Differential expression of the functional marker candidate *AOX* is related to stress-induced reprogramming of growth behavior in a primary culture of *Daucus carota* L.

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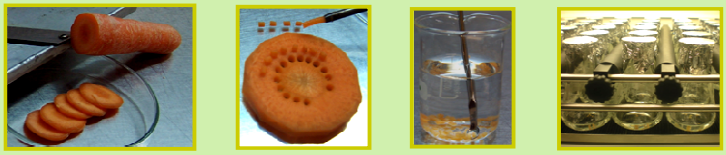
ABSTRACT

Alternative oxidase (AOX) is suggested to play a crucial role in efficient cell reprogramming under stress (Clifton et al., 2006 and Arnholdt-Schmitt et al., 2006). In order to focus on the dynamics of gene expression during development and growth, a carrot primary culture experimental approach was chosen. *AOX* expression is being studied under controlled temperature conditions after inoculation of differentiated secondary root phloem explants in an auxin and cytokinin-containing nutrient solution that induces tissue redifferentiation to callus growth. This poster presents the studies by qRT-PCR in order to search for *AOX* differential gene expression between individual plants.

MATERIALS AND METHODS

1. PRIMARY CULTURE SYSTEM



Slices from the third upper part of carrot tap roots of nine individual plants (cv.Rotin) were cut and the explants (2-4 mg) of the secondary phloem were inoculated in NL liquid medium (Neumann 1966) (see pictures above). The cultures were incubated under continuous rotation (90 rpm) at continuous light (95-100 µmol m^{-2} s^{-1}, Philips) for 14 days at 21ºC and 28ºC. Samples for *AOX* expression analysis were collected after desinfection (T0) and 14 days after inoculation (T14). At harvest (T14) the fresh weight of each *callus* was determined.

2. RNA EXTRACTION AND cDNA SYNTHESIS

Total RNAs were extracted using the RNeasy Plant Mini Kit (Qiagen), with on-column digestion of DNA with the RNase-Free DNase Set (Qiagen). Two µg of total RNA were used to synthesize cDNA with the RETROscript kit (Ambion) using the oligo d(T) primer.

3. qRT-PCR

cDNA samples were diluted (1:20) in water and 4 µl were used as template. In order to identify optimal reference genes for qRT-PCR, specific primers for five carrot genes were tested (see table bellow). Specific primers for *DcAOX1a* and *DcAOX2a* (Frederico et al., 2009) were used to analyze the expression levels of both carrot *AOX* genes.

qRT-PCR were performed with the Maxima SYBR Green q-PCR Master Mix (Fermentas) on a 7500 Real Time PCR System (Applied Biosystems). Four-point standard curves of a 4-fold dilution series (1:1-1:125) (run in triplicate) from pooled cDNA were used for PCR efficiency calculation. The samples were run in duplicate and no template controls were included in all plates.

Evaluation of expression stability of reference genes was done using the statistical application *geNorm* (Vandesompele et al., 2002).

Gene	Acc nº
translational initiation factor 1 ***(TIF1)***	DQ898156
heat shock protein 70 ***(HSP70)***	X60088
beta-tubulin 2 ***(βTub2)***	U63927
elongation factor 1-alpha ***(EF1α)***	GQ380566
Actin	X17526 and X17525

RESULTS AND DISCUSSION

1. PRIMARY CULTURES OF NINE PLANTS

Root weight (g)	*Callus* fresh weight (mg)				
	n	28ºC	n	21ºC	
298,90	50	42,4	53	39,9	**R1**
120,20	55	64,1***	47	24,8	
173,56	62	62,2***	76	26,3	
187,83	38	28,7	35	25,5	
126,63	77	55,0***	64	21,4	**R2**
54,19	62	28	65	32,8*	**R3**
76,07	36	28,5***	34	9,0	
108,24	61	25,7	65	23,6	
128,85	60	21,8***	60	14,6	

*, *** significantly higher at p<0,05 and 0,001

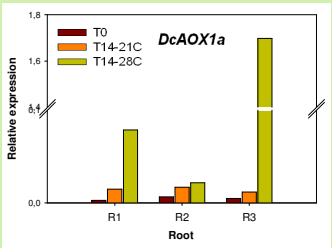
- Calli FWs of five plants were higher at 28ºC than at 21ºC.
- Three plants did not show differences in calli FWs between temperatures.
- Calli FWs of one plant showed higher values at 21ºC than at 28ºC.
- Due to the different growth behavior **R1**, **R2** and **R3** were selected for qRT-PCR expression analysis.

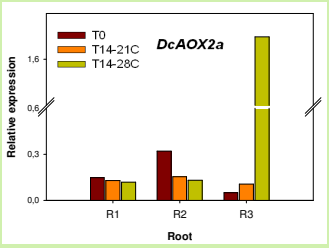
2. VALIDATION OF HOUSEKEEPING GENES

DcEF1α and ***DcTIF1*** were selected by *geNorm* as the most stable and suitable gene pair for the primary culture studies.

Both genes were therefore used to normalize *DcAOX1a* and *DcAOX2a* genes expression.

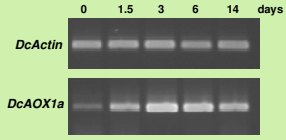
3. *DcAOX1a* AND *DcAOX2a* GENE EXPRESSION ANALYSIS BY qRT-PCR





- ***DcAOX1a*** is up-regulated during primary culture induction. Increased levels of *DcAOX1a* transcript accumulation can be observed at 28ºC in two of the three roots. However, its expression seems not to be related to the growth rate (R2 shows the highest growth rate) but instead with the induction of growth.

- Campos et al. (2009) refer early activation of *DcAOX1a* during growth induction, showing a clear up-regulation of expression with a peak after three days, still a high level of expression at the end of the lag-phase (day 6) and a decline at day 14 (see gel below).



-***DcAOX2a*** demonstrates differential expression between plants.

REFERENCES

Arnholdt-Schmitt et al. (2006) Trends in Plant Sci, 11:281-287; Campos et al. (2009) Physiologia Plantarum, 137:578-591; Clifton et al. (2006) Bioch et Bioph A, 1757:730-741; Frederico et al. (2009) Physiologia Plantarum, 137:498-508; Neumann K (1966) Phytohorm Organogen, 38:95-102; Vandesompele et al. (2002) Genome Biol, 3: RESEARCH0034.

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