Gene Amplification from Cryopreserved *Arabidopsis thaliana* Shoot Tips

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Abstract
Cryopreservation is a way to store elite quality plant germplasms. The exact mechanism of stress tolerance during cryopreservation is unknown. Unavavailability of a detailed protocol for understanding the moelcular genetics of plant cryostress is a major obstacle in plant cryobiology research. This paper describes the methods of extraction of total RNA from cryogenically stored plant tissues accompanied by successful amplication of cDNAs by reverse transcriptase PCR. The whole process can be completed in two to three days. Through this protocol, several genes were indentified which were differentially expressed during cryostress. This protocol will help researchers to pursue further research in the field of molecular genetics of plant cryostress. Interesting genes identified via these processes can be cloned and plants can be transfomred for the purpose of trait enhancement and modification.

Introduction
Cryopreservation refers to storing of plant materials at low temperature (-196°C). At this low temperature all cellular and biochemical activities, including the pathways leading to cell death, are essentially stopped. Vitrification (use of cryoprotectants) is used to inhibit the formation of intracellular ice crystals, as ice crystals will ultimately lead to cell death. Cryopreservation is an important part of germplasm preservation and enhancement of agriculturally important crops. We do have a good understanding of genomics of plant cold stress (Thomashow, 2001), but, unfortunately, there are not much previous reports to understand the molecular genetics of cryostress in plants.

It has been reported that chloroplast targeted cor 15A protein has some cryoprotective activity (Lin and Thomashow, 1992). Hightower et al. (1991) reported production of transgenic tobacco and tomato plants expressing high levels of afa3 antifreeze proteins. Inhibition of ice recrystalization was observed in some of the transgenic tissues expressing the afa3 proteins. Harding (1991) studied ribosomal gene (rDNA) expression in cryopreserved potato (*Solanum tuberosum* cv. Golden Wonder). It was reported that rDNA expression was unchanged in cryopreserved potatoes. Elleuch et al. (1998) transformed opium poppy (*Papaver somniferum*) by sam1 gene from *Arabidopsis thaliana.* They also concluded that the sam1 expression was unchanged in opium poppy even when stored at -196°C for cryopreservation. It was also reported that neomycinphosphotransferase II (npt II) gene was found to be stably expressed after cryopreservation in transgenic lines of hybrid aspen (*Populus tremula* L.×*Populus tremuloides* Michx.) (Jokipii et al., 2003). Blanc et al. (2005) reported that two cycles of cryopreservation of rubber tree (*Hevea brasiliensis*) increased the transformation efficiency in transgenic rubber plants. Hao et al. (2004) assessed the GUS gene expression from cryogenically stored pear (*Pyrus pyrifolia* cv. Okusankichi). They reported functional GUS gene expression in transgenic pears even after cryopreserved for 1 year. Similar types of observations were reported by Tereso et al. (2006). They also reported that transgenic maritime pine (*Pinus pinaster* Sol. ex Aiton) continued to express GUS gene after recovered from cryopreservation.

The above reports describe some of the efforts by researchers to understand molecular genetics of plants under cold stress, specifically cryostress. However, an in depth protocol, which could be used for gene amplifications in cryogenically stored *Arabidopsis thaliana* shoot tips is still needed. This paper describes detailed step-by-step procedure for RNA extraction from cryopreserved *Arabidopsis thaliana* plants followed by reverse transcriptase PCR to amplify few cryostressed genes. I hope that the data presented here will open doors to further research in the field of molecular genetics plant cryostress. Understanding the molecular and genetic basis of plant responses to cryostress will also open doors to further research in many aspects of plant abiotic stress tolerance.

Materials and Methods

Plant materials
Arabidopsis thaliana seeds (cv. Columbia) were surface sterilized and grown in standard tissue culture media (Towill et al., 2006) the for seven days. Plant shoot tips were treated in the following ways:

Treatment 1: Shoot tips were collected from 7-day-old *Arabidopsis* plants, immersed in 0.3 M sucrose overnight and then flash-frozen in liquid nitrogen for RNA extraction. This treatment will serve as the 'control' in our experiments.

Treatment 2: Shoot tips were collected from 7-day-old *Arabidopsis* plants, immersed in 0.3 M sucrose overnight, treated with 2 M glycerol + 0.4 M sucrose for 20 minutes at 22°C, 15 minutes PVS3 (50% glycerol, 50% sucrose in water) at 0°C for 15 minutes, and then flash-frozen in liquid nitrogen for RNA extraction.

Treatment 3: Shoot tips were collected from 7-day-old *Arabidopsis* plants, immersed in 0.3 M sucrose overnight, treated with 2 M glycerol + 0.4 M sucrose for 20 minutes at 22°C, 15 minutes PVS3 (50% glycerol, 50% sucrose in water) at 0°C for 15 minutes, flash-frozen in liquid nitrogen and held in liquid nitrogen overnight, then thawed in 1.2 M sucrose at 22°C for 20 minutes and plated onto MS medium (Murashige, and Skoog, 1962). The shoot tips were harvested for RNA extraction after 6h or 16h recovery on the medium.

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The treated plant materials (described above) were kindly provided by Dr. Gayle Volk, USDA National Plant Germplasm Preservation Center, Fort Collins, USA.

RNA extraction

Total RNA from *Arabidopsis* shoot tips were extracted using Plant RNA Extraction Kit (Agilent Technologies, USA) following manufacturer's protocol with minor modification. 100 mg. of *Arabidopsis* shoot tissue was ground in liquid nitrogen in RNAase free mortar and pestle. 1 ml. of extraction solution (provided with the kit) and 10 ul. of beta mercaptoethanol were added to the mortar with the plant tissue sample and ground thoroughly. The tissue mix was allowed to thaw for few seconds and transferred to a mini prefiltration column and centrifuged for 3 min. at 16000g. Equal volume of isopropanol was added to the filtrate and incubated for 5 min. at room temperature. The filtrate was transferred to a mini isolation column and centrifuged for 30 min. at 16000g. The flow through was discarded and the column with bound RNA was put back onto the same collection tube. 500 ul of wash solution was added to the mini isolation column and centrifuged for 1 min. The flow through was discarded again and the isolation column was put back onto the same collection tube. 500 ul of wash solution was added to the mini isolation column and centrifuged for 1 min. The flow through was discarded and the column was put back onto a fresh collection tube and the empty column was centrifuged again for 1 min. to remove any excess ethanol that might have trapped in the collection tube. There may be some residual ethanol flow through in the collection tube. The collection tube along with some residual ethanol was discarded. The empty column with bound RNA was transferred to a 1.5 ml. microfuge tube and 50 ul of nuclease free water was added directly into the column. After waiting for 1 min. the column was centrifuged at 16000g for 1 min. The eluted RNA was checked for quality using a NanoDrop spectrophotometer (NanoDrop Technologies, USA).

RNA extraction was performed from two separate batches of plant samples for each of the three treatments (two sets of biological replications as described in Fig. 1) and after cDNA synthesis (described below), cDNAs from both replications were pooled and reverse transcriptase polymerase chain reaction (RT PCR) reactions were performed.

First strand cDNA synthesis

cDNA synthesis was performed using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA) following manufacturer's protocol as follows. As a first step, the following components (cDNA synthesis mix) were added into a thin-walled 0.5 ml. PCR tube: 1 ug total RNA (from each of the three treatments), 1 ul 50 uM oligo $(dT)_{20}$, 1 ul 10 mM dNTP mix and DEPC (diethylpyrocarbonate) treated water to bring to volume of 10 ul.

The above mix was incubated at 65°C for 5 min. and placed on ice for 1 min. Then the following components were added in the order indicated below (as recommended by the manufacturer). The amount for each component is for 1 reaction only which can be scaled up according to need: 2 ul 10X RT buffer, 4 ul 25 mM $MgCl_2$, 2ul 0.1M DTT, 1 ul RNaseOUT (40 U/ul), 1 ul Superscript III RT (200 U/ul), and 10 ul of cDNA synthesis mix from above.

The above mix was briefly centrifuged and incubated at 50 °C for 50 min. The reaction was completed with an incubation at 85 °C for 5 min. followed by incubation in ice for 1 min. The reaction mix was briefly centrifuged and 1 ul. of RNase H was added to the tube and incubated at 37 °C for 20 min. The synthesized cDNAs were stored at -20 °C.

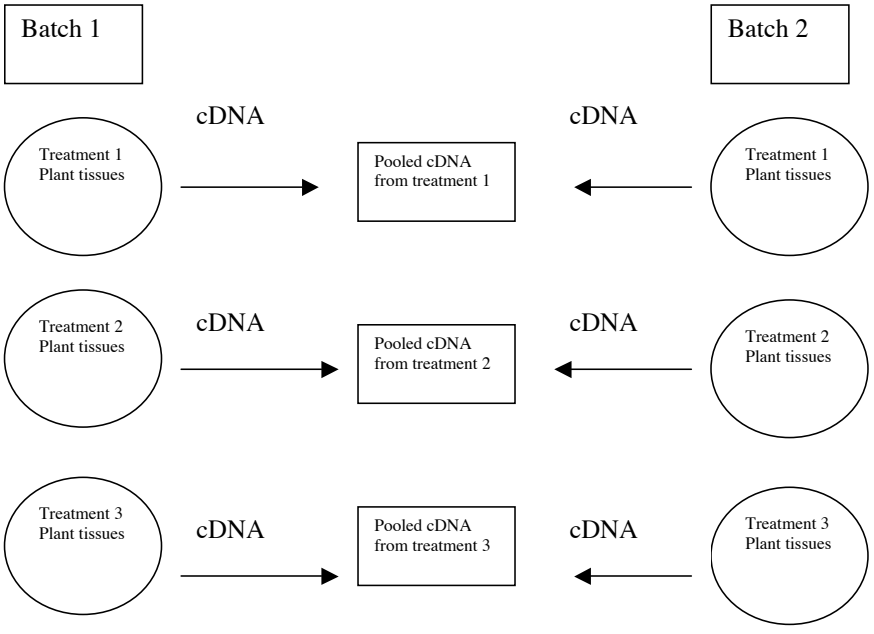

Fig. 1. Schematic representation of the experimental design.

Primer design

The following cold and drought induced genes were chosen from previous work by scientists and from GenBank database (http://www.ncbi.nlm.nih.gov/): **AT1G27730, AT2G33380, AT3G53110, AT4G20260.** The sequences of these genes were used to design primers for the RT PCR. Primers were ordered from Invitrogen, USA and primers were designed at www.invitrogen.com. 200 ng of cDNA was used for RT PCR. The primer sequences for the above mentioned genes are as follows:

AT1G27730: AGTCGAGCACTGGACAAAGGGTAA (5 → 3')
TTGTGACCACCGAGAGAGCTTGGTAA (3' → 5')
AT2G33380: GTGCCATCACCATTATTGCCGGTT (5' → 3')
GTCTTGTTTGCGAGAATTGGCCCT (3' → 5')
AT3G53110: ACCATATGCTTGCTACGGATGGGT (5'→ 3')
AACCAAATTCACCCGCTGTTGGTC (3' → 5')
AT4G20260: CGCTGCAAAGAAGCTCGTGAAAGA (5' → 3')
AGATGGTCCAAACCGGGTAAACGA (3' → 5')

Reverse transcriptase PCR

The cDNAs synthesized from three different treatments were amplified using the primers (described above). PCR was performed as follows. The amount for each component is for 1 reaction only which can be scaled up according to need: 12.5 ul 2X PCR master mix (Fermentas, USA), 1 ul forward primer (1 uM), 1ul reverse primer (1 uM), 200 ng cDNA and nuclease free water up to 25 ul.

25 ul of this PCR mix was loaded onto a MJ Mini thermal cycler (BIO-RAD, USA) with the following conditions: Cycle 1: 94°C for 2 minutes, Cycle 2: 95°C for 30 seconds, Cycle 3: 56°C for 30 seconds, Cycle 4: 72°C for 30 seconds, Cycle 5: cycles 2-4 were repeated 40 times, Cycle 6: 72°C for 3 minutes, Cycle 7: hold at 4°C. The RT PCR products were run in 1% agarose gels (stained with SYBR safe, Invitrogen, USA for nucleic acid visualization) at 65 volts. A negative control (water) was also used for PCR besides the cDNAs from three treatments.

Critical steps in the procedure

- RNA extraction: All tools including pipettors, mortars and pestles, including hands (with gloves on) were sprayed with Rnase away (Molecular Bio Products, USA). All water used must be either DEPC treated or nuclease free. All pipette tips must be certified nuclease free.
- cDNA synthesis and PCR: It is advisable to spray the pipettors and hands (with gloves on) with DNA-ExitusPlus (AppliChem, Germany) to avoid any cross contamination with DNA. All pipet tips must be cerified nuclease free. Either nucelase free or DEPC treated water must be used. Try to avoid loading too much DNA for the PCR reaction (200 ng of DNA should be more than enough to amplify the genes).

Troubleshooting

Problem

RNA yield is low

Possible solution

Cryogenically stored plant tissues may contain glycerol and other cryo protectants which may clog the filters of the columns used for RNA extraction. Be sure to use liquid nitrogen to grind the tissue very well. This helps in less clogging of the columns.

Problem

False positive band in PCR

Possible solution

The water or primer may be contaminated. Use fresh water. Use the primers to amplify a negatve cotrol sample (water) and if you still see bands then your primers may be contaminated, so they need to be replaced with new ones.

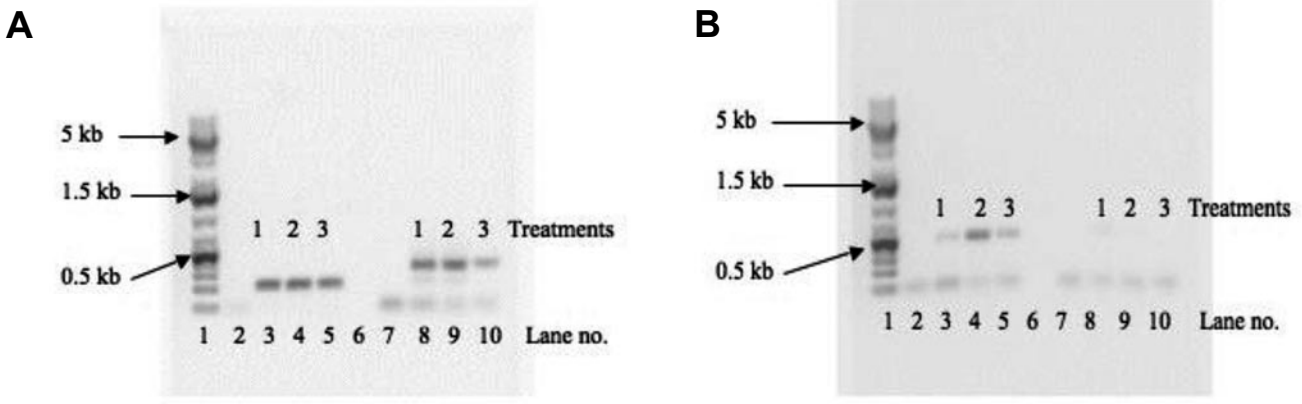

Fig. 2: Reverse transcriptase PCR of several cryostressed genes in *Arabidopsis thaliana*. Lane 1 of both the gels was loaded with GeneRuler™ 1kb DNA Ladder Plus (Fermentas, USA). (**A**) Lanes 2-5: RT PCR of **AT1G27730** gene, lane 2: negative control (PCR of water with **AT1G27730** primers). lane 6: blank, Lanes 7-10: RT PCR of **AT2G33380**, , lane 7: negative control (PCR of water with **AT2G33380** primers). (**B**) Lanes 2-5: RT PCR of **AT3G53110** gene, lane 2: negative control (PCR of water with **AT3G53110** primers). Lanes 7-10: RT PCR of **AT4G20260**, lane 6: blank, lane 7: negative control (PCR of water with **AT4G20260** primers).

Results and Discussion

This paper demonstrates isolation of good quality RNA followed by cDNA synthesis and RT PCR to amplify cryostress induced genes. As expected, cold and drought induced genes were found to be differentially expressed (Fig. 2A and 2B). From the RT-PCR reactions (Fig. 2A and 2B), it is clear that **AT2G33380** and **AT3G53110** were differentially-expressed during cryopreservation of the *Arabidopsis* shoot tips.

AT1G27730 was equally expressed in all three treatments (Fig. 2A). It encodes a zinc finger protein (Zhang et al., 2002) and is induced by a precursor of jasmonic acid (Taki et al., 2005).

AT2G33380 is a calcium ion-binding protein and responsive to desiccation in *Arabidopsis thaliana* (Source: GenBank). **AT2G33380** was reported to be differentially expressed in plants under salt stress (Taji et al., 2004). Liu et al. (2005) reported **AT2G33380** was expressed in *Arabidopsis* due to hypoxia. **AT2G33380** was also shown to be upregulated in presence of NaCl, and manitol in genome-wide microarray experiments in *Arabidopsis thaliana* (Kreps et al., 2002). **AT2G33380** was also reported to be a Ca^{2+} binding protein in *Arabidopsis thaliana* (Day et al., 2002) and has a MYB and MYC transcription factor recognition sequence (Abe et al., 2003). **AT2G33380** is also expressed in petals and stamens during the late stage of developments (Zik and Irish, 2003) and is induced by abscisic acid (Carter et al., 2004). We observed **AT2G33380** to be upregulated in both treatments 1 and 2. It was found to be weakly expressed in treatment 3 (Fig. 2A).

AT3G53110 is strongly upregulated in treatment 2 and was weakly expressed in either treatments 1 or 2 (Fig. 2B). **AT3G53110** is also known as los4 (low expression of osmotically responsive genes) and an ATP-dependent helicase (Source: GenBank) in *Arabidopsis thaliana*. Mutations in **AT3G53110** affect mRNA export, and lead to cold-responsive gene expression (Gong et al., 2002; Gong et al., 2005). Los4 mutants were shown be hypersensitive to cold treatment compared to wild type plants (Gong et al., 2002). LOS4 also regulates the expression CBF gene expression (Gong et al., 2002) and it is documented that CBF imparts freezing tolerance in plants (Jaglo-Ottosen et al., 1998).

AT4G20260, a sugar responsive gene (Lee et al., 2004) was not expressed in any one of the treatments (Fig. 2B). **AT4G20260** is also a drought-responsive plasma membrane polypeptide family protein in *Arabidopsis thaliana* (Source: GenBank).

From these results it can be noticed that some drought induced genes were expressed when *Arabidopsis* plants were cryostressed. It is interesting to note that salt, drought, hypoxia and cold induced genes are expressed in cryopreserved *Arabidopsis* tissues. Several types of abiotic stresses (*e.g.* heat, cold, drought, salt etc.) may induce many common genes (Bray et al., 2002). From the data presented here, it is evident that cryogenically stored plant cells also share some common biochemical pathways with other types of abiotic stresses. However, more research is needed to elaborate the mechanisms of tolerance of cryostress in plant cells.

Variation in gene expression within and among populations has been previously documented (Oleksiak et al., 2002). As high biological variation is expected in *Arabidopsis* populations especially during microarray (Zhu and Wang, 2000) or other genomic expression profiling experiments, it is recommended (Zhu and Wang, 2000) to pool samples from individual plants (Thimm et al., 2001). For the experiments reported here, cDNA samples from two different replicates were pooled before performing RT PCR.

Conclusion

The application of the tools of molecular biology in the field of plant cryobiology to identify abiotic stress-induced genes is definitely a novel approach. Although plants are not cryostressed in nature, cryogenically-stored plant tissues undergo osmotic stress, oxidative stress, dehydration stress, and cold stress (Sakai, 2004). This paper was an attempt to elaborate the experimental details of gene amplification protocols in cryogenically stored *Arabidopsis thaliana* shoot tissues. Differential expression of genes in cryogenically stored *Arabidopsis thaliana* shoot tissues was documented. Understanding the molecular mechanisms of cryostress will help us to understand how plants respond to various types of abiotic stresses in nature.

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References

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) Function as Transcriptional Activators in Abscisic Acid Signaling, Plant Cell *15*, 63–78.

Blanc, G., Baptiste, C., Oliver, G., Martin, F., and Montoro, P. (2006) Efficient Agrobacterium tumefaciens-mediated transformation of embryogenic calli and regeneration of *Hevea brasiliensis* Müll Arg. Plants. Plant Cell Rep *24***,** 724-733.

Bray, E., Bailey-Serres, J., Weretilnyk, E. Responses to Abiotic Stresses. (2002) In *Biochemistry and Molecular Biology of Plants.* B. B. Buchanan, W. Gruissem, and R. L. Jones, eds. (John Wiley and Sons) pp. 1158-1201.

Carter, C., Pan, S., Zouhar, J., Avila, E.L., Girke, T., and Raikhel, N.V. (2004) The Vegetative Vacuole Proteome of *Arabidopsis thaliana* Reveals Predicted and Unexpected Proteins. Plant Cell *16*, 3285–3303.

Day, I.S., Reddy, V.S., Ali, G.S., and Reddy, A.S.N. (2002) Analysis of EF-hand-containing proteins in *Arabidopsis,* Genome Biol **3**, research0056.1–0056.24.

Elleuch, H., Gazeau, C., David, H., and David, A. (1998) Cryopreservation does not affect the expression of a foreign sam gene in transgenic *Papaver somniferum* cells. Plant Cell Rep *18***,** 94-98.

Gong, Z.Z., Dong, C.H., Lee, H., Zhu, J.H., Xiong, L.M., Gong, D.M., Stevenson, B., and Zhu, J.K. (2005) A DEAD box RNA helicase isessential for mRNA export and important for development and stress responses in Arabidopsis, Plant Cell *17***,** 256–267.

Gong, Z.Z., Lee, H., Xiong, L.M., Jagendorf, A., Stevenson, B., and Zhu, J.K. (2002) RNA helicase-like protein as an early regulator of transcription factors for plant chilling and freezing tolerance, Proc Natl Acad Sci USA. *99*, 11507–11512.

Hao, Y., Cheng, Y., and Deng, X. (2005) Stable maintenance and expression of a foreign gene in transgenic pear shoots retrieved from *in vitro* conservation. J Plant Physiol *162***,** 237-243.

Harding, K. (1991) Molecular stability of the ribosomal RNA genes in Solanum tuberosum plants recovered from slow growth and cryopreservation. Euphytica *55***,** 141-146

Hightower, R., Baden, C., Penzes, E., Lund, P., and Dunsmuir, P. (1991) Expression of antifreeze proteins in transgenic plants. Plant Mol Biol *17*, 1013-1021.

Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O., and Thomashow, M.F. (1998) Arabidopsis CBF1 Overexpression Induces COR Genes and Enhances Freezing Tolerance. Science *280***,** 104 – 106.

Jokipii, S., Ryynänenb, L., Kallioc, P.T., Aronenb, T., and Häggman, H. (2004) A cryopreservation method maintaining the genetic fidelity of a model forest tree, *Populus tremula* L. × *Populus tremuloides* Michx. Plant Sci *166***,** 799-806.

Kreps, J.A., Wu, Y., Chang, H.S., Zhu, T., Wang, X., and Harper, J.F. (2002) Transcriptome Changes for Arabidopsis in Response to Salt, Osmotic, and Cold Stress, Plant Physiol *130***,** 2129–2141.

Lee, E.J., Koizumi, N., and Sano, H. (2004) Identification of genes that are up-regulated in concert during sugar depletion in *Arabidopsis.* Plant, Cell Env *27***,** 337-345.

Lin, C., and Thomashow M.F. (1992) A cold-regulated *Arabidopsis* gene encodes a polypeptide having potent cryo-protective activity. Biochem Biophys Res Comm *183***,** 1103-8.

Liu, F., VanToai, T., Moy, L.P., Bock, G., Linford, L.D., and Quackenbush, J. (2005) Global Transcription Profiling Reveals Comprehensive Insights into Hypoxic Response in *Arabidopsis.* Plant Physiol. *137***,** 1115–1129.

Murashige, T., and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant *15*: 473-497.

Oleksiak, M.F., Churchill, G.A., and Crawford, D.L. (2002) Variation in gene expression within and among natural populations. Nature Gen *32*, 261 – 266.

Sakai, A. (2004) Plant Cryopreservation. In *Life in the frozen state*. Editors: B.J. Fueller, and N. Lane, and E.E. Benson, eds. (CRC Press, New York, NY) pp. 329-346.

Taji, T., Seki, M., Satou, M., Sakurai, T., Kobayashi, M., Ishiyama, K., Narusaka, Y., Narusaka, M., Zhu, J., and Shinozaki, K. (2004) Comparative Genomics in Salt Tolerance between Arabidopsis and Arabidopsis-Related Halophyte Salt Cress Using Arabidopsis Microarray. Plant Physiol *135***,** 1697-1709.

Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K., Shibata, D., Kobayashi, Y., and Ohta, H. (2005) 12-Oxo-Phytodienoic Acid Triggers Expression of a Distinct Set of Genes and Plays a Role in Wound-Induced Gene Expression in *Arabidopsis*. Plant Physiol *139*, 1268–1283.

Tereso, S., Miguel, C., Zoglauer, K., Valle-Piquera, C., and Oliveira, M.M. (2006) Stable Agrobacterium-mediated transformation of embryogenic tissues from *Pinus pinaster* Portuguese genotypes. J Plant Growth Reg *50***,** 57-68.

Thimm, O., Essigmann, B., Kloska, S., Altmann, T., and Buckhout, T.J. (2001) Response of Arabidopsis to Iron Deficiency Stress as Revealed by Microarray Analysis. Plant Physiol *127*, 1030-1043.

Thomashow, M.F. So what's new in the field of plant cold acclimation? Lots! (2001) Plant Physiol *125*, 89-93.

Towill, L.E., Bonnart, R., and Volk, G.M. (2006) Cryopreservation of Arabidopsis thaliana shoot tips. CryoLetters *27*, 353-360.

Zhang, B., Ramonell, K., Somerville, S., and Stacey, G. (2002) Characterization of Early, Chitin-Induced Gene Expression in Arabidopsis. Mol Plant Pathogen Interaction. *15***,** 963–970.

Zhu, T., and Wang, X. (2000) Large-Scale Profiling of the Arabidopsis Transcriptome Variation in gene expression within and among natural populations. Plant Physiol *124*, 1472-1476.

Zik, M., and Irish, V.F. (2003) Global Identification of Target Genes Regulated by *APETALA3* and *PISTILLATA* Floral Homeotic Gene Action, Plant Cell. *15* 207–222.

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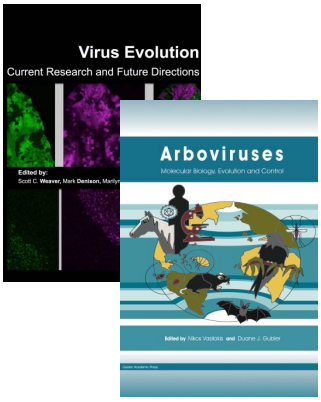

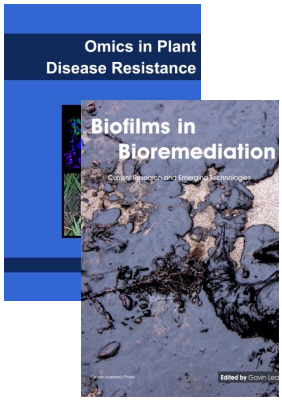

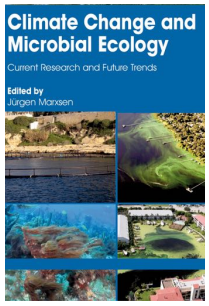

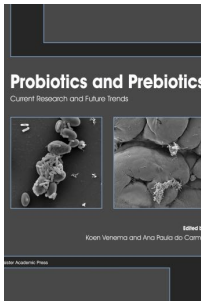

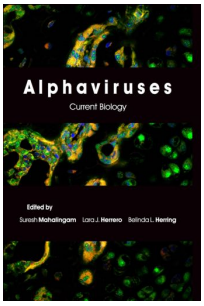

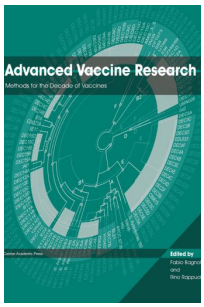

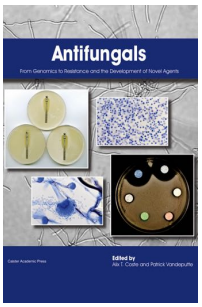

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