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Expression of the ICE1 Genes of the Aronnik Korolkov Plant: Determination of Resistance to Freezing

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ABSTRACT

The impact of low temperature on the Korolkov Aronnik plant triggers the expression of the CBF family of transcription factors, which, in turn, activate many downstream genes that confer plant resistance to cold and frost. The article will consider the identification of the ICE1 gene (inducer of CBF 1 expression) by Aronnik Korolkov, an upstream transcription factor that regulates the transcription of CBF genes during cold. A mutation in the wild Aronnik Korolkov blocks the expression of CBF3 and reduces the expression of many genes located downstream of CBFs, which leads to a significant decrease in plant resistance to cold and freezing. The ICE1 genes of the Aronnik Korolkov plant encode the MYC-like transcription activator BHLH. ICE1 binds specifically to MYC recognition sequences in the CBF3 promoter.

KEYWORDS: Cold exposure, resistance to freezing, Aronnik Korolkova, research, DNA, ICE1.

ARTICLE DETAILS

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INTRODUCTION

Cold is an environmental factor that limits the geographic distribution and growing season of many plant species, and this often negatively affects crop quality and productivity. Most temperate plants can acquire resistance to freezing temperatures by prior exposure to low, non-freezing temperatures, a process known as cold acclimatization. Plants of tropical and subtropical origin are sensitive to low temperatures (0°C–10°C) and are incapable of cold acclimatization. Many studies have shown that the expression of cold-regulated genes is critical in plants for both cold tolerance and cold acclimatization. Cold-sensitive genes code for a wide range of proteins, such as enzymes, involved in respiration and metabolism of carbohydrates, lipids, phenylpropanoids and antioxidants; molecular chaperones, antifreeze proteins, and others thought to be responsible for resistance to freeze-induced dehydration[1].

Many of the cold- and dehydration-sensing genes have one or more copies of the DRE/CRT cis element in their promoters, which has the core sequence CCGAC. A family of transcription factors known as CBFs or DREB1s binds to this element and activates the transcription of downstream genes sensitive to cold and dehydration. Interestingly, the CBF/DREB1 genes themselves are induced by low temperatures. This induction is temporary and precedes the induction of downstream genes with a cis DRE/CRT element. Therefore, there is a transcriptional cascade leading to the expression of DRE/CRT class genes under cold stress. Ectopic expression of CBFs/DREB1s in plants turns on downstream genes sensitive to cold even at warm temperatures and provides improved freeze tolerance.

MATERIALS AND METHODS

Genetic analysis of Arabidopsis plants expressing the firefly luciferase reporter gene revealed several mutants with unregulated expression of cold-sensitive genes. The hos1 mutant (high expression of osmotically sensitive genes) shows enhanced cold induction of CBFs and their downstream cold sensitive genes. HOS1 encodes a protein that is present in the cytoplasm at normal growth temperatures but accumulates in the nucleus upon cold processing. Transcription of CBF genes is also subject to

feedback suppression by its own gene product or its subsequent target gene products. This was revealed as a result of studies of the los1 mutant, which is defective in the translational lengthening factor 2 gene. The LOS1 mutation blocks the cold induction of genes with the CRT/DRE element, but causes over-induction of CBF genes. It was shown that protein synthesis in LOS1 mutant plants is disrupted precisely in the cold. Therefore, cold-induced CBF transcripts cannot be translated to activate downstream genes, and feedback suppression leading to superinduction of CBF transcripts cannot occur.[2].

Expression of ZAT10 is rapidly and temporarily induced by cold in ARONNIK Korolkov, and this induction is stronger and more stable in the los2 mutant. Therefore, LOS2 can control the expression of delayed cold response genes through transcriptional repression of ZAT10. The Arabidopsis LOS4 locus is involved in the accumulation of CBF transcripts upon cold treatment. LOS4-1 mutant plants are sensitive to cold stress, and cold sensitivity can be reduced by ectopic expression of CBF3.

Scientists conducted genetic screening to identify components of cold signaling upstream of CBF proteins. A cold sensitive bioluminescent Arabidopsis plant was constructed by expressing the firefly luciferase (LUC) coding sequence under the control of the CBF3 promoter. Homozygous CBF3-LUC plants were subjected to chemical mutagenesis, and mutants with altered expression of CBF3-LUC induced by cold were isolated by fluorescent imaging. Here we report an ICE1 mutant that has impaired CBF3-LUC cold induction and is defective in cold acclimatization. ICE1 encodes the MYC-like transcription activator bHLH, which binds to the CBF3 promoter. Thus, ICE1 plays a key role in regulating the expression of cold-sensitive genes,

As noted above, the Korolkov Aronnik plant containing the CBF3–LUC transgene emits bioluminescence in response to cold stress. Homozygous CBF3-LUC plants (here referred to as wild type) were mutagenized with ethyl methanesulfonate and the resulting M2 population was screened for mutants with aberrant bioluminescent responses to cold stress using a low light imaging system. Several mutants have been isolated showing abnormal cold regulation of CBF3-LUC expression. One of these mutant lines, designated as ICE1, practically blocks the expression of CBF3-LUC in the cold. Wild plants showed strong luminescence in response to 0°C treatment, while the ICE1 mutant showed very little luminescence induction throughout the cold treatment period.

The ICE 1 mutation blocks cold induction of CBF3 and affects the expression of other cold-sensitive genes. Luminescent images of plants were collected after 12 hours of cold treatment (0°C). (B) Quantification of the luminescence intensity of wild type seedlings (solid circles) and ICE 1 (open circles) in response to different durations of cold treatment. Transcript levels of CBFs and their downstream target genes in wild-type and ice1 plants in response to cold treatment. Seedlings were either not treated (0 h) or cold treated (0°C) for the specified time (h). As determined by fluorescent imaging, all F1 plants showed reduced cold-induced CBF3-LUC expression similar to that of ICE 1[3].

The wild plant Aronnik Korolkova demonstrated CBF3 induction after 1 h of cold stress, and the peak of expression occurred at 6 h. On the contrary, CBF3 induction was almost abolished in ICE units. The level of induction of CBF2 was slightly lower in ICE 1 after 1 hour of cold treatment, while after 6 and 12 hours the level of induction was higher in the mutant. Cold induction of downstream target genes of CBFs has been investigated. Expression levels of RD29A, COR15A, and COR47A during cold stress were lower in ICE 1 than in wild type, while KIN1 induction was lower in ICE 1 only after 48 h of cold stress.

Consistent with these RNA blot results, microarray analysis using Affymetrix near full genome genechips showed that of 306 genes induced threefold or more in wild type by 6-hour cold treatment, 217 were either not induced in the ICE 1 mutant or their induction is 50% or less of that of the wild type. For 87 of the 306 cold-induced genes, their wild-type and ICE 1 induction levels differ by less than a factor of two (Supplementary Table 1B). Interestingly, two genes show higher levels of cold induction in the ICE1 mutant. The ICE 1 mutation impairs cold and freeze tolerance.[4].

The advantages of using assays for DNA isolation over other methods are that they are quick and easy, no harmful chemicals such as phenol or chloroform are used in the assay, the DNA, once bound to the membrane, can be washed and cleaned of contaminants, and then eluted from the column (membrane) with water.

At normal growth temperatures, ICE1 seedlings and wild Aronik Korolkov seedlings were similar in size.

Plant material and experimental conditions.

Three-month-old medicinal plants Aronnik Korolkov, listed in the Red Book, were selected for the study. They were grown artificially (from grains) (collection of Southclinical & Genetic Laboratory JSC): a total of 37 pieces were selected. shoots of plants of each genotype that were grown in 2 liters of brown forest soil (pH= 5.0). Prior to exposure of these seedlings to low temperatures, Aronnik Korolkova was kept for a month at a temperature of 20 ± 2 °C with the appropriate irrigation regime, and was illuminated with fluorescent lamps. Then the leaves were selected for analysis. Then Aronnik Korolkov was placed in a cold chamber for research. The impact of cold stress was facilitated by the temperature down to 0…+2 °C during the week (cold stress), then the temperature gradually decreased to –4–6 °C for several days (freezing), and the light supply mode was left as before. For laboratory analyses, the leaves located third and fourth in a row from the top were used. This was done to isolate RNA and to perform physiological analyses. In order to conduct analyzes and obtain RNA, a mixed sample was obtained from

the leaves of Aronnik Korolkov. The cold resistance test was carried out according to the method [5].

Picture 1. Aronnik Korolkova. Raised in laboratory conditions by grain germination (collection of South clinical &Genetic Laboratory).

Phenotyping of cold tolerance

Using the conductometric method, the degree of electrical conductivity of the tissues of this plant (%), the resistance of membrane cells, and the extent to which the tissues of the leaf itself of this plant were damaged (%) were determined using a ST300C portable conductometer (Ohaus). 200 mg of a fresh leaf of Aronnik Korolkov was immersed in 150 ml of deionized water, then the electrical conductivity of the plant was gradually determined immediately after immersion (L0), and after 2 hours (L1), they began to boil in a water bath for 60 min at 100 °C. Thus, the electrical conductivity was determined - after the solution had cooled (L2). The relative electrical conductivity was calculated using the following formula:

REC (%) = L0/L1 × 100.

Cellular membrane stability (CMI, %) was determined using the following formula:

CMI = (1 - (L1/L2)) / (1 - (C1/C2)) × 100

where C1 and C2 are the average electrical conductivity of the process control before and after boiling.

How much tissue damage was assessed on a scoring scale based on the ratio of 100 - CMI. The data obtained were processed using one-way analysis of variance[6].

RESULTS OF THE GENETIC STUDY

Low temperature is one of the most important environmental stressors. Low-temperature exposures were carried out at the vegetative and generative stages. Aronnik Korolkov was used as a tolerant genotype. Aronnik seeds were germinated and grown in a mixture of peat and perlite.

Cold stress is an important environmental factor that limits the agricultural productivity of plants grown in hilly areas. Many plant species are injured or die due to low temperatures.

Wilting and dryness caused by cold stress in sensitive plants are the result of loss root water conduction. In addition, plants lose their turgor at low temperatures, and the loss of stomata control leads to further increasing water loss. The second is photo-oxidative damage that occurs on leaves with simultaneous exposure to cold and direct sunlight.
rays. Photooxidative damage means that at low or high temperature, which limits plant growth, cell destruction caused by environmental stressors.

In recent years, both in classical breeding research and in molecular and physiological research, the development of new
Increasing attention is paid to genotypes resistant to low temperature stress. Some components that make up the structure of a plant
necessary for Aronnik Korolkov to perceive cold stress and for resistance to cooling (0-15°C).

Recently, cold-sensitive miRNAs have been found in several plant species.

The purpose of this study is to determine the effect of low-temperature stress exposure on the Korolkov Aronnik plant.

The index of cold tolerance of generative growth (TIGG) was determined by the number of seeds of genotypes, cold-treated, not treated (control), and analyzed statistically. Statistical analysis of the cold hardiness index of genotypes,

performed using vegetative and generative tests, was carried out using the Jump program.

When the number of seeds obtained from the control treatment was considered, it became clear that the application of cold led to
a decrease of almost 50%. In some genotypes, the number of seeds decreased by 80%. The genotype of wild plants took first place in the plant tolerance index.

This study was carried out in order to determine the resistance of Aronnik Korolkov to the effects of cold at low low temperatures. When identifying resistance to cold stress, the index of cold resistance was calculated taking into account the norm of the number of seeds. Cold resistance of Aronnik Korolkov's genotypes is insufficient to explain the changes in antioxidant systems only.

Each plant species has its own range of optimal temperatures, and deviation from this optimum is stressful for the body. The damaging effect of cold stress is different under the action of: (1) low positive temperatures and (2) temperatures below 0°C.

The screening results showed (Table 2) that the cold tolerance genes ICE1 and ICE2 were present in all the studied Aronnik Korolkov plants. Although the presence of these genes were much different with the expressivity on cold stress.

Table 1. Screening indicators

	Number of plants studied	Number of plants with the ICE1 gene	Number of plants with ICE2 gene	Number of plants expressing the ICE1 gene	Number of plants expressing the ICE2 gene
Quantity	37	37	7	34	4
%	100%	100%	18.9%	91.9%	57.1%

As can be seen from 37 tested seedlings, the expression of ICE1 and ICE2 genes was much different in the genome of Aronnik Korolkov. In the genome of Aronnik Korolkov, the ICE1 gene was found in all seedlings with expression (91.9%), while for the ICE2 gene these indicators were much lower than expected, given the gene was found in 7 seedlings with expression (57.1%). These data indicate that the index of the ICE2 gene in Aronnik Korolkovka plants with freezing sensitivity is low.

Studies have shown that cold exposure (0 ... +2 °C) did not lead to significant changes in the electrical conductivity of leaf tissues. During freezing (–4…–6 °C), the relative level of electrical conductivity increased significantly, and the stability of cell membranes decreased, which indicates an increase in the release of electrolytes from tissues (Fig. 3).

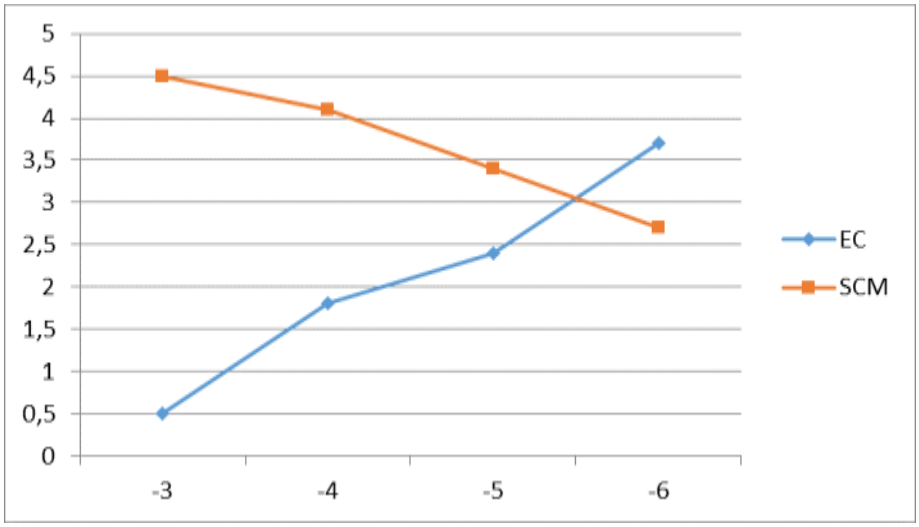

Figure 2. (1-5) Measurement time in hours, (-3... - 60C) temperature, EC – electrical conductivity, SCM – stability of cell membranes

As can be seen from this figure, for 3-month-old artificially grown (based on grains) plants of Aronnik Korolkovfrost is detrimental. Thisindicates a low level of frost resistance of this plant and the detrimental effects of frost.

When conducting an experiment as part of writing an article and determining the susceptibility of genes to cold/frost, significant differences were observed in the expression of the ICE1 and ICE2 genes. No differences in the cold/frost response were observed for the ICE2 gene. Whereas for the ICE1 gene, the expression level showed high activity (Fig. 3)[7].

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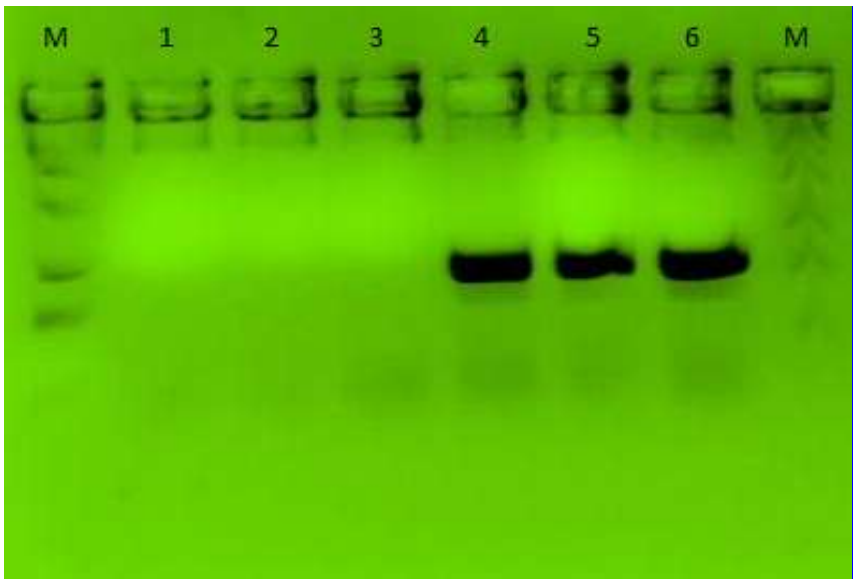

Figure 3. M - Molecular weight marker, (1-3) geneICE2,(4-6) geneICE1

The relative expression level of the ICE1 gene included in the experiment increased upon induction of low temperature stress (Fig. 4).

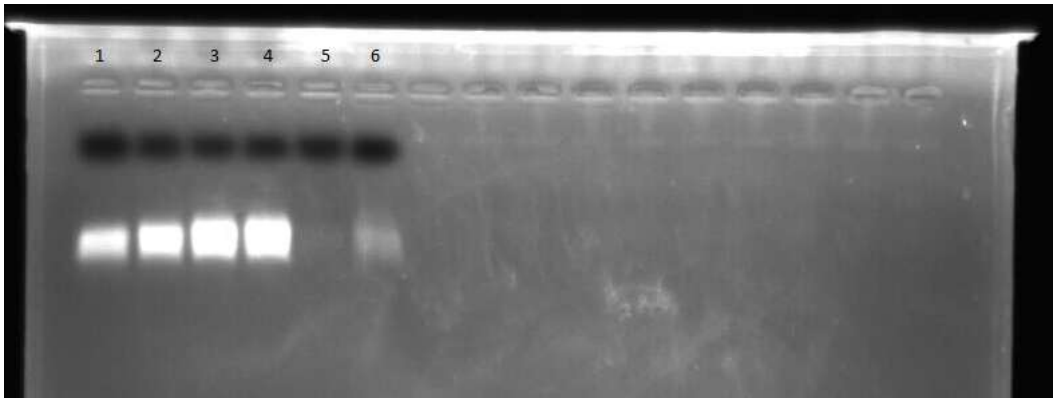

Figure 4. Gene expression levelICE1,measured in hours (1-6 hours).

Received on pfigures 3 and 4resultsindicate that the ICE1 gene exhibits a high level of expression in Aronnik Korolkov. Expression of the cold response regulator gene ICE1 in the resistant genotype significantly increased by 1.5–1.8 times upon stress induction.

The environmental factor has certain quantitative indicators: intensity and range of action. The actions of the factor are characterized by its amplitude (Fig. 5).

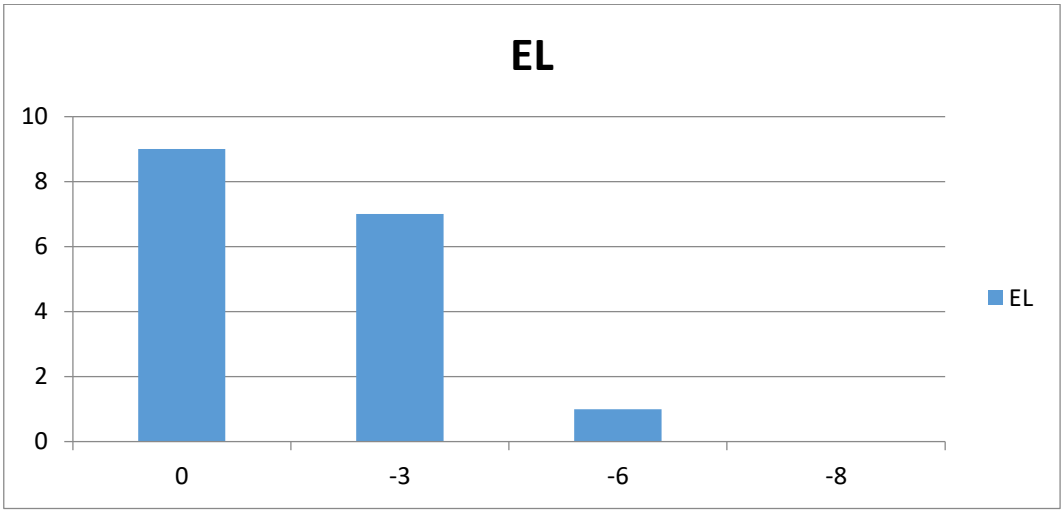

Figure 5. Scheme of the impact of the environmental factor on the life of Aronnik Korolkov: (1-(10%)– 10-(100%)) – Intensity of reactions of Aronnik Korolkov to cold stress; EL - Endurance limit of Aronnik Korolkov within 4 days; 0 - -8 Temperature regime.

According to the tests, the survival rate when frozen below -6 degrees for 4 days of Aronnik Korolkov officinalis was practically equal to zero. The survival of the studied plant after the tests did not reach 5 days, i.e., according to our data, Aronnik Korolkova is not frost-resistant, and temperatures below -60C affect this plant detrimentally.

The conducted studies showed that the ICE1 cold-resistance gene is present in this plant, and the level of its expression of Aronnik Korolkov officinalis is observed at 100%.

The obtained results show that Korolkov's Aronika officinalis has the ICE2 frost resistance gene, but the expression of this gene is insignificant. The immediate response of this gene does not keep up with the expression of a sharp drop in temperature below -6 ° C and has a detrimental effect on Aronnik Korolkov.

Thus, Aronika Korolkova is stress sensitive due to the insignificant expression of the ICE2 gene. The more important role of the gene in the process under study requires additional research with this plant species.[8].

DISCUSSION

In the course of research within the framework of the article, it was revealed that low temperature stress leads to a violation of the stability of cell membranes, changes in the lipid, protein, and enzyme balance of the cell. It was found that the ICE1 gene was more actively expressed in Aronnik Korolkov, and its expression was significantly higher already at the stage of cold acclimatization, i.e., at the first stage of stress induction. These results are consistent with data from published studies, in which the increased resistance of tea plants is due to a faster response to cold stress. Conducting additional studies with different genes will help verify the data obtained.

CONCLUSION

Thus, increased expression of the ICE1 gene is shown. In general, the stable ICE1 genotype is characterized by an earlier response to stress. However, only two genes were analyzed in our work, therefore, for further verification of the resistance of Aronnik Korolkov to cold, it is necessary to involve more genes. For further research, it is important to study the expression of all known genes in different organs of Aronnik Korolkov plants at different strengths of low temperature exposure.

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