 2). Immediately after the onset of oxygen shortage in the nutrient solution the photosynthetic rates decreased with a stronger drop in the flooding sensitive *S. jacobaea*. The decrease was intensified by the addition of 50mM sucrose

Table 1. Content of water soluble carbohydrates in the shoots and roots of flooding tolerant *Senecio aquaticus* and flooding sensitive *Senecio jacobaea* following one or two days of hypoxia without (C+24h H, C+ 48h H) or with additional 50mM sucrose in the nutrient solution (C+24h H+S, C+ 48h H +S), wsC - water soluble carbohydrates (fructose+glucose+sucrose + fructan).

	<i>Senecio jacobaea</i>					<i>Senecio aquaticus</i>				
sugars (mg/g FW)	C	C+24h H	C+48h H	C+24h H+	C+48h H+	C	C+24h H	C+48h H	C+24h H+	C+48h H+
				S	S				S	S
Shoots										
fructose	1,25	3,29	3,51	4,35	7,06	1,55	3,26	1,83	2,56	1,91
	0,26	1,18	0,6	0,61	0,99	0,28	1,48	0,32	0,67	0,58
glucose	1,26	4,75	5,07	6,79	8,47	2,37	4,24	3,61	4,08	3,87
	0,18	0,83	1,86	1,35	2,16	0,43	0,84	0,55	0,03	0,67
sucrose	4,93	9,26	11,80	15,48	18,40	5,32	15,70	11,29	15,48	22,74
	1,08	0,41	1,70	1,56	1,80	1,04	2,17	2,29	1,87	3,50
fructan	6,75	8,50	10,20	11,60	14,10	9,21	26,15	22,65	21,04	26,41
	0,45	1,41	0,89	1,56	2,32	1,32	3,77	1,53	4,09	2,43
starch	3,19	4,66	3,86	5,23	9,13	5,09	5,92	6,25	3,00	4,44
	0,50	0,90	0,30	0,93	1,13	0,04	0,78	0,22	0,30	0,60
wsC+starch	17,37	30,46	34,44	43,45	57,16	23,54	55,27	45,63	46,16	59,36
Roots										
fructose	1,30	1,39	1,41	3,25	5,72	0,89	1,22	1,37	2,13	3,01
	0,46	0,35	0,33	1,09	2,33	0,04	0,17	0,24	0,09	0,58
glucose	3,44	3,24	3,97	8,77	7,78	0,88	2,86	2,96	5,04	5,61
	0,83	0,48	1,17	1,11	1,85	0,26	0,16	0,42	1,03	1,28
sucrose	4,89	4,24	8,31	9,03	12,46	6,15	7,68	11,96	15,53	12,12
	0,58	1,54	2,65	2,41	3,06	1,55	0,61	3,07	3,62	3,42
fructan	4,21	5,76	8,42	8,06	9,2	8,8	9,9	16,5	18,8	21,45
	0,63	1,58	2,28	2,18	2,67	0,89	1,53	1,66	3,18	4,16
starch	1,48	1,64	1,53	1,98	2,18	2,19	3,03	2,73	1,20	1,45
	0,20	0,29	0,22	0,10	0,2	0,38	0,03	0,44	0,41	0,07
wsC+starch	15,32	16,27	23,64	31,09	37,34	18,90	24,69	35,52	42,70	43,64

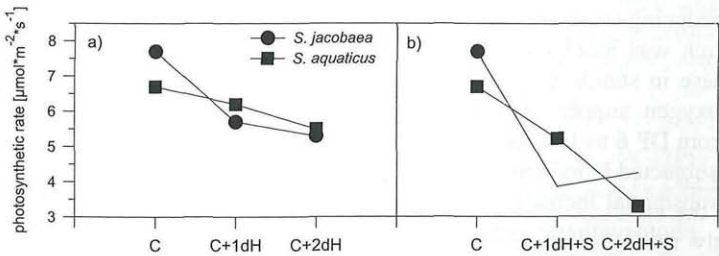


Fig. 1. Photosynthetic rates of *Senecio jacobaea* and *Senecio aquaticus* after the onset of hypoxia in the nutrient solution (a) - without sucrose and (b) - with 50mM sucrose. Each data represents the means of at least 8 independent experiments. (SD < 20 %).

to the hypoxic nutrient solution. In that case the drop in CO₂ fixation was nearly 50% in both species.

Discussion

One day after hypoxic treatment the reducing sugars and fructans increased although photosynthetic rates were reduced. The accumulation of fructans in shoots and roots during oxygen shortage indicates a rate of carbon fixation that exceeds the rate of utilisation (ALBRECHT & al. 1993). Under hypoxia both processes are restricted. Immediately after the onset of hypoxic treatment there is a more significant drop in the carbon fixation in the flooding sensitive species (*S. jacobaea*) compared with its tolerant counterpart (*S. aquaticus*). The average value for the decrease in the photosynthetic rate is nearly 25 % in the tolerant and 35 % in the intolerant species.

The accumulation of sugars could be caused by a lesser sensitivity of photosynthesis and a high sensitivity of growth to restricted oxygen supply in their root environment. Growth has been shown to be a good indicator of hypoxic stress (THOMSON & al. 1990). Apparently roots stop their elongation very soon after transfer from aerated to nitrogen-flushed nutrient solution. Acclimation of the roots to hypoxia over 9 days resulted in a resumed elongation of the roots (ALBRECHT & al. 1993). Reduced growth seems to be imposed by strongly restricted respiration due to inadequate supplies of oxygen. Nevertheless, sufficient energy must be available to enable plants to survive. It seems reasonable to suppose that part of the energy supply comes from the induction of glycolytic and fermentative enzymes, since there is no other known mechanism by which ATP can be generated. Glycolysis is highly controlled by the activity of phospho-fructokinase (PFK). In *Senecio aquaticus* the activity of this enzyme was not increased under hypoxic conditions (SMITH & APREES 1979).

The energy rich sucrose, synthesized in shoots, is transported to the oxygen deprived tissues of the roots. Sucrose degradation occurs via invertase and sucrose synthase (SS). The results of several authors (CHOUREY & al. 1991, RICARD & al. 1991) suggest, that under anoxia / hypoxia the SS pathway plays a major role. This is supported by a decreased activity of alkaline invertase activity under anoxia and a strong increase of SS activity (GUGLIELMINETTI & al. 1995). Moreover, the flow of carbon from sucrose to hexose monophosphates through the SS pathway results in saving ATP compared with the invertase pathway. Afterwards the UDP-glucose is converted to glu-1-P, which is a branching point between catabolic (glycolysis) and anabolic (starch synthesis) carbon pathways. It has been shown above, that the carbon flux channelled to the glycolytic pathway is not at all or even slightly accelerated (ALBRECHT & WIEDENROTH 1994a, ANDREWS & al. 1994). Pyruvate as the end product of glycolysis can hardly be used in respiration if the tissue is deprived of oxygen. To counteract the decreased ATP production in the absence of oxygen pyruvate is partially channelled to ethanolic fermentation.

Radical changes to other metabolic pathways do not seem to be a realisable strategy of adaptation to hypoxia for they would be very costly and therefore appear phylogenetically restricted. However, when intact plants are flooded a number of compounds have been reported as increasing in concentration as for example shikimic acid, oxalic acid, succinic acid and alanine (CRAWFORD 1992).

(12)

Further higher levels of glutathione (ALBRECHT & al. 1994b) and ascorbate (unpublished data) have been measured under restricted oxygen supply and following readmission of oxygen. A high level of both antioxidants are of importance to cope with varying oxygen tension and the endangering by post-hypoxic injury. The carbon at least, as the important building material for the synthesis of antioxidants, seems not to be limited following hypoxia.

A c k n o w l e d g e m e n t s

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