SHORT COMMUNICATION

DO EXTERNAL OXYGEN LEVELS INFLUENCE SUCROSE METABOLISM IN THE SUGARCANE STALK?

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Abstract

Comparison of gene expression and metabolite profiles amongst internodes of different developmental status has provided indirect evidence of a transition from normal to low O_2 conditions during internode maturation. The inference is based on high abundance in mature internodes of transcripts of anoxia-responsive genes and low levels of tricarboxylic acid cycle enzyme transcripts and metabolites. To investigate this correlation further, the current study determined the effects of anoxic, normoxic and hyperoxic conditions on the levels of selected sucrose metabolites and the activity of SuSy, UDPGlcPPase and three invertase isoforms. The results are discussed in terms of the possible influence that hypoxic conditions may have on sucrose storage in the stalk.

Keywords: sugarcane, sucrose metabolism, hypoxia, sucrose synthase, invertase

Background

Comparison of gene expression and metabolite profiles amongst internodes of different developmental status has provided indirect evidence of a transition from normal (normoxic) to low (hypoxic) O_2 conditions during internode maturation. The inference is based on high abundance in mature internodes of transcripts of anoxia-responsive genes (e.g. alcohol dehydrogenase) (McCormick, 2003; Watt *et al.,* 2005) and low levels of tricarboxylic acid cycle enzyme transcripts (e.g. malate dehydrogenase) and metabolites (e.g. malate) (Figure 1). Although hypoxic internal environments are common in bulky plant organs (e.g. tubers) (Bologa *et al*., 2003) and dense, metabolically-active tissues (e.g. in seeds) (Rolletscheck *et al*., 2002*)*, this is the first indication that such conditions may occur in mature sugarcane internodes. Hypoxia has several effects on metabolism, including the restriction of glycolytic and respiratory activity and the induction of energetically-conservative pathways (Geigenberger *et al*., 2002). Restricted O_2 availability in the mature internode may result in the repression of the invertases (EC 3.2.1.26) (2 ATP molecules utilised per sucrose [Suc] molecule) in favour of cleavage via the energetically less expensive pathway of sucrose synthase (SuSy) (EC 2.4.1.13) and UDP-glucose pyrophosphorylase (UDPGlcPPase) (EC 2.7.7.9) (1 pyrophosphate molecule per sucrose molecule) (Figure 1). A correlation between internode maturity and expression level of these genes has been confirmed, with transcript abundance of a vacuolar acid invertase gene and a SuSy gene decreasing and increasing, respectively, with internode maturity (Figure 1).

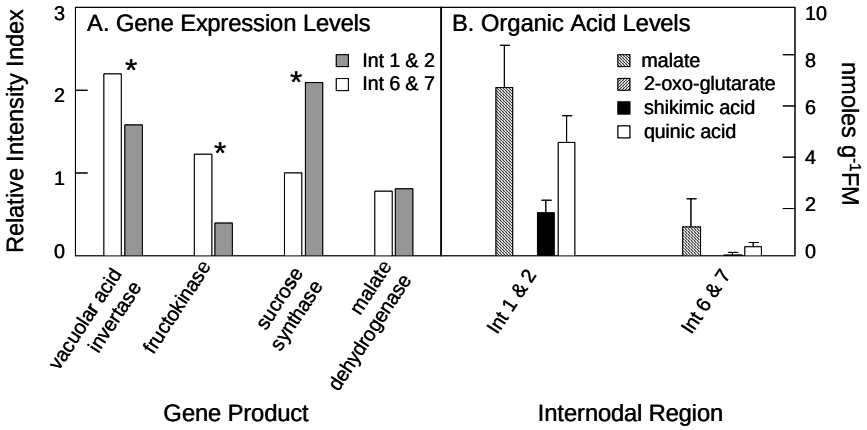

Figure 1. Variations in gene transcript and metabolite levels associated with culm development in N19. Gene transcript levels (A) were determined by cDNA array analysis and the abundance data are expressed as a Relative Intensity Index (McCormick, 2003). Metabolite concentrations (B) were measured by means of Gas Chromatographic-linked Mass Spectroscopy. Values represent the means of at least three replicates, and significant differences ($P<0.05$) in transcript levels (A) between culm regions are indicated by an asterisk.

Objective

The objective of this preliminary investigation was to assess whether *in vitro* variations in external O_2 availability to excised internodes influenced metabolism in a manner similar to previously observed correlations to internode maturity. To this end, excised mature internodes (internode 12) of mature (12 to 14 month-old), field-grown *Saccharum* spp. hybrid cv. N19 (N19) were subjected to 24-hour anoxic (0% O_2), normoxic (21% O_2) and hyperoxic (99% O_2) treatments. After harvest, the levels of malate, lactate, Suc, glucose (Glc) and fructose (Fru) were determined, as was SuSy activity in both the synthetic and cleavage directions.

Results

- Decreased external O_2 levels resulted in an increase in lactate concentration and a decrease in malate levels, verifying the capacity of the *in vitro* treatments to perturb carbon metabolism (Table 1).

- Variations in O_2 concentration did not result in significant changes in Suc, Glc and Fru levels, although there was a general increase in the Fru:Glc ratio in response to low external oxygen (Table 1).

- SuSy activity in the Suc cleavage direction declined with increasing external O_2, while the synthetic activity of the enzyme was not affected by the treatments applied (Table 1).

Table 1. Effect of external O_2 on levels of selected organic acids, sugars and SuSy activity. Metabolite levels (lactate, malate, Suc, Glc, Fru) and SuSy activity were determined in excised internode 12 of N19 exposed to 0%, 21% and 99% O_2 for 24 h. The control represents the metabolite and SuSy activity levels prior to the application of the O_2 treatments. For metabolites, the values represent the mean of five to six replicates, while the SuSy activity data are the average of at least two determinations (ND = not determined).

	Treatment			
	Control	0% O_2	21% O_2	99% O_2
Organic Acids:				
Lactate (μmol/g DW)	2.395 ± 0.292	3.660 ± 0.380	1.331 ± 0.523	1.953 ± 0.929
Malate (μmol/g DW)	0.552 ± 0.235	0.364 ± 0.315	1.060 ± 0.689	1.912 ± 0.224
Sugars:				
Sucrose (mmol/g DW)	1.543 ± 0.091	1.434 ± 0.167	1.543 ± 0.085	1.417 ± 0.263
Glucose (μmol/g DW)	4.415 ± 1.987	7.220 ± 4.478	6.887 ± 4.108	14.018 ± 3.246
Fructose (μmol/g DW)	4.606 ± 2.053	9.984 ± 2.714	7.309 ± 3.602	8.956 ± 4.206
Fru:Glc Ratio	1.1	1.4	1.0	0.6
Sucrose Synthase Activity:				
Synthesis (μkatal/mg protein)	12.770 ± 3.84	6.210 ± 4.18	ND	14.560 ± 6.83
Cleavage (μkatal/mg protein)	30.750 ± 4.70	27.830 ± 6.91	ND	12.290 ± 8.67

Discussion and Future Work

Rates of SuSy activity in the cleavage direction were similar in the controls (30.75 ± 4.70 μkatal/mg protein) and internodes subjected to anoxic conditions for 24 h (27.83 ± 6.91 μkatal/mg protein) (Table 1). However, in internodes exposed to 99% O_2, the Suc cleavage activity of the enzyme was lower (approximately 12.29 μkatal/mg protein) (Table 1). This decline in SuSy activity in response to elevated O_2 suggests that the previously reported decrease in activity (Botha and Black, 2000) and gene expression (Figure 1) (Watt *et al*., 2005) of this enzyme during internode development may be associated with the onset of hypoxia in mature internodes. The similar malate levels observed during internode maturation (Figure 1B) and exposure to low O_2 levels (Table 1) further supports the notion of a possible link between internode maturation and hypoxia. Investigation of the activity of the three invertase isoforms under these experimental conditions, currently in progress, will be necessary to confirm whether the route of Suc cleavage is influenced by prevailing O_2 levels.

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