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Evaluation of the expression of induced genes in response to dehydration stress of Camelina (*Camelina sativa*) calli

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ABSTRACT

Plants are constantly exposed to various biological and non-biological stresses that endanger their lives. Drought stress is one of the abiotic stresses that have a great impact on the yield and life of plants and is one of the main causes of reduced crop yields. Reducing the effects of environmental stresses such as drought using methods such as irrigation, fertilizer application and appropriate planting methods is limited. Therefore, genetic modification of plants is an important effort to minimize the effect of environmental stresses. in this research, Twenty disinfected camelina seeds were cultured on the MS medium containing 3% sucrose, 0.8% agar and pH 5.8 under a laminar hood. After 14 days, the cotyledon explants (about 1 cm) were separated from the seedlings and placed on the callus induction medium. The MS callus induction medium containing 0.5 mg / l kinetin, 2 mg / l -2,4 D, 3% sucrose, 0.8% agar and pH 5.8. Samples were subcultured every two weeks to the same medium and calli were formed after 4 weeks. Then the calli were transferred to the medium containing a concentration of 30% PEG. To study gene expression, first callus samples were treated with liquid nitrogen and to study the effect of drought stress on gene expression, this sample was sent to Zagros Bioidea Company located in the Razi University Incubator. Gene expression was performed through microarray technology. The results showed that seven different genes whose expression increased by almost six times the control value can be mentioned, including Cold-acclimation protein (CAP160), NAC10, Abscisic acid (ABA), ABF4, CRK3, lysM domain receptor-like kinases (LYKs) and Basic/helix-loop-helix(bHLH130-like). Drought tolerance is not a genetically simple trait, but a quantitative and complex trait with various aspects that require the use of molecular methods to investigate the relevant mechanisms. This study aimed to investigate the expression of different genes of callus tissues of the Camelina plant under stress and non-stress conditions by microarray method.

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Introduction

Drought is one of the most important factors in reducing plant yield in arid and semi-arid regions in the world. The rate of yield reduction varies depending on the intensity of stress during the growing period and the tolerance of the crop (1). Increasing air temperature during the growing season, especially in the summer months, increases the severity of dehydration stress in the plant. Therefore, selecting cultivars that are more tolerant of dehydration will ensure higher yields. The need for oilseeds is constantly evolving around the world. Among the crops, oilseeds are of special importance and have rich reserves of fatty acids. Camelina is an oily plant belonging to the Brassicaceae family and a hexaploid plant, and 89,418 genes encoding the protein have been identified (2). Due to the close genetic relationship that Camelina has with the Arabidopsis model plant, Arabidopsis genes have been used for resistance and dehydration in the Camelina (3). Camelina is a plant that can adapt to cold and dry environments and is also found in warm regions and can also tolerate water shortages as a stress in the early growing season (4). About 80% of camelina oil is used in health products and food, and about 14% of the oil is used in industrial raw materials such as cosmetics, detergents, fuels, plastics and adhesives (5). Camelina can be used as an antifungal or insecticide in agricultural products. Inhibits the growth of fungal hypha when 1 to 5% camelina is added to the soil. A fungus that causes cotton and alfalfa root rot and can be used to protect against fungal attacks (6). Camelina has a unique protein, so it is widely used in the pharmaceutical, agricultural, nutritional and biofuel industries (7). Plants such as soybeans, peanuts, canola, camellia, etc. are rich sources of oil. These species are commonly used for cooking oil, but camellia is an exception and is considered in the discussion of fuel and food compared to other plants. Camelina has many important biological compounds (8). Biological components such as phenols, glucosinolates, tocopherols, unsaturated fatty acids and polysaccharides have been reported to play a role in the defense mechanism and reduce the risk of cancer and other vital activities of living organisms (9). Differences in stress resistance may be due to differences in plant reactions in terms of stress perception, signal transduction, proper gene expression, or other new metabolic pathways that are unique to resistant plants (10). One of the most important methods

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to reduce the effect of environmental stresses is to find genotypes that have desirable genes and traits in this field (11). For this purpose, one of the most important research goals is to understand the molecular mechanisms associated with dehydration in plants. With recent advances in molecular genetics, hundreds of stress-induced genes have been identified and used as candidate genes for genetic engineering. Abiotic stresses increase or decrease the expression level of relevant genes by altering the pathway of carbohydrate metabolism (12). Therefore, identifying the relevant candidate genes in tolerant and sensitive lines by evaluating the changes in gene expression in the stress conditions compared to the non-stress conditions to implement programs to increase plant resistance to drought stress can be a very good starting point. Alteration in gene expression is one of the most important events that occurs in stressed plant cells and leads to biochemical and physiological responses to stress (13). There are several methods for studying gene expression, one of the most important of which is a microarray. A microarray test examines the expression levels of thousands of genes under an experimental condition. Most studies, including multiple microarray tests, cover a range of conditions. For example, microarray studies often compare the expression levels of genes in different growths at different stages of development or in healthy and stressed samples (14) . Myeloblastosis (MYB) proteins are a diverse class of DNA-binding proteins involved in regulating the transcription of plant genes responsible for responding to environmental stresses such as salinity, cold, and drought (14). The MYB family is one of the largest gene families of transcription factors that control stress in most plants. So far, more than 100 genes have been identified in the Arabidopsis family, another 80 in rice and 78 in maize (15). In many transcription factors of Bzip, MYC, bHLH proteins(16), DREBs (17) and NAC (18) play the most important role in responding to abiotic stresses (19). A number of hormones and transcription factors are actively involved in regulating plant tolerance to stress. Among the hormones of abscisic acid, salicylic acid and jasmonic acid, abscisic acid has the most important role in controlling the expression of stress-related genes (20).

Materials and Methods

Seed culture and callus induction

The camelina seeds (Soheil cultivar prepared by Biston Shafa Company, Iran) were disinfected with 2% sodium hypochlorite for 10 min. Then, they were rinsed with sterile distilled water three times. Twenty disinfected camelina seeds were placed on the MS medium containing 3% sucrose, 0.8% agar and pH 5.8 under the laminar hood. The cultured seeds were incubated at 25 ° C at 16 h photoperiod and 8 h dark conditions. After 14 days, the cotyledon explants (about 1 cm) were separated from the seedlings and placed on the callus induction medium. The MS callus induction medium containing 0.5 mg / l kinetin, 2 mg / l -2,4 D, 3% sucrose, 0.8% agar and pH 5.8. Samples were subcultured every two weeks to the same medium and calli were formed after 4 weeks. Then the calli were transferred to the medium containing a concentration of 30% PEG.

Microarray analysis

Autoclave and DEPS were used to extract RNA during the following steps. For this purpose, 100 mg of the sample tissue was poured into a mortar and converted into a powder using liquid nitrogen. the prepared powder was transferred to a 2 ml microtube and immediately (before removing the powder from the freezing state) added 1 ml of extraction buffer (RNx-plus) (shake the microtube gently for 5 to 10 seconds to buffer. Then mix thoroughly with powder). Samples were placed at room temperature for 5 minutes. Then 200 µl of chloroform was added to the mixture and the microtubes were shaken slightly to mix the ingredients thoroughly. The microtubes were placed on crushed ice for 5 minutes. The microtubes were centrifuged at 12,000 rpm and 4 °C for 15 minutes. After removing the microtube from the centrifuge, the supernatant phase was separated and transferred to a new 1.5 ml microtube and 1 ml of isopropanol was added. The microtube was shaken gently to mix the ingredients and finally place in the freezer at -20 °C for 20 minutes. The samples were centrifuged at 12000 rpm and 4 °C for 15 minutes. The supernatant was poured and 1 ml of 75% ethanol was added to the precipitate. Slightly vortex the microtube to dissolve the sediment formed at the bottom of the microtube in ethanol. The samples were centrifuged at 7500 rpm and 4 °C for 8 minutes. After removing the microtube from the centrifuge, the supernatant phase was separated and transferred to a new 1.5 ml microtube and 1 ml of isopropanol was added. Shake the microtube gently to mix the ingredients and finally place it in the freezer at -20 °C for 20 minutes. The samples were centrifuged at 12000 rpm and 4 °C for 15 minutes. The supernatant was poured and 1 ml of 75% ethanol was added to the precipitate. Slightly vortex the microtube to dissolve the sediment formed at the bottom of the microtube in ethanol. The samples were centrifuged at 7500 rpm and 4 °C for 8 minutes. The supernatant was out and leave it at room temperature to dry slightly. 50 µl of sterile distilled water treated with DEPS was added to the sample. The microtube was placed at 5 °C in Ben Marie for 5 minutes to dissolve the precipitate in water. At this point, the RNA was ready to use. After this step, the obtained sample was mixed with the loading buffer material and transferred to the electrophoresis wells and electric current was passed through it and a photo of the created bands was taken with a gel docking device. To make a single-stranded cDNA, the Vivantis two-step kit from Sina Clone Company was used according to the following steps: 10,000 ng of the prepared RNA sample was poured into the tube. 1 microliter of oligodT primer was added to the RNA sample. Then one microliter of dNTP was added to the previous volume. The volume of the solution was increased to 11 µl with deionized and nuclease-free distilled water. The above solution was placed at 65 °C for 5 minutes, then placed on crushed ice for 2 minutes. To synthesize cDNA, 2 µl of Buffer M-Mulv, 0.5 µl of M-Mulv R-transcriptase and 16.5 µl of Nuclease-free Water were added to the sample and placed at 42 °C for 60 minutes. It was then placed at 85 ° C for 5 minutes to stop the reaction. The resulting product was a single-stranded cDNA that could be used directly for the PCR reaction. To study gene expression, first callus samples were treated with liquid nitrogen and to study the effect of drought stress on gene expression, this sample was sent to Zagros Bioidea Company located in the Razi University Incubator. Gene expression was performed through microarray technology. The microarrays

were designed using Imaxio, which is accelerated by agile technologies. These technologies have been approved for microarrays. For this purpose, 60 nucleotide probes were used to study the expression of genes. All statistical items such as data distribution were observed for statistical analysis of gene expression. The normal distribution of the medians allowed for statistical analysis.

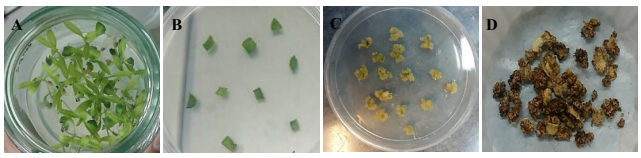

Figure 1. Steps of preparation callus sample, Germinated seeds (A), cotyledon pieces explants (B), callus induction (C) and Dried calli (D).

Results

Figure 1 shows the results of different steps of culture plant cultivation and preparation of callus samples for microarray analysis.

The results of DNA microarray experiments showed that many genes in drought stress conditions in the Camelina plant significantly increased expression. Seven different genes have increased expression almost six times as much as the control, including CRK3, ABF4, Basic / helix-loop-helix (bHLH130-like), Cold-acclimation protein (CAP160), lysM domain receptor-like kinases (LYKs), NAC10 and Abscisic acid (ABA) (Table 1).

Discussion

Microarrays have been one of the first high-powered tools for transcriptome analysis over the past two decades (21). In this way, creating thousands of profiles of genes simultaneously, improves the study of transcript analysis (22). Genes encoding transcription factors are an important group of genes that play a key role in the development of resistance to biological and abiotic stresses in plants. Studies show that ATAF, CUC and NAM genes are expressed in response to drought and salinity stress and transcription factors play a role in regulating these genes (22). The cDNA sequence for CAP160 is an acidic protein that is detected in spinach by adaptation to cold and increases with exposure to temperature and water stress Research by Kaye et al showed an increase in the expression of this gene in dehydrated conditions in tobacco plants (23). Abscisic acid is an important phytohormone for plant growth and development and plays an important role in integrating a variety of stress signals and controlling stress responses. Plants must regularly adjust ABA levels according to changing physiological and environmental conditions (24). ABA mediates stress-induced modulation in plant development, including inhibition of root growth, leaf aging, and stomatal closure, allowing the plant to adaptively shorten its life cycle to escape drought. Stress-responsive transcription factors play an important role in abiotic stress response and stress tolerance (25). Among them, the ABF4 factor increases potency by inducing a suppressor of overexpression of constans1 (SOC1) transcription under drought stress conditions. In the research of Wang et al (26)., In a study on *Arabidopsis thaliana* under drought stress conditions, they saw a significant increase in the ABF4 factor. CRK3 protein kinase plays an important role in controlling cell cycle progression in G2 / M phase transfer (27). LYKs are essential for the detection of pathogens and the activation of immune responses (28). Stress in the plant increases, which is in accordance with the results of research by Zhang et al. In the study of drought stress in citrus (29).

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Interest conflict

The authors declare that they have no conflict of interest.

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Table 1. Genes with the highest expression under drought stress in microarray analysis.

Gene_description	Control	X expression
CRK3	-2.763032787	6.788217481
ABF4	-2.763032785	6.788217471
bHLH130-like	-2.734032785	6.653127978
CAP160 protein	-2.719807917	6.587850959
lysM domain receptor-like kinases (LYKs)	-2.653032784	6.289881269
NAC10	-2.622032788	6.156168788
Abscisic acid (ABA)-dependent signaling	-2.614032786	6.122126208

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