Gene Expression in Cold-stressed Barley as Detected by Microarray Analysis

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Introduction and Goal

Abiotic stresses, such as drought, cold or high salt, influence cereals plant growth, productivity and quality (e.g. freezing and dehydration represent osmotic stress and destabilize the cell membranes (Wang, 2003)). When plants are exposed to these stresses, they pass through physiological and biochemical adaptations. Variety of genes are expressed under adverse conditions in plants (Thomashow, 1999). During cold stress, the expression of many genes is up- or down-regulated. Advantage of microarray technology is that provide high throughput tool for analyzing a great number of genes.

Goal of this work is to find genes in spring and winter barleys that are influenced by cold stress and to analyze their expression profiles during stress. A few of experiments reported influence of temperature on the leaves and crown nodes, that is why we focus our study also on crown nodes by microarray technology.

Materials and Method (scheme shown in **Figure 1**)

♠ PLANTS AND COLD STRESS

barley cultivars: spring ´Atlas68´ (USA), winter ´Igri´ (DEU) and ´Kos´ (PL) with moderate hardiness, winter ´Okal´ (CZE) with extraordinary one → germination and cultivation in box (Tyler): at 18 °C with 12 h/12 h light/darkness at 400 µmol m^{-2} s^{-1} in nutritive solution Hydropon (2% P_2O_5, 4% K_2O, 3% N and trace elements) into a phase of 3 leaves → cold stress: at 3 °C for 12 h, 24 h and 96 h, control plants taken before cold stress, leaves and crown nodes were collected from plants

♠ TARGET PREPARATION

331 EST barley clones (ISC Fiorenzuola d´Arda, Italy) obtained from a cDNA library (leaves of cv. Nudinka) → amplification with T3/T7 or M13 universal primers (annealing 50 °C or 58 °C, 35 cycles) → control by gel electrophoresis → purification by Qiagen Kit → 10 µl 400 ng/µl in 10 µl spotting solution Corning printed on Corning GAPS™ II amino-silane coated slides by microarray printing system (MicroGrid II) → crosslinking (Ultra Lūm) at 250 mJ for 10 min. → prehybridization at 42 °C/4 h using prehybridization solution (5x SSC, 0.1% SDS, 10 mg/ml BSA), washing: 2x dH_2O and 2x 95% EtOH, drying

♠ PROBES PREPARATION

♣ RNA Isolation: 500 mg of plant crown nodes and leaves by modified procedure according Atienza (2004) → purification RNeasy Plant Mini Kit (Qiagen)

♣ Labelling probes: reverse transcription from 15 µg of total RNA and fluorescently labelled with the fluorochromes cyanine-3 (Cy3) and cyanine-5 (Cy5), duplicating labelling and hybridizaton by swapping the fluorescent dyes (Atienza, 2004)

♠ HYBRIDIZATION, SCANNING AND DATA EVALUATION

manual hybridization in chambers in water bath at 42 °C for 16 h using hybridization buffer ArrayIt Hyb2, washing: 3x (3x SSC + 0,01% SDS) and 1x (1xSSC), drying → scanning by red and green laser (GeneTAC UC4) using GT UC program → data evaluation by applied GeneTAC Integrator 4.0

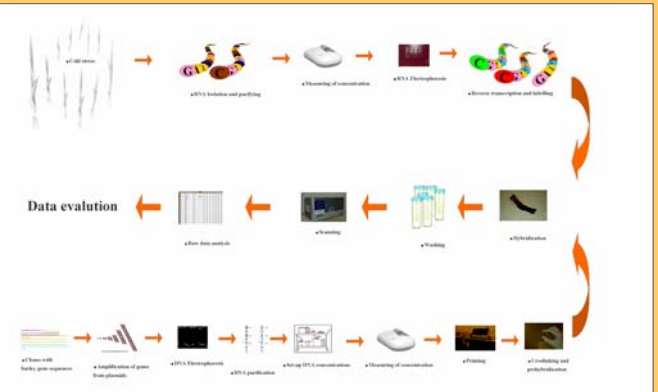


Figure 1 Operating sequence of gene expression study using microarray technology

Results and Discussion

♠ Crown nodes and leaves were used to analyze gene expression in barley cultivars spring ´Atlas68´ (USA), winter ´Igri´ (DEU), ´Kos´ (PL) and ´Okal´ (CZE) with different hardiness levels: before (as control sample) and after cold stress (12 h, 24 h, 96 h).

♠ 331 EST barley clones as targets were amplified and checked by gel electrophoresis (some DNA target shown in **Figure 2**) → now we are looking for suitable data normalization approaches to compare them with parameter Fold Change from the program GeneTAC Integrator 4.0. (shown in **Figure 3**).

$$Fold\ Change = \frac{Experimental\ Channel}{Control\ Channel} * Norm\ Factor$$

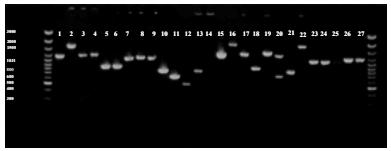


Figure 2 Agarose gel of some barley clones prepared to printing on slides

♠ We present partial results of crown nodes of the cultivar of ´Atlas´ (details shown in **Table 1**).

Clone No. ISC002.A02F990329 (dehydrin 5, TC5600-20KDa photosystem I subunit) had Fold Change grater than 2 after 12 h and 96 h stress, behaves as up-regulated gene but after 24 h as down-regulated.

Clone No. ISC005.G03F990617 (cold acclimation protein Blt101) and clone of Oxalate oxidase are down-regulated genes after 24 h but up-regulated genes after 96 h stress → similar results but under different conditions were published by Pearce (1998), Faccioli (2002) and others.

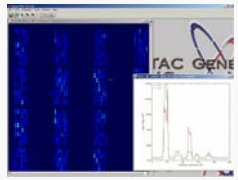
Table 1 Some clones with interesting expression changes at spring cultivar ´Atlas´ during 12 h, 24 h and 96 h cold stress

Stress 12 h				
Clone ID	Mean (control Cy3)	SD	Mean (control Cy5)	SD
ISC001.C04F990317	-1.98	0.06	-1.12	0.05
HVSMEk05J07	-1.71	0.14	-1.27	0.06
ISC008.A05F990804	1.54	0.11	2.49	0.51
HVSMEa22P10	1.73	0.31	2.33	0.12
ISC005.G03F990617	1.88	0.11	2.13	0.17
ISC005.G02F990621	2.30	0.15	1.85	0.06
ISC002.A02F990329	3.03	0.42	3.77	0.19

Stress 24 h				
Clone ID	Mean (control Cy3)	SD	Mean (control Cy5)	SD
ISC002.A02F990329	-11.49	1.35	-7.80	1.06
ISC008.A05F990804	-3.63	0.30	-2.92	0.43
ISC005.G03F990617	-2.97	0.18	-2.77	0.65
HVSMEa22P10	-3.16	0.19	-2.61	0.20
HVSMEe25M19	-2.97	0.12	-2.21	0.11
Oxalate oxidase HvOxOa	-2.02	0.26	-1.15	0.02
Cab (LHCII type I - cl27)	1.46	0.10	1.98	0.05
Cab (LHCII type I - cl538)	1.96	0.12	2.06	0.18
ISC007.D10F990713	1.88	0.25	2.37	0.19
ISC005.B12F990613	1.21	0.07	2.43	0.16
Cab (LHCII type I - cl32)	1.86	0.24	2.55	0.22

Stress 96 h				
Clone ID	Mean (control Cy3)	SD	Mean (control Cy5)	SD
ISC005.B12F990613	-4.71	0.34	-2.19	0.09
Cab (LHCII type I - cl27)	-3.82	0.27	-2.28	0.32
ISC007.D10F990713	-3.69	0.25	-2.93	0.38
Cab (LHCII type I - cl32)	-3.63	0.56	-2.98	0.43
HVSMEe08P23	-3.52	0.25	-1.94	0.13
ISC007.G02F990714	-3.45	0.37	-2.00	0.05
Cab (LHCII type I - cl538)	-3.32	0.32	-3.06	0.22
ISC003.B05F990518	-2.73	0.26	-1.63	0.12
ISC003.G07	-2.70	0.12	-2.05	0.10
Cab (LHCII type I - cl514)	-2.63	0.10	-1.69	0.07
Oxalate oxidase HvOxOa	1.81	0.10	2.73	0.20
HV_CEb01J01	1.87	0.08	2.11	0.16
HVSMEe25M19	1.96	0.18	2.08	0.36
HVSMEa22P10	1.99	0.07	2.26	0.34
HVSMEb19P16	2.20	0.21	3.00	0.34
HVSMEt24P20	2.49	0.04	2.53	0.35
HVSMEt21B07	2.56	0.05	2.49	0.13
ISC007.F01F990706	2.58	0.28	2.44	0.15
ISC005.G03F990617	4.95	1.11	3.20	0.05
ISC002.A02F990329	8.39	0.79	9.11	0.50

3a



3b

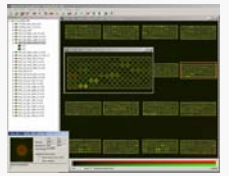


Figure 3 Raw scan of slide with spring cultivar ´Atlas68´ during 96 h cold stress (control Cy3) above (**a**) and same slide in the evaluation program below (**b**)

Conclusion and Future

♠ More clones were expressed after 96 h stress than after 24 h or 12 h.

♠ Some interesting candidate genes will be confirmed by quantitative real-time PCR in the future.

We have to find better data evaluation by more precisely statistic method to confirm these results and to compare them with gene expression profiles in other barley cultivars.

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