

The ICE-CBF-COR Pathway in Cold Acclimation and AFPs in Plants

¹Fu Zhang, ¹Yue Jiang, ¹Li-Ping Bai, ¹Liang Zhang, ¹Li-Jing Chen,
¹Hao Ge Li, ¹Yu Yin, ¹Wen-Wen Yan, ²Ying Yi and ¹Zhi-Fu Guo

¹School of Biosciences and Biotechnology, Shenyang Agricultural University, China

²School of Agronomy, Shenyang Agricultural University, China

Abstract: Low temperature adversely affects agriculture production, especially in the quality and yield of crops. The great mass of crops, such as wheat, corn, rice and other main crops are planted in the temperate climate and cold climate in the areas of the northern hemisphere. Every year, because of low temperature disasters that main crops loss as high as thousands of dollars, with the increasing environmental degradation, climate anomalies, low temperature would made an more bad attack winter crops, at that time the loss would be more severe. How to improve crop resistance to low temperature capabilities and to improve crop quality and yield has become a focus of the study. Currently, the frost resistance and plant molecular biology has made great progress, a large number of studies have found that after cold acclimation may be related to cold-induced plant gene expression and other regulatory factors, thereby increasing the plant's frost resistance. The pathway that ICE-CBF-COR is one of major ways, play an important role in plant resistance to cold and is well studied. In this paper characterization of ICE-CBF-COR mechanism in plant resistance to cold and antifreeze proteins (AFPs) have been discussed.

Key words: Low temperature • Frost resistance • ICE-CBF-COR • Antifreeze proteins (AFPs)

INTRODUCTION

Cold acclimation process can lead to increase plant freezing tolerance. Low temperatures affect membrane structure and composition, metabolic rates, induce transcriptomic changes, cellular dehydration, inhibits photosynthesis, disturb functioning of ion channels, cellular signaling etc [1-8].

The plant cells and plant membrane system have closely related to frost resistance. Although the characteristics of cell wall pores are important in protection against freezing, fewer or smaller pores can prevent the extracellular ice from penetrating the cells [9]. When chilling comes or suddenly encountered, cell membrane phase transition occurs in non-frost resistance plant and low frost resistance plant. The liquid crystal phase would turned into a gel state and the structure of the membrane damaged, resulting in the formation of a non-lipid pore that has greater permeability on plasmalemma and cause a lot of electrolyte external flow across the channel and in the end cause plant death. Many plants suffer from the cold front after a slow cooling process, the cold resistance will gradually increase, a process known as cold acclimation. Cold

acclimation in essence is the process that plant regulator itself to suit the environment by the low temperature signal transduction pathway. To some extent, cold acclimation along with the changes in a series of physiological responses in plants by the ambient temperature factor receptors to stimulate the plant to the relevant signal receptors, through the plant's own unique signal transduction system and regulating plant antifreeze proteins and other regulatory proteins expression, non-protein factor (IP3, ABA, etc.) content, thereby enhancing the ability of plant antifreeze. In the process of cold acclimation, according to the expression of gene encoding protein product the protein could be divided into two categories, one is a functional protein directly protect cells from stress damage; the other is regulatory proteins for signal transmission and regulation of gene expression have been shown in Figure 1 [10].

There are many shortcomings in Traditional plant breeding methods to improve frost resistance, which directed the use of transgenic technology to improve a plant's function is an important method of modern breeding, crop breeding research is currently a hot research.

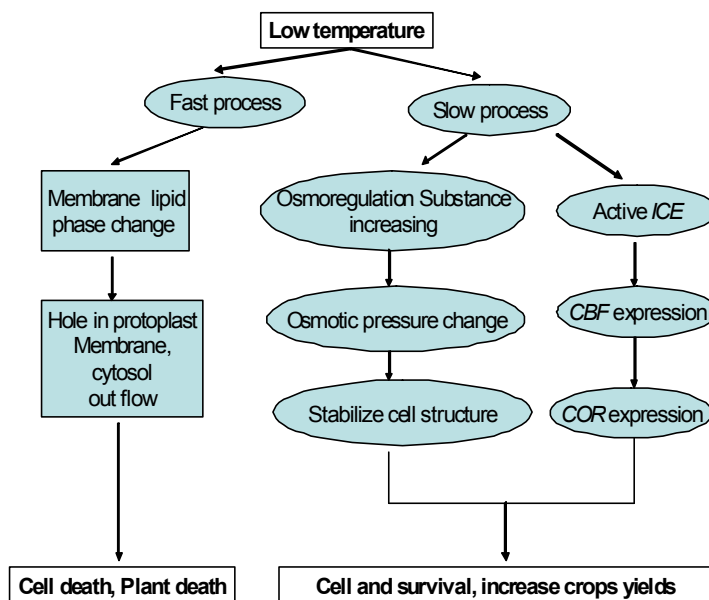


Fig. 1: Plant response to cold

There are two mainly ways could account for plants against frost: First, to avoid intracellular ice formation and prevent ice crystal growth; the second is to maintain cell membrane stability and protein structure of the biological activity of nucleic acids. Therefore, the work from molecular biology research is to transform crop antifreeze genetic characteristics around these two aspects. Antifreeze proteins (AFPs) and its genes are well studied for decades, for AFPs can causing the ice crystals form change and inhibit ice crystal growth and this effect in different directions on the strength of the points [11].

Fast process: non-frost resistance plant encounter low temperature, freezing temperatures lead to extracellular ice formation, membrane lipid phase changes (liquid crystal phase into gel state), form hole in membrane, cytosol out flow and cause cell death. Slow process: Plant adapt to cold environment, the ICE-CBF-COF pathway was activated and other regulated osmoregulation substance were induced to protect plant from cold damage.

The ICE-CBF-COR Pathway in Plant to Cold Acclimation: In the process that plant adapt to cold stress, the plant in vivo would undergo a series of chemical reactions in molecular level. These reactions occur in accordance with an orderly sequence of space-time and interconnected, forming a low-temperature response of the molecular mechanisms. It is very

important that understand the molecular mechanisms of plant to cold responses, to breeding cold-resistance crops. In the molecular mechanisms of plant to cold responses, ICE-CBF-COR pathway is in-depth study.

Cold-Regulated Genes (COR): A large number of protein synthesis can be induced by cold acclimation, most of which respond rapidly to low temperature and can be induced by abscisic acid (ABA) and water stress, these genes encoded proteins called cold-induced gene (cold-induced genes) or cold-regulated genes (cold-regulated genes or cold-responsive genes, COR). Up to now, many cold-induced genes have been isolated and identified in *Arabidopsis thaliana*, alfalfa, rape, spinach, potatoes, barley, wheat, rice, rye and other plants[12].

Among common commercial crops, potato (*Solanum tuberosum*), rice (*Oryza sativa* L.) and corn (*Zea mays*) originate from tropical and sub-tropical regions and are chilling sensitive, while others like oat (*Avena sativa* L.) are chilling tolerant but freezing sensitive. Other common crops, including barley (*Hordeum vulgare* L.), common wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) are more cold hardy and therefore are well adapted to survive winters with freezing temperatures.

The promoter region of cold-regulated genes (COR) contains a 5 bp core sequence CCGAC dehydration responsive element (Dehydration responsive element, DRE), as a cis-acting promoter elements, can activate the

expression of COR gene at low stress [13]. A COR19 cDNA representing unique cold-induced sequences has been cloned from the hardy citrus relative *Poncirus trifoliata* and the expression of COR19 mRNA in response to cold acclimation [14]. Twelve cold induced cDNA cloned (*Ssci1*, *Ssci2*, *Ssci3*, *Ssci4*, *Ssci5*, *Ssci6*, *Ssci7*, *Ssci8*, *Ssci9*, *Ssci10*, *Ssci11*, *Ssci12*) have been isolated by differential screening of a cDNA library prepared with mRNA from cold resistant potato (*Solanum sagarrnzdinum*)[15]. A cold-induced gene (*MpRCI*) from plantain (*Musa paradisiaca* L.; ABB Group) has been cloned and expression of an *MpRCI*::GFP fusion protein in onion epidermal cells showed localization of the protein product in the plasma membrane [16].

C-Repeat-Binding Factor (CBF): Transcription factor, also called trans-acting factors, is the protein combine to upstream of certain gene-specific nucleotide sequence, translocation from the cytoplasm to the nucleus after activated, identify and combine to the promoter cis-acting element, activate and regulate gene expression. A typical plant transcription factors, including: DNA-binding domain, the location of oligosaccharides, transcription regulation domain and a nuclear localization signal [17]. Research for transcription factor has lasted for a long time, but the transcription factor CBF study in recent years and research on CBF in plant stress resistance have a breakthrough.

Transcription factor CBF is an important transcription factor in the process of cold acclimation, whose role is to respond to the cold signal, bind to the DRE sequence of COR genes and quickly start downstream COR gene expression to protein. CBF studies of plant cold acclimation is an important part of the study, therefore, the transcription factor CBF are regarded as a switch to activate some COR genes when face low temperature [18].

CBF with CRT (c-repeat) / DRE (Dehydration Response Element) element binding. The two motifs are defined as 5' TGGCCGAC 3' and 5' TACCGACAT 3' respectively with the shared motif 5' CCGAC 3'. The core sequence is called low temperature-responsive Element(LTRE)[19].The core sequence commonly found in cold-induced genes and dehydration-induced genes in promoters, for the inducible expression of these genes are necessary [20]. CBF transcription factor is widely present in Arabidopsis, maize, tomato, rapeseed, tobacco, rice and wheat and many species of plants and participate in a variety of gene expression, the gene family members contain about 60 amino acid residues of the very

conserved DNA-binding domain (ie AP2/ERF domain), these proteins can be divided into five categories according to structure: AP2 class, DREB class, ERF class, RAV and other kinds of types. The different plant species in the regulation of gene expression and functional CBF studies, more comprehensive coverage of the Arabidopsis, cotton and other crops on a little research relatively [21].

As the CBF transcription factor can regulate multiple functional genes expression in plant to the drought, high salt and related low temperature tolerance, the use of CBF transcription factors to improve plant resistance is a better access to the combined effect of ideal than simply use a single gene to change the plant resistance, which provides a new technical approaches to genetically engineered plant resistance and has broad application prospects [22].

Inducer of CBF Expression (ICE): Study showed that expression and regulation of CBF gene is a complex process, CBF protein translation requires the participation of other factors, therefore, ICE factors was found. ICE is a kind of transcription factors in the low temperature-induced expression of CBF genes family, it can bind to promoter sequence of CBF particularly at low temperature, to induce the expression of CBF, then CBF bind to its downstream target gene promoter sequences DRE COR gene, induced COR gene expression, thereby increasing the plant frost resistance (Fig. 2) [23]. In the study of ICE, ICE1 (inducer of CBF expression1) is the upstream signal that directly regulate CBF expression. Viswamthan ICE, who isolated the transcription factor ICE1 (inducer of CBF expression1) from ICE. [24]. ICE1 coding region contains a bHLH (basic helix-loop-helix) domain, DNA-binding characteristics of the MYC, MYB-like protein, while the protein through the bHLH domain and interaction of MYCR / MYBR component, induce CBF gene expression [25].

Antifreeze Protein (AFP): Low temperature-induced proteins in plants are a series of proteins induced under stress conditions, antifreeze proteins (AFPs) is one of them [26]. After a lot of analysis to body fluids of Antarctic fish in different groups, AL DeVries finally separated a special protein, to some extent, can stop solution into ice and called antifreeze proteins (AFPs) [27]. AFP itself is a class of proteins that inhibit the growth of ice crystals, lower the freezing point of aqueous solutions by non-colligative properties and has little effect on the melting point, leading to the solution

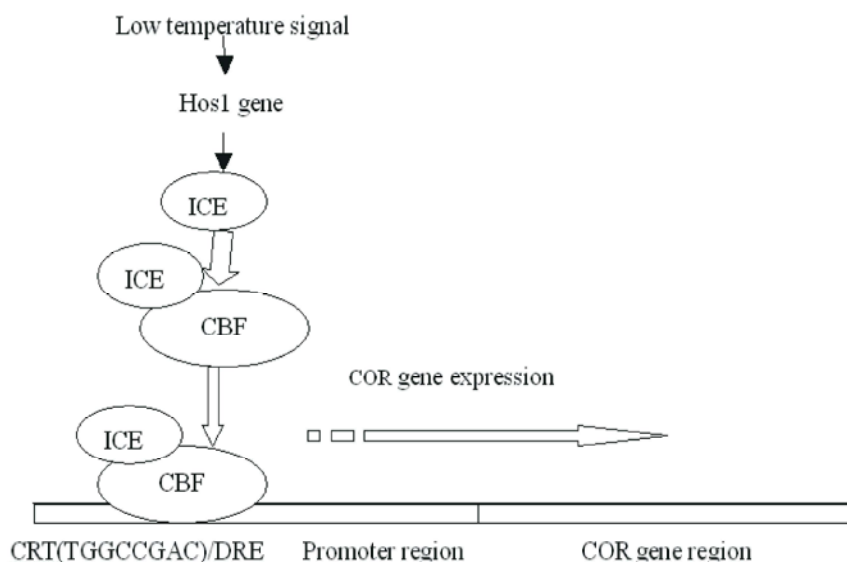


Fig. 2: ICE-CBF-COR pathways in plant to cold acclimation in ABA-independent regulatory system

difference between melting point and freezing point, this difference is called thermal hysteresis activity (THA) or antifreeze activity, the greater the difference, the greater the anti- Frozen activity [28], so the antifreeze protein is also known as thermal hysteresis proteins or temperature hysteresis protein. Some studies have shown that in addition to antifreeze activity of AFPs, but also has some activity and antibacterial, insect activity. Antifreeze protein (AFPs) found in the initial from the Antarctic fish and more in-depth research in fish and insects. The AFPs research in plant has started for the past few years. Until 1998, Daw in York University (Britain) found AFPs and related genes in carrots [29]. Griffith, etc. [30] pointed out clearly that endogenous protein is produced in the cold acclimation of wheat (*Triticum aestivum*). The presence of a variety of AFPs in plants was found by Urrutia [31]. The antifreeze protein genes has been cloned in plants, such as 1117kD of AFP cDNA in ryegrass [32]; 3117kD of AFPs of the CHT gene, 2418kD the AFPs of CHT46 gene in winter rye [33] and two antifreeze proteins genes from winter rye have been transformed into *E. coli*, the test results show that the expressed protein with antifreeze activity.

With the research deepened in freezing mechanism of plant, now, many plant cold protein synthesis has been observed, synthesized proteins such as 47 kD antifreeze protein in *Arabidopsis* [34], 39kD antifreeze protein in wheat [35] etc, all of them have common properties: thermal stability, glycine-rich, low-aromatic amino acids and high hydrophilic amino acids, the protein can

maintain a high degree of scalability to protect the cells due to low temperature-induced dehydration. A 1099 bp of the gene in seeding of carrot was cloned by PCR and identified as antifreeze protein gene. The gene was linked to CaMV 35S promoter and the pCAMBIA2300 vector, which was transferred to tobacco. The damage of transgenic tobacco lower than contrast tobacco significantly by observing after 0°C treatment [36]. This result indicates that plants are similar to fish to reduce the freezing point, modified form of ice crystals, and inhibit the modification of the ice re-crystallization and other features, these features play an important role raising the anti-icing capability of cell in frozen stress. A antifreeze protein gene mpAFPI49 of turtle was transfer into tobacco [37], the frost resistance of transgenic tobacco was significantly better than the wild type after two days at low temperature condition. When the temperature returns to room temperature, the recovery of transgenic tobacco growth, while wild-type tobacco were stop growing because of irreversible low-temperature damage.

After cold acclimation, LpIRI-a and LpIRI-b re-crystallization in inhibitor protein (IRI) enhances the freezing ability of the host cell's [38]. In addition to these two types of antifreeze proteins associated with the plant freezing tolerance, protein over-expression of *ACBPs* also lead to reduce two undersaturated glyceride lecithin and increase plant frost resistance increase [39]. Currently, antifreeze proteins isolated from plants and can be used for transgenesis is increasing and the DNA of antifreeze protein in different plants and amino acid sequences show

no clearly detectable homology. Therefore, research about isolated and cloned plant antifreeze protein gene will be a hotspot to enhance crop frost resistance [40]. Frost resistance of plants is the result of long-term evolution, different plants its cold climate and different cold tolerance of plants. The CBF1 content in leaves increased after low temperature-treated by real-time reverse transcription-polymerase chain reaction (RT - PCR method), and at the same time, the content of other genes transcription and translation of proteins also have significant changes, indicating that low temperature can induce gene transcription and translation and a series of complex links [41].

Conclusion and Perspective: The ICE-CBF-COR signaling pathway of stress is not only one in plants to cold environment, but also a important pathway and in-depth study response to cold. Due to environmental deterioration, declining arable land, more complex climate change and erratic cold weather, how to increase agricultural yield and quality has always been a research hotspot. Therefore, improvement of plant frost resistance, frost crop varieties for cultivation of great significance in agriculture.

These implementations need to be based on genetic engineering, need for more scientific and technical personnel. Although plant genetic engineering theory and technology system about plant frost resistance has been basically established, huge crops yield loss is still exist. Even so, we believe that under the joint efforts of researchers, the mechanism of respond to cold, antifreeze proteins and related genes and other factors affecting the relationship between each other will be further clarified in the near future.

ACKNOWLEDGMENTS

We express our gratitude to the anonymous reviewers for helpful comments to improve the manuscript. This work was supported by the National Natural Science Foundation of China (Grant No. 30900894), the Startup Foundation for Doctors of Liaoning province in China (Grant No. 20091069) and the Funding (A type) from Research Program of Education Department in Liaoning province (Grant No. L2010486).

REFERENCES

1. Levitt, J., 1980. Responses of plants to environmental stresses, vol. 1, 2 edn. New York: Academic Press, USA.
2. Dowgert, M.F. and P.L. Steponkus, 1984. Behavior of the Plasma Membrane of Isolated Protoplasts during a Freeze-Thaw Cycle. *Plant Physiol.*, 75(4): 1139-1151.
3. Furbank, R.T., C.H. Foyer and D.A. Walker, 2003. Regulation of photosynthesis in isolated spinach chloroplasts during orthophosphate limitation. *Biochemica Biophysica Acta*, 894(3): 552-561.
4. Govindachary, S., N.G. Bukhov, D. Joly and R. Carpentier, 2004. Photosystem II inhibition by moderate light under low temperature in intact leaves of chilling-sensitive and -tolerant plants. *Physiol. Plant.* 121(2): 322-333.
5. Griffith, M.M. and W.F. Yaish, 2004. Antifreeze proteins in overwintering plants: a tale of two activities. *Trends in Plant Sci.*, 9(8): 399-405.
6. Gulick, P.J., S. Drouin, Z. Yu, J. Danyluk, G. Poisson, A.F. Monroy and F. Sarhan, 2005. Transcriptome comparison of winter and spring wheat responding to low temperature. *Genome*, 48(5): 913-923.
7. Heather, A.V.B. and M.F. Thomashow, 2006. Arabidopsis transcription factors regulating cold acclimation. *Physiol. Plant.* 126: 72-80.
8. Uemura, M., R.A. Joseph and P.L. Steponkus, 1995. Cold Acclimation of Arabidopsis thaliana (Effect on Plasma Membrane Lipid Composition and Freeze-Induced Lesions). *Plant Physiol.*, 109(1): 15-30.
9. Rajashekar, C.B. and A. Lafta, 1996. Cell-Wall Changes and Cell Tension in Response to Cold Acclimation and Exogenous Abscissic Acid in Leaves and Cell Cultures. *Plant Physiol.*, 111(2): 605-612.
10. Xiao-juan, Z., 2004. Freezing cold-induced plant gene research. Shaoxing University. Press, China, 24(9): 14.
11. Min-an, Y., C. Hong-Wen, F. Dai-Ming and G. Li, 2001. Antifreeze protein in plant genetic engineering application. *Acta Bot. Boreali-Occidentalia Sin.*, 21(1): 110-114.
12. Fowler, D.B. and R.J. Carles, 1979. Growth, Development and Cold Tolerance of Fall-acclimated Cereal Grains. *Crop Sci.*, 19: 915-922.
13. Novillo, F. and J.M. Alonm, 2004. Cold acclimation of Arabidopsis thaliana: Affect on plasma membrane lipid composition and defreeze-induced lesion. *Plant Biol.*, 11: 13985.
14. Qinyin, C. and A. Gloria, 1995. Moore and Charles L. Guy. An unusual Group 2 LEA gene family in citrus responsive to low temperature. *Plant Mol. Bio.*, 29(11): 11-23.

15. Tadeusz, R., W. Irzykowski and W. J. Grygorowicz, 1997. Identification and expression of novel cold induced genes in potato (*Solanum sogarandinum*). Plant Sci., 124: 69-78.
16. Dong-Ru, F., B. Liu, W. Li, Y. He, K. Qi, H. Wang and J. F. Wang, 2009. Over-expression of a cold-induced plasma membrane protein gene (MpRCI) from plantain enhances low temperature-resistance in transgenic tobacco. Environ. Exp. Bot., 65: 395-402.
17. Liansen, L., J. Michae, H. Thornas and H. MacRae, 1999. Eur. J. Biochem., 262: 247-257.
18. Wen-Gong, H., K. Yin and Z. Gao, 2006. Research in genetic engineering of plant antifreeze. Biotechnology Bulletin, pp: 2.
19. Chawade, A., 2011. Unravelling the complexity of cold acclimation in plants. Edita Västra Aros AB. Göteborg.
20. Li, X. and H. Li, 2009. CBF transcription factors in plant stress resistance in the role of Anhui Agricultural Sci., 37(32): 15749-5751.
21. Yun-Fei, C., H. Zhang and K. Xiao, 2007. CBF transcription factor-mediated plant low-temperature signal transduction in cotton. J. Cotton Sci., 19(4): 304-311.
22. Chun-Bo, X., Y. Wang, H. Zhao and X.Y. Li, 2010. The CBF transcription factor used in genetic engineering research in China-Anhui Agricultural Sciences. J. Anhui Agn. Sci., 38(14): 1723-1726.
23. Michael, F., S. Thomashow, J. Gilmour, J. Eric and J. Stockinger, 2001. Physiol Plant, 112: 171-175.
24. Viswanathan, C., A. Asarno and J.K. Zhu, 2003. Regulator of cold induced transcriptome and freezing tolerance in Arabidopsi. Gene and Develop., 14: 1043-54.
25. Chinnusamy, V., M. Ohta and S. Kanrar, 2003. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes and Develop., 17: 1043-1054.
26. Yang, Y., Z.H. Lei and F.R. Peng, 2007. Cold-induced proteins and cold tolerance and plant research, Northwest Plant Sci., 27(2): 14-21.
27. Arthur, L., D. Vries and E. Donald, 1969. Freezing resistance in some antarctic fishes. J. Sci., 163: 1073-1075.
28. Sidebottom, C., S. Buckley, P. Pudney, S. Twigg, C. Jarman, C. Holt, J. Telford, A. McArthur, D. Worrall, R. Hubbard and P. Lillford, 2000. Phytochemistry: heat-stable antifreeze protein from grass. Nature, 199: 40-42.
29. Griffith, M. and S.C.D. Yang, 1992. Antifreeze protein produced endogenously in winter rye leaves. Plant Physiol., 100(2): 593-596.
30. Urrutia, M.E., J.G. Duman and C.A. Knight, 1992. Plant thermal hysteresis proteins. Biochem. Biophys. Acta, 11(21): 199-206.
31. Hu, A., T. Qiu and J. Zheng, 2001. Antifreeze protein and its application in food industry-Science and Technology of Food Industry.
32. Michael, W., W. Robert and B. Ron, 1999. Arabidopsis 22-kilodalton peroxisomal membrane protein. Physiol. Plant. 105: 600-606.
33. Sidebottom, C. and D. Worrall, 2000. Heat-stable antifreeze protein from grass. Nature, 406: 256-2638.
34. Xin, Z., A. Mandaokar and J. Chen, 2007. Arabidopsis, ESK1 encodes a novel Regulator of freezing tolerance, Plant J., 49(5): 786-799.
35. Zhang, C., Z. Feias and S. Warnke, 2009. Identification of genes associate with cold acclimation in perennial ryegrass. J. Plant Physiol., 166: 1436.
36. Fan, Y., B. Iju and H. Wang, 2002. Cloning of an antifreeze protein gene from carrot and its influence on cold tolerance in trartsgenic tobacco plants. Plant Cell Rep., 21: 296-301.
37. Wang, Y., M. Li and W. Xie, 2008. Insect antifreeze protein gene into tobacco cold resistance. Crop Sci., 34(3): 397-402.
38. Zhang, C., F. Shui-zhang, A. Rajeev and J.D. Hannapel, 2010. Ice recrystallization inhibition proteins of perennial ryegrass enhance freezing tolerance. Planta, 232: 155-16.
39. Zhi-Yan, D., X. Shi, Q. Chen and M. Chye, 2010. Depletion of the membrane-associated acyl-coenzyme a-binding protein ACBP1 enhances the ability of cold acclimation in arabidopsis. Plant Physiol., 152: 1585-1597.
40. Wang, D., J. Cang, Y. Mou, Y. Zeng, P. Xu and J. Yu, 2009. Plant Gene Research cold-Northeast Agricultural University in October 2009, 40(101): 134-138.
41. Peter, A., W. Gorsuch, A. Sargeant and S.D. Penfield, W.P. Quick, K. Owen and K. Atkin, 2010. Mg protoporphyrin monomethylester cyclase deficiency and effects on tetrapyrrole metabolism in different light conditions. Plant Cell Physiol., 51(7): 1488-1498.