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IDENTIFICATION OF THE SPECIFIC GENES OF ISOCITRATE LYASE FROM PLANT *AMARANTHUS CAUDATUS* L.

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Abstract

Isocitrate lyase is considered as an enzymatic indicator of a cycle diagram and occupy different functions in the plant cell metabolism. Objective: The objective of this study was to identify Isocitrate lyase genes and to development the specific primers. Methods: Using primers prefixes cloned of metamorphic genes Isocitratase lyase that were able to find the presence of two genes within the gene of *Amaranthus* namely (icl_1, icl_2), which come on mentioned later in relays products by the polymerase chain reaction (PCR). The results: The results of this study showed the total RNA extract was two types of mRNA of Isocitrat lyase genes of the A. *thaliana* genes named ICl_2 and ICl_1. The presence of two genes icl_1 and ICL_2 in the *Amaranthus* genome showed the fragment of the ICl_1 gene of *arabidopsis* was 53% fragmented of the icl_2 gene is 447 bp long and its homology with the icl_1 gene of *arabidopsis* was 36%. On the basis of sequences, specific to isocitrate lyase genes were developed, which made it possible to investigate their expression activity at the initial stages of *Amaranthus* development.

Key words : Isocitrate lyase, *Amaranthus*. Arabidopsis, metabolism, primers.

Introduction

At present, doing research and analysis the performance systems of various plant enzymes are widely used and applying for research systems of Isocitratase lyase plants as well as microorganisms and animals. Isocitrate lyase is considered as an enzymatic indicator of a cycle cells programs (Akubugwo *et al.,* 2007). The main physiological role of plants is to participate in the manufacture of sugar that involves the growth of a particle organism in the conditions of feeding and maintain the organic materials accessible of the plant. It was showed the presence of Nucleosome forms and calories are involved in the process recovery of glycosides. On this basis, the plant cells type (ICL) showed number of molecules of isoforms which possessed different types of sites of propagation in developing seeds that grow especially oily and contain a large amount of fat (Azhar-ul-Haq *et al*., 2006), other researchers have demonstrated two similarities of isocitratase lyase plants (Biswas *et al*., 2013; Clemente *et al*., 2011).

Isocitrate lyase is a glyoxysomes enzyme template (Azhar-ul-Haq *et al*., 2006) in the cells may be at the same time inside the nucleosomes of ICL-xls, which occupy different functions in plant cell metabolism (Biswas *et al*., 2013). Moreover, there is information of data through experiments conducted on the acquisition of the nucleosum ICL sites, that has a specific enzyme of the green tissue which plays a major role and function in the cytoplasm which ensures recycling in the reaction that takes place during the light breath for hexadecylates (Eastmond *et al*., 2000; Davis *et al*., 1994) then ICL can be considered a catalyst in direct or reverse the activity which shows cleavage of isocitrate and the activity that lead to generate glyoxylate and succinate) (Egamperide, 1990; Azhar-ul-Haq *et al*., 2006).

Agricultural products produce a large amount of protein, amino acids, Vitamins, micro and micro elements. This is what makes marigold of a promising future agricultural marketing. The study of the biology of molecules and the properties of enzymes are regulated the process especially the high quality food meal. Therefore, the objective of this study was to identify the Isocitrate lyase genes and to development the specific primers.

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Materials and Methods

Research materials in this work were obtained and worked at the State University of Varonish, Russia for the period from 2017 to 2018. Seeds of marigold plant *Amaranthus caudatus* L. where planted in the hydroponic at 25°C for 10 days. Plant cells RNA were extracted by phenol-chloroform with the following modifications in order to inhibit RNAase in the phase of obtaining the PI cells that used 42.6% substance of guanidine thiocyanate and cleaning process of the RNA using lithium chloride. Good qualitative analysis for RNA conducted that kept the nature of the mucous membranes with electrical relay at 1% volts / om in gel acarose at 7 volts/cm (Eprintsev *et al*., 2007). Reverse cloning was done using reverse transducer revert Aid M- MuLV and applied the manufacturer's instructions of Lithuania. In the seed quality analysis using primer Oligo- (DT) 20 that process of selectively the primers comparison with amino acids in their sequence of isostrates. Plants primers were carried on basis base http://www.ncbi.nih.gov/) (NCBI, CWA).

The polymer interaction was achieved with the group of materials in the Amplisence Helicon/ Russia. While the PCR analysis was selected the principles based on the sequence of acid amino acids direct -5'-T G Y G G N C A Y A T G G G N G G - 3 ' - 5 ' - DCRCARTTRTANGC-3' (Egamperide, 1990). However, conducted the PCR reaction in the tertsik and biometra-DNA devices personal cycler (Biometer Germany) and the process of finding serial nucleotides that conducted it on the regulator (Automatic Sequencer USA), CEQ2000XL (Beckman Conlter). According to the manufacturer's activities, genetic activity of UbQMPAH for bp, the PCR conducted on a DNA Engine Thermal Cycler (Chromo4) (Bio-Rad - USA using the formula of SYBR green I. The process of selection specific primates and their relationship to genes, the isostratizes were conducted on the basis of the sequences which were obtained from this work using the program Primer 3 (Clemente *et al*., 2011). Either primers for PCR-PB as a result of analysis were obtained ICL1 directly as 3'5'-GCCACTTGAATAGTT, on the contrary, the ACATAAGGCAACTTC-3'-5', where the temperature was 54° C for ic_2 -GGATAGGACTTACTA-3'-5' and vice versa-AGCTTGTCCTTAAA-3 '5' the temperature was56°C (Biswas *et al*., 2013).

Results and Discussion

The results of this study obtained a high quality and non-deformed RNA products from plant tissue, which was determined by the method of conducting electrical transfer of 1% of the substance. the results obtained by the electric transfer procedure show that it is a quantity 28R rRNA predominates above 185 rRNA material and this indicates the absence of deformed products Under the influence of RNAase (Fig. 1). These results are agreed with previous report (Eprintsev *et al*., 2007), also results agreed with previous study confirmed the experimental methods using phenol-chloroform extract it became clear to us the concentration of the total tissue of the RNA from the seed to the plankton (Clemente *et al,*. 2011) is carried out in the selection process Holotropic agent used guanidine-isothiocyanat and using the substance interconnectedness alkaloz gel (Akubugwo *et al.,* 2007).

The results of this study showed the reaction steps of polymers in molds kDNA and extracted from growth Plush Category (Red) per day of third growth indicators 1- fruits amplification with extract of primates. The isostrates showed the presence of two gel tapes -

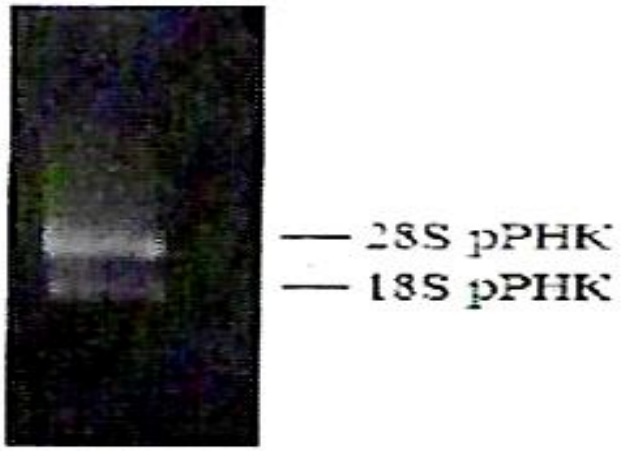

Fig. 1 : The electrical transfer of the RNA tissue group from the velor seeds of 1% of the gel Gelatinous substance with a substance ethidium.

electrical relay as shown in the fig. 2. The first step manufacturing integrated DNA use recombinant reverse transcription Moloney virus that proliferating the white blood cells of the M-MuLv in the mice and the enzyme mentioned Consists of only one sub-unit and shows' → 5' 3' primer and depends on polymerization activity (RNA) as a form of immunization Primer Oligo- (dt) 20 (Eprentsev *et al.,* 2010). The reverse transcription process extracted a substance RNA and then PCR material and DNA material analysis with a preliminary extract of genes. This indicates the presence of these genes (Kraujalis *et al*., 2013).

The real time PCR of this study showed the later period of using primers with specific properties of icl_2 and icl_1 genes with the maximum expression of the gene

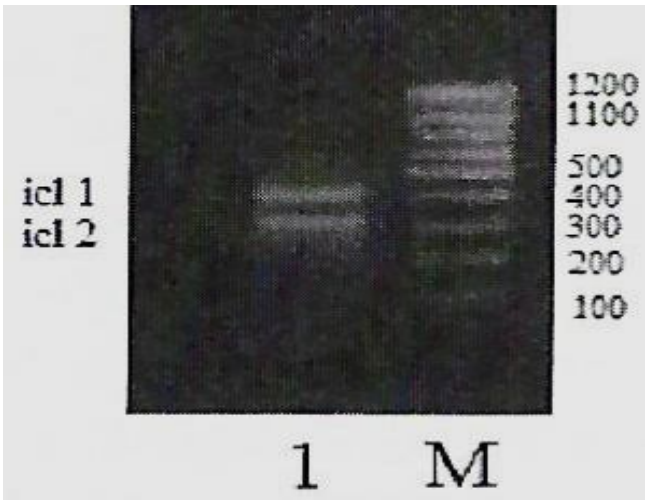

Fig. 2 : The results of the reaction steps Polymers in molds kDNA and extracted from growth Plush Category (Red) per day Third of growth, indicators 1- fruits amplification with extract of primates.

that observed them during a 1-2 days period of growth and germination. The presence of two genes icl_1 and icl_2 in the *Amaranthus* genome showed the fragment of the icl_1 gene of *arabidopsis* was 53% fragmented of the icl_2 gene is 447 bp long and its homology with the icl_1 gene of *arabidopsis* was 36%. On the basis of sequences, specific to isocitrate lyase genes were developed, which made it possible to investigate their expression activity at the initial stages of *Amaranthus* development. The gene activity of icl_1 over time was gradually decreased and the ICL_2 was begun reduced then starts to increase (Fig. 3). The results of the PCR in real time with the primers with specific properties agreed with previous reports (Khanam and Oba, 2013; Stintzinga *et al*., 2004). Using PCR analysis, of selected primers comparison in the results obtained when there is ammonia acid from the molecules proteins of ICL, which extracted

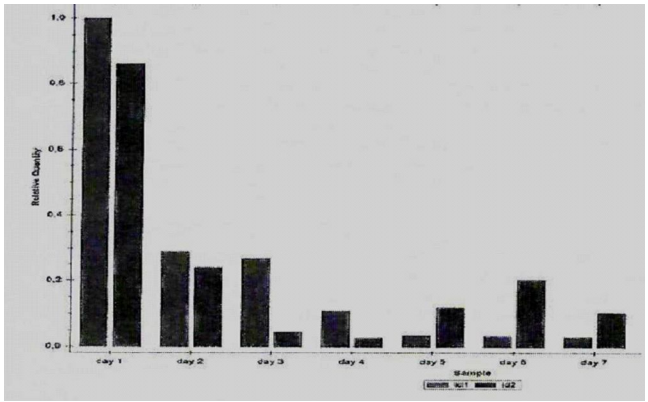

Fig. 3: The relative level of expression of Isocitrate lyase genes in the growing marigold plant.

from different plant types of taxonomic groups. There was a need to be fully convinced that the PCR that was separated the combination of different genes which is known as isocitrate lyase. Therefore, the PCR products that obtained were extracted from the gel which came in accordance with the work (Eprentsev *et al.,* 2009). The cleaned (cleaned) nucleotides in sequence have been sequenced and compared with the database Genetic (Kraujalis *et al*., 2013). The data showed that the amplicons have a genetic similarity with mRNA genes of the isostratylase of thaliana A. As well as for the length amplicons (339 bp) had identified a sexual similarity with the genes of icl1 of the plant Arabidosis (Kumar *et al*., 2011).

Comparison of the analysis results in the second fruit product using the PCR with the obtained from the primers of isostratylase showed us that its genetic similarity with icl2-A-thaliana genes (AB442085.1) is less than the first amplicon and is 36%. The data obtained during the amplification process with extracted primers using amplicons mRNA for both genes Isocitrat lyase (Tzachi, 2003). A genetic similarity to the nucleotides in its gene sequence of thaliana- A explained and explained in Gene Bank. Therefore, the germination and seed growth of the marigolds express two types of genes that denote Isocitrat lyase (Rucht and Windmer, 1986). The genetic reported two differed enzymatic enzyme profiles. On the basis of rise and follow-ups had been tested on the basis of fertile primers specific recipes e.g. sequence primers directly to icl1 3'5'- GCCACTTGAATAGTT- and reverse 5'-ACATAAGGAAACTTC-3 'degree Heating temperature 54°C for ICL2 -GGATAGGACTTACTA-3 '-5', direct 3 '5' - AGCTTGTCCTAGAA - at 56 ° C this corresponds to (Sharma *et al*., 2012).

Conclusion and Recommendation

The results of this study concluded that the total RNA extract there are two types of mRNA of Isocitrate lyase genes shown in their symmetry with *A. thaliana* genes icl_2 and icl_1. The gene expression of the same type of icl when the growth with the plush showed the dynamic activity of ICL in the growth of the seeds of the shellfish.

Conclusion

The results of this study concluded that the total RNA extract there are two types of mRNA of Isocitrate lyase genes shown in their symmetry with *A. thaliana* genes icl_2 and icI_1. The gene expression of the same type of icl when the growth with the plush showed the dynamic activity of ICL in the growth of the seeds of the shellfish.

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