CONTRIBUTION OF CYTOCHROME *C* AND ALTERNATIVE OXIDASE PATHWAYS TO RESPIRATORY SUCROSE LOSS IN POSTHARVEST SUGARBEET (*BETA VULGARIS* L.) ROOTS

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ABSTRACT

It is estimated that respiration is responsible for approximately 70% of the sucrose loss that occurs during postharvest storage of sugarbeet roots. Respiration provides the metabolic energy and carbon substrates needed to maintain healthy tissue during storage, heal wounds acquired during harvest and defend against pathogens. Two respiratory pathways, the cytochrome *c* oxidase pathway and the alternative oxidase pathway, contribute to total respiration. In sugarbeet, little information is available on the role of these two pathways in sucrose utilization and postharvest losses. This information, however, would improve our understanding of this physiological process and may provide insight into methods to reduce postharvest respiratory sucrose loss. Analyses of the changes in total respiration and the contribution of the two pathways in sugarbeet roots subjected to different storage conditions and durations, and in response to typical harvest stresses are in progress. Initial results indicate that the cytochrome *c* respiratory pathway predominates in healthy unwounded and wounded sugarbeet roots, and that the relative capacities of the two pathways change little in response to wounding, time in storage, or storage temperature. Respiration was approximately 8-fold higher at the root surface and 1.5-fold higher in the internal tissue of the crown than in the root internal tissue.

INTRODUCTION

Respiration is the major cause of sucrose loss during postharvest storage of sugarbeet roots and is responsible for annual revenue losses in the millions of dollars. It is estimated that respiration is responsible for approximately 70% of the sucrose loss that occurs under favorable storage conditions (WYSE, R.E., 1973). Despite the economic importance of postharvest respiration to the sugarbeet industry, little is known about the enzymes and metabolic pathways

involved in sugarbeet root respiration or the physiological, biochemical or molecular factors that regulate them.

Two pathways contribute to respiration in plants. A cytochrome *c* oxidase and an alternative cyanide insensitive oxidase catalyze the final oxidative reaction in respiration, resulting in the reduction of molecular oxygen and the release of carbon dioxide. In nearly all plants, the cytochrome *c* oxidase pathway predominates and the alternative oxidase pathway is a minor contributor to total respiration. Stress conditions often encountered during sugarbeet harvest and postharvest storage, such as chilling, wounding or pathogen attack, however, have been demonstrated to increase respiration through the alternative oxidase pathway in other plant species (MOORE, A.L. *et al.*, 2002). The effect of these stresses on the alternative oxidase pathway in sugarbeet root is unknown.

The relative contribution of cytochrome *c* oxidase and alternative oxidase pathways to total respiration was examined in sugarbeet roots. The contribution of the two pathways was examined in different portions of the root at time of harvest, and the effects of wounding and time in storage on the relative capacities of the two pathways were determined at two storage temperatures.

MATERIALS AND METHODS

Sugarbeet hybrid VDH66156 (Van der Have, Netherlands) was greenhouse grown in Sunshine Mix #1 (Sun Gro Horticultural Products, Canada) in 15-liter pots with supplemental light under a 16-h light/8–h dark regime. Plants were hand harvested 16 to 18 weeks after planting and gently hand washed. Respiration was measured in different regions of the root immediately after harvest. For storage studies, roots were stored from 1 to 13 days after harvest at 1° and 10° C. Roots were placed into storage without treatment (unwounded) or bruised by tumbling in a pilot scale beet washer for 30 min (wounded). Respiration was measured as O_2 consumption at 25°C using an oxygen electrode (Hansatech, King's Lynn, UK). Tissue respiration measurements utilized the reaction conditions of Moore & Whitehouse (1997), using 0.8 mM KCN to inhibit cytochrome *c* oxidase and 10 mM or 15 mM salicylhydroxamic acid (SHAM) to inhibit alternative oxidase in surface or internal tissues, respectively. Mitochondria were isolated (DAY, D.A. & WISKICH, J.T., 1975) and the capacities of cytochrome c oxidase and alternative oxidase were assayed (VANLERBERGHE, G.C. *et al.* 2002) using 0.8 mM KCN and 1.5 mM SHAM to inhibit cytochrome *c* and alternative oxidases, respectively.

RESULTS AND DISCUSSION

Respiration rate was variable in different portions of the sugarbeet root (Table 1). Surface tissue respiration was approximately eight-fold greater than the internal tissues of the root. Surface tissue included the epidermis and one to two millimeters of underlying tissue; internal tissue was taken near the center of the root, but did not include tissue from the central vascular cylinder. Crown tissue respired at a rate approximately 1.5-fold greater than internal tissue. Crown tissue was sampled at the shoulder of the crown, one centimeter below the epidermis.

Differences in the relative capacities of the two respiratory pathways were observed. The cytochrome *c* oxidase pathway predominated in all regions of the root and accounted for 76, 89 and 68% of the respiratory capacity of the root internal tissue, crown tissue and surface tissues, respectively, based on preliminary studies using pathway specific inhibitors and whole tissue sections. Alternative oxidase capacity was greatest in the root surface tissues. Ongoing research will confirm the relative capacities of cytochrome *c* oxidase and alternative oxidase with isolated mitochondria.

Table 1. Total respiration and capacities of the cytochrome c oxidase and alternative oxidase pathways as a percent of total respiratory capacity in different areas of the sugarbeet root. Tissue was sampled 1 cm below the epidermis of the crown (internal crown tissue), 1 cm from the longitudinal center of the root at the widest portion of the root (internal root tissue) and at the outermost 1 to 2 mm of the root at its widest portion (surface tissues). Data are the mean of ten replicates ± SE.

	total ($nmol\ O_2\ min^{-1}\ g^{-1}$)	cyt. c (%)	AOX (%)
internal crown tissue	13.3 ± 1.5	88.5 ± 3.7	11.5 ± 3.7
internal root tissue	8.8 ± 1.3	76.4 ± 6.3	23.6 ± 6.3
surface tissues	73.5 ± 8.1	68.4 ± 3.2	31.6 ± 3.2

The effect of wounding and temperature on total respiration and the relative capacities of cytochrome *c* oxidase and alternative oxidase were determined with wounded and unwounded roots stored at 10° and 1° C for up to thirteen days. Total respiration was determined using whole tissue, and the relative capacities of cytochrome *c* oxidase and alternative oxidase were determined using isolated mitochondria. Tissue was sampled from the widest portion of the root, approximately 1 cm below the epidermis. At 10° C, the respiration rate of unwounded roots was nearly unchanged during thirteen days of storage (Table 2). Wounded roots, however, exhibited increased respiration throughout the duration of the experiment. Two days after harvest, the respiration rate of wounded roots increased three-fold from the respiration rate at harvest. After the second day in storage, the respiration rate of wounded roots declined, but remained higher than the unwounded control roots for the remainder of the experiment.

A significant increase (Fisher's LSD, " = 0.05) in relative capacity of the cytochrome *c* oxidase pathway and a corresponding decrease in the alternative oxidase pathway was observed during the first two days in storage at 10° C in both wounded and unwounded roots. The relative contribution of the two pathways returned to initial values by the fourth day in storage and remained unchanged for the remainder of the experiment.

At 1° C, wounded and unwounded roots exhibited similar changes in respiration during thirteen days in storage (Table 3). During the first week in storage, the respiration rates of wounded and unwounded roots were nearly unchanged, except for a small transient increase in respiration one day after harvest. With additional time in storage, the respiration rate of wounded and unwounded roots increased, and by the thirteenth day in storage, the respiration rate of all roots had increased approximately three-fold from the respiration rate at harvest.

The relative capacities of cytochrome *c* oxidase and alternative oxidase were relatively unchanged at 1° C in response to wounding or time in storage. A statistically significant increase in cytochrome *c* oxidase capacity, however, was observed in unwounded roots after six days in storage (Fisher's LSD, " = 0.05).

Table 2. Total respiration and capacities of cytochrome c oxidase and alternative oxidase pathways in greenhouse grown roots stored at 10° C with and without bruising. Total respiration is expressed as the percent change in respiration rate relative to the respiration rate at harvest (day 0). Capacities are expressed as the percent of total respiratory capacity. Data are the mean of four replicates ± SE. n.d., not done.

days after harvest	unwounded			wounded		
	total (% change)	cyt. c (%)	AOX (%)	total (% change)	cyt. c (%)	AOX (%)
0	100 ± 10	88.1 ± 1.1	11.9 ± 1.1	100 ± 10	88.1 ± 1.1	11.9 ± 1.1
1	139 ± 15	97.6 ± 0.9	2.4 ± 0.9	186 ± 29	97.4 ± 0.7	2.6 ± 0.7
2	113 ± 22	95.1 ± 2.1	4.9 ± 2.1	333 ± 62	96.2 ± 2.3	3.8 ± 2.3
3	104 ± 31	n.d.	n.d.	240 ± 26	n.d.	n.d.
4	127 ± 29	91.1 ± 0.3	8.9 ± 0.3	177 ± 29	90.7 ± 1.3	9.3 ± 1.3
7	144 ± 46	89.3 ± 1.2	10.7 ± 1.2	233 ± 48	89.6 ± 3.9	10.4 ± 3.9
13	120 ± 26	n.d.	n.d.	191 ± 29	n.d.	n.d.

Table 3. Total respiration and capacities of cytochrome c oxidase and alternative oxidase pathways in greenhouse grown roots stored at 1° C with and without bruising. Total respiration is expressed as the percent change in respiration rate relative to the respiration rate at harvest (day 0). Capacities are expressed as the percent of total respiratory capacity. Data are the mean of four replicates ± SE. n.d., not done.

days after harvest	unwounded			wounded		
	total (% change)	cyt. c (%)	AOX (%)	total (% change)	cyt. c (%)	AOX (%)
0	100 ± 10	88.1 ± 1.1	11.9 ± 1.1	100 ± 10	88.1 ± 1.1	11.9 ± 1.1
1	180 ± 31	84.0 ± 2.7	16.0 ± 2.7	135 ± 11	88.1 ± 3.4	11.9 ± 3.4
2	112 ± 20	n.d.	n.d.	99 ± 13	n.d.	n.d.
3	106 ± 15	87.1 ± 1.1	12.9 ± 1.1	108 ± 8	92.5 ± 1.9	7.5 ± 1.9
6	162 ± 34	94.3 ± 0.3	5.8 ± 0.3	110 ± 18	94.0 ± 0.6	6.0 ± 0.6
11	209 ± 55	90.5 ± 1.0	9.5 ± 1.0	190 ± 5	90.8 ± 1.3	9.2 ± 1.3
13	310 ± 31	88.5 ± 3.3	11.5 ± 3.3	283 ± 58	91.6 ± 0.7	8.4 ± 0.7

CONCLUSIONS

The cytochrome *c* oxidase pathway is the predominant respiratory pathway in sugarbeet root, as it is in nearly all plant species. In sugarbeet roots, the cytochrome *c* oxidase pathway accounted for approximately 85 to 95% of the total respiratory capacity of internal root tissues (Tables 2 & 3). Although a lower relative capacity for the cytochrome *c* oxidase pathway was observed in internal root tissue (76%) using whole tissue sections (Table 1), this data requires confirmation with isolated mitochondria and should be viewed as preliminary. Preliminary data also suggest that surface tissues differ from internal root tissue in the relative capacities of the two respiratory pathways with

the alternative oxidase pathway contributing more to total capacity at the root surface than in internal tissue. This observation will be confirmed in future research using isolated mitochondria.

Few changes in the relative capacities of cytochrome *c* oxidase and alternative oxidase were observed in response to wounding, storage temperature or duration in storage. Where differences were noted, the relative capacity of cytochrome *c* oxidase increased relative to alternative oxidase. This contrasts with other plant species in which an induction of alternative oxidase in response to wound and chilling has been documented (MOORE, A.L. *et al.*, 2002).

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