Greener Journal of Biochemistry and Biotechnology

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Research Article

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ABSTRACT

Gamma rays have sufficient energy to ionize matter and therefore can damage living cells. The damage produced in the cell or tissue is proportional to the number of ionizing paths produced in the absorbing material. This investigation was carried out to determine the effects of gamma radiation on the specific gene (arginine decarboxylase (ADC)) of pistachio to choose the best one under various stresses. These results show that the cobalt-60 radiation source which its energy was 1.3M.ev destroy ADC gene. Gamma radiation treatments may be used to examine their effects on germination and survival percentage, growth traits and morphological variation of plants and then isolated the gene of interest to transfer it to other plants.

Keywords: Gamma ray, arginine decarboxylase, pistachio, cobalt-60.

ABBREVIATIONS

CTAB: cetyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, extraction buffer
ADC: arginine decarboxylase
SAM: S-adenosylmethionine
EtBr: Ethidium bromide

INTRODUCTION

The genus Pistacia consists of eleven species which is commercially important (Zohary, 1952). There are also two other wild species in Iran; Pistacia atlantica subsp. Mutica and Pistacia khinjuk (Kafkas and Perl-Treves, 2002). Pistachio originated in central Asia (Whitehouse, 1957) but now it is planted in many countries around the world, Asia, southern Europe, South Africa, Australia and America. The main world producers of pistachio nuts are Iran, USA, Turkey and Syria (FAO, 2006).

Scientists tend to think of biological effects in terms of the effect of radiation on living cells, as a matter of fact, ionizing radiation, by definition, interacts only with atoms by a process called ionization. Thus, all biological damage effects begin with the consequence of radiation interactions with the atoms forming the cells. If radiation interacts with the atoms of the DNA molecule, or some other cellular component critical to the survival of the cell, it is referred to as a direct effect. Such an interaction may affect the ability of the cell to reproduce and, thus, survive. If enough atoms are affected, such that the chromosomes do not replicate properly, or if there is a significant alteration of the information carried by the DNA molecule, then the cell may be destroyed by "direct" interference with its life-sustaining system.

Gamma radiation is very high-energy ionizing radiation. Gamma photons have about 10,000 times as much energy as the photons in the visible range of the electromagnetic spectrum.

To examine the effects of radiation on plants is a complex and broad field. Gunckel and Sparrow (1961) found that the Gamma irradiation increases the plant growth and development by some changes in cells and tissues depending on the irradiation level. It is one of the most important physical agents used to improve the characters and productivity of many plants (Sharma and Rana, 2007). The gamma ray had an adverse effect on

plants and this depended on plant species and the dose of irradiation (Artk and Peksen, 2006). El Sherif et al. (2011) suggested that increasing doses of gamma irradiation caused severe effects on the plant development.

These effects contain changes in metabolism and cellular structure of the plants (Wi et al., 2005). Many of the complications of a phenotypic or biochemical based assay can be done through direct identification of genotypes with DNA based assays (Mengoni et al., 2000).

Two alternative pathways appear to have specific roles in development and growth in plants. Despite ornithine decarboxylase ODC seems to be implicated in the regulation of the cell cycle in actively dividing cells and meristematic zones, arginine decarboxylase is the primary enzyme for putrescine synthesis in non-dividing elongating cells, secondary metabolic processes and in cells under various stresses (Gerner and Meykens, 2004 reviewed in Alcazar et al., 2006).

MATERIAL AND METHOD

Plant Material

In the present study, we collected pistachio leaf samples from 18 Fandoghi trees in commercially cultivated pistachio cultivars in Kerman, Iran.

DNA Extraction

The collected fresh leaf samples were washed and dried and quickly frozen in liquid nitrogen then ground using mortar and pestle. Genomic DNA was extracted from leaf tissue by the modified CTAB method (Kafkes and Perl-Treves, 2002). The DNA concentration and purity was determined using a spectrophotometer and DNA was diluted to 5µg/µl for PCR reactions.

PCR Amplification

The ADC primer was tested to amplify the isolated DNA. Primer sequence and annealing temperature show in Table 1. PCR was performed using ADC primer and amplification reactions was carried out in an Eppendorf Master cycler (Eppendorf Netheler-Hinz, Hamburg, Germany), The apparatus was programmed to execute the following conditions,1 cycle: a denaturation step of 5 min at 94°C, followed by 30 cycles composed of 30 s at 94°C, 90 s at the annealing temperature 60°C. A final extension of 72°C for 5 min was included.

Table1: primer sequence and annealing temperatures

Primer Sequence (5'-3')	Anealing Temprature
GATCCGTCATAATCGATACC	60°C
ATGCTTGATTGCTTCCAGC	60°C

The ADC primer was tested to amplify the isolated DNA. PCR was performed using ADC primer and amplification reactions was carried out in an Eppendorf Master cycler.

PCR reactions were carried out in a volume of 25 µl with 30 ng of genomic DNA, 2 µM primer ,1 U of Taq polymerase, 0.2 mM dNTPs, 10 mM Tris–HCl (pH 8.3), and 2.5 Mm MgCl2. After amplification, the gels were stained with 0.5 µg/ ml ethidium Bromide solution and visualized by illumination under UV light. Reproducibility of the patterns was checked by running the reactions in duplicates.

Gamma emitting radionuclides are the most widely used radiation sources. The penetrating power of gamma photons has many applications. However, while gamma rays penetrate many materials, they do not make them radioactive. The three radionuclides by far the most useful are cobalt-60, cesium-137, and technetium-99m.

We used cobalt-60 radiation source which its energy was 1.3M.ev. After that we repeated the PCR reactions with exactly the same condition. We used cobalt-60 radiation source just in the laboratory experiments not in the field and the high research council and relevant committees approved the study.

RESULT

The DNA concentration and purity was determined using a spectrophotometer and the ratio (A260/A280) between 1.7- 1.9 corresponded to good quality DNA and it was used for PCR reactions.

The purpose of our study was to find the best sequence of the ADC gene for resistance to stress conditions after using Gamma ray and then growing the transgenic pistachio in each condition. It means we should save the entire genome of pistachio except the gene of interest (A D C) . We wanted to make different

transgenic pistachio using different ADC sequences and find the more resistance one under various stresses. However, we amplified the ADC sequence and then treated the DNA using cobalt-60 radiation source which its energy was 1.3M.ev. After that we repeated the PCR reactions with exactly the same condition. Using 1% agarose gel and let the gel run for 55 minutes from negative to positive. Once the gels were done, staying in enough Ethidium bromide (EtBr) to slightly cover the gel. The Ethidium bromide stains the DNA. Nevertheless, there were no PCR products and we couldn't find the changes in ADC gene sequence after using cobalt-60 radiation source, it is possible that the Gamma ray changes the primers attached sites on the. DNA strands and therefore there was no PCR product (Figure-1).

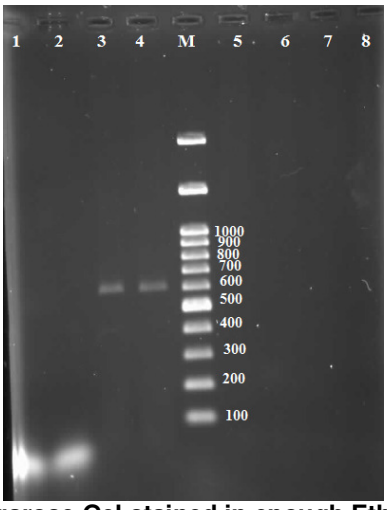

Figure-1: 1% Agarose Gel stained in enough Ethidium bromide.

Genomic DNA amplification pattern in pistachio, with ADC primer. 1,2: Control (primers), 3,4: ADC PCR product before Gamma ray treatment M: molecular weight marker, 5, 6: ADC PCR product after Gamma ray treatment.7:Negative control (PCR without DNA), 8: Negative control (PCR without primers).

DISCUSSION

Gamma ray is a kind of ionizing radiation and are the most energetic form of such electromagnetic radiation, having the energy level from around 10 kilo electron volts (keV) to several hundred keV. Hence, they are more penetrating than other types of radiation such as alpha and beta rays (Kovacs and Keresztes, 2002). Several energy rays are widely used in mutation breeding, such as X-, β-, and γ-rays, and neutrons, and protons. Gamma rays were reported to be the most efficient ionizing radiation of creating mutants in plants as they can induce high mutation numbers in plants (Kovacs and Keresztes, 2002). Gamma radiation can interact with atoms and molecules to produce free radicals in cells that are able to modify important components of plant cells (Wi et al.,2005).

Peng and Zhou, (2008), found that, the flavonoid content of soybean seedlings exposed to radiation treatment during the stress period was enhanced at the beginning and then decreased in comparison with that of the control. Kim et al.,.(2004), reported that, when the seeds of red pepper were first gamma-irradiated, the resultant plant growth was stimulated at 2 to 8 Gy but was scarcely affected at 16 Gy. In contrast, Ling et al.(2008) found that plant growth was stimulated at 10 Gy and that inhibition occurred at radiation levels above 10 Gy.

The results of the experiments of Minisi et al,.(2013), indicated that higher dosage of gamma radiation reduced number of surviving plants, germination percentage and plant height.

Arginine decarboxylase belongs to the family of lyases, specifically the carboxy-lyases, which cleave carbon-carbon bonds. In plants and some bacteria,putrescine can be synthesized from arginine via arginine decarboxylase (ADC) through the intermediate agmatine (Hanfrey et al. 2001). Putrescine is further converted into spermidine and spermine by spermidine synthase and spermine synthase, respectively. These add aminopropyl groups generated from S-adenosylmethionine (SAM) by SAM decarboxylase (Bagni and Tassoni 2001). In plants, ADC is the primary enzyme for putrescine synthesis in non-dividing elongating cells, secondary metabolic processes and in cells under various stresses (Gerner and Meykens ,2014 reviewed in Alcazar et al., 2006). Studying plant stress responses is an important issue in a world; stress conditions cause extensive losses of agricultural production worldwide. Individually, stress conditions such as salinity, drought or heat have been the subject of intense research. Plant tolerate to conditions such as excessive or inadequate light, water, salt and

temperature, and resistance to pathogens. Not only is plant physiology known to change under stress, but changes in the genome have also been identified (Hahn et al.,2013). There is no doubt that Iranian pistachio has the best taste and maximum nutrition. The special climate of Kerman province is the best place for growing pistachios and Iran exports 150,000 to 200,000 tons of pistachios annually (Ardekani et al., 2009).

From the obtained data noticed that gamma ray had a highly significant impact on plant DNA. In conclusion, it is suggested that, plants are exposed to gamma rays and then selected for resistance to stress and isolated the gene of interest to transfer it to other plants.

The results of the experiment indicated that this dose of gamma irradiation caused severe effects on the plant DNA.

The ultimate plan of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait and selection of economically important mutants. For breeding purposes mutagenic treatments with low physiological effects and strong genetic effects are desirable.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ali Negarestani for his assistance and also we thank all staff of the Iranian Pistachio Resource Institute for their help in collecting the plant materials.

Conflict of Interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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