International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Molecular Cloning of Flavone Synthase I Gene from Cumin *(Cuminum Cyminum)*

Kobra Yousefi [1], Farzaneh Razmju [2], Aziz Ahmad Osmani [3]

[1, 3]Departement of Biotechnology and Seed Thecgnology, Agricultur Faculty, Kabul University, Jamal Mina, Kabul, 1006, Afghanistan

[2]Department of Forestry and Natural Resourses, Agriculture Faculty, Kabul University, Jamal Mina, Kabul, 1006, Afghanistan

Abstract: ***Cuminum cyminum. L is one of the member Apiaceae family. This plant hasanticancer and antioxidant traits. The main purpose of this research is cloning of flavone synthase I gene from Caraway Cumin (Cuminum cyminum) indigenous in Iran.In order to cloning FNSI after extraction of total RNA, cDNA Library were constructed using oligo dT primers and gene amplification of FNSI was performed. After cutting the amplified gene cloned in expression plasmid PET28a and was transferred to the DH5α bacterial utilizing by heat shock method. Screening of transformed bacteria was done on medium containing kanamycin and at least to confirm the gene cloning method for determination of the cloned genes, gene amplified of the gene using specific primers and cutting plasmid by specific restriction enzymes and analyzed of the products on agarose gel was used. In this experiment, after all the cloning steps, the 6kb and 1kb bands were observed on the electrophoreses gel plasmid and the flavone synthase I gene, respectively, confirming the cloning of this gene in pET28a plasmid. Given that flavones are important in plants as well as being of high pharmaceutical value, having a cloned set of flavone synthase genes can be of high pharmaceutical and commercial value.***

Keywords: *Cuminum cyminum, Flavone synthase I, Cloning, Secondary metabolites*

1. Introduction

Cumin *(Cuminum cyminum L).*oneof the Apiaceae family is rich in secondary metabolites. Cumin extract has anti-oxidant [1], anti-bacterial [2] and anti-cancer [3-4] effects. The essential oils of this plant include compounds such as tannin, resin, allure, cement, phellandrene and caron. The main constituent of the essential oil comprises aldehyde cuminique or couinol [5].Secondary metabolites are a diverse group of molecules that help the plant to adaptation, especially under environmental stress [4]. Among these compounds, there are flavonoids and anthocyanins which, in addition to their structural roles in protective tissues, their role in attracting pollinating insects, act as molecular signals for plants to interact with the environment and acting as biological markers have been established in chemotaxis studies [6]. Most of the enzymes genes involved in the synthesis of secondary metabolites have been identified and sequenced[7]. Concurrent with the biosynthesis of flavonoids, other various compounds such as flavonoids, isoflavones, anthocyanins are synthesized by the activity of pathway enzymes [8].Flavones are one of the most important derivatives of flavonoids that have special roles in plants and are of great importance in the treatment and health of humans [9]. These compounds are essential in plants for protection against ultraviolet light, flower staining, intra-species interaction, plant defense and plant strength [10]. Flavones are a subgroup of flavonoids that are highly diverse [11]and are important in the protection of plants against UV [7],in the interaction of plants with other microorganisms [12-13] and in the treatment of human's disease. Their most important therapeutic properties include antioxidant, anti-cancer, antifungal, anti-inflammatory and anti-clotting veins [9]. Flavones are synthesized by the precursor of flavanones in the biosynthetic pathway of flavonoids (Figure 1). The biosynthetic pathway of flavones is caused by twoindependent enzymatic systems of flavone synthase I and flavone synthase II in different plants that are not found simultaneously in one plant. Flavone synthase I is a soluble dioxygenase that was first reported in the parsley in 1981 in the Apiaceae family [14] and the first reported outside the Apiaceae family was in rice [15]in the gramineae family. However, in most plants FNS is type II, which is a cytochrome P450 membrane band [8].Gene cloning can be used to cut a single gene sequence and to multiply it by insertion it in a bacterium that enables it to replicate. With Cloning, a single piece of DNA allows to obtain many copies of the original molecule. Gene modification and manipulation can be defined: TheFormation of new compounds from hereditary material by the incorporation of nucleic acid molecules that are produced by various methods outside the cell into a virus, bacterial plasmid, and or any other vector system that allows them to be isolated in the host genome, so that these compounds are not naturally present in the host butafter isolation canbe replicate continuously in the host [16]. By cloning the flavone synthase I gene, an enzyme that produces anti-cancer and antioxidant compounds (flavones), in bacteria, the product of this enzyme can also be extracted directly from bacteria for drug productionand indirectly we can provide the context for transferring this gene to a suitable plant (preferably an edible plant). So that medicine can be given to humans through diet. In this study, cloning of the flavone synthase I gene from the native *Cuminum cyminum* of Iran was transferred to DH5α by heat shock method after cutting the amplified gene fragment in pET28a expression plasmid.

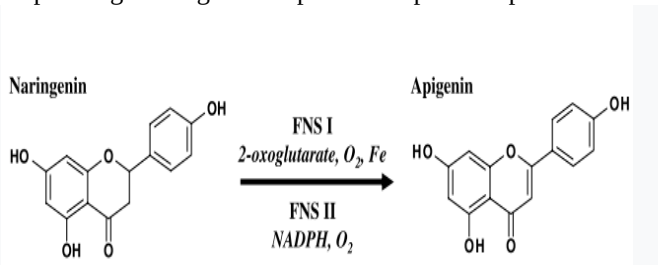

Figure 1: Flavon formation reaction from flavonone: This image shows the formation of epigenin from narangine by the flavone synthase enzyme.

2. Materials and Methods

2.1. Cultivation and preparation of plantlets

Cumin seeds (Pakan Seed Isfahan Co.) in plastic pots (20 15 15 cm) containing a mixture of clay, perlite and peat moss (1: 1: 1) were cultivated and then were transferred in a greenhouse at 22 ±2 centigrade degreeand relative humidity. 70% and irrigated once every two days. After 21 days, the plantlets were collected and washed and were prepared for Molecular analysis.

2.2. Molecular analysis

To investigate the expression of flavone synthase I gene, total RNA was extracted from freeze-dried plantlet using RNA X Plus kit (Sina gene co., Catalog number: RN7713C). The extraction steps were performed according to the manufacturer's recommended instructions. It should be noted that all procedures were performed in ice-free RNAase conditions. Thenfrom extracted RNA was constructed cDNA libraryby RNA Dependent DNA polymerase (RT) (MMuLV) from Fermentas Co.). The amplification of the flavone synthase I and tubulin genes (as internal controls) was performed using the cDNA library and the *Taq* polymerase enzyme in the presence of the specific primers listed in Table 1. The amplification reaction of this gene involved primary denaturation stage at94 ºC for 4 min and 30 replication cycles including (denaturation at 94 ºC for 1 min, annealing of primers for the amplification of the flavone synthase I gene and tubulin at 51 ° C and 57 ° C, respectively for 1 min, extending stage at 72 ° C for one minute) and one final extension at 72 ° C for 10 minutes. Equal amounts of PCR product were loaded on 1% agarose gel to investigate the expression of flavone synthase I gene compared to the internal control. To investigate the expression of flavone synthase I gene by semi-quantitative RT-PCR method and amplified by band intensity which was separated on 1% agarose gel. After normalizing to the corresponding amplified tubulin gene, it was performed by Gene tools software .

Table 1: Sequences of Primers for the Flavone synthase I Gene, Tubulin, Tm, and GC Percent

Content GC (%)	Tm (˚C)	Sequence *	Primer name
40.9	64.1	´-ATGGCTCCAACAACAATTACTG-3´5	F-FNSI
38.1	59.2	´5-CTAAGCTAAAATTCCATCTGC-3´	R-FNSI
45.5	63.7	5´-GCTTTCAACACCTTCTTCAGTG-3´	F-Tubulin
50	63.3	5´-CTTTCTCAGCTGAGATCACTGG-3´	R-Tubulin

*Sequence of Flavone synthase I Gene Based on Sequence of this gene of Cumin Plant with Accession DQ683349.1 Registered in Gene Bank and Tubulin Primers Sequence Based on Tubulin Gene Sequence Based on Wheat Tubulin Gene Record Accession DQ435671.1 in Gene Bank was designed and manufactured by MWG Germany Co.

2.3. Cloning

2.3.1. Bacterial culture and plasmid extraction

In order to extract the plasmid, the bacteria containing the desired plasmid must first be cultured (pET28a plasmid Fig.2). Materials needed for bacterial culture include Tryptone (10 g / l), NaCl (10 g / L) and yeast extract (5 g / L) for preparation of culture medium, 100 ml was prepared and autoclaved. After partial cooling of the culture medium, 25 µg / ml of antibiotic was added. After the bacteria were added to the incubator, the medium was incubated at 37 ° C and was shacked at 200 rpm for 10 h. To prepare competent cells, 5 ml of the culture medium was first inoculated with the bacteria. After 7 hours, the culture medium containing the bacteria was completely transferred to the culture medium to reach 0.8 OD under the same conditions.

The following materials were prepared for plasmid extraction and then manually was extracted plasmid.

50 mM glucose, 25 mM EDTA, 10 mM Tris-HCl (ingredients for preparation of solution 1), these materials were prepared from a basic stock and lysozyme (4 µg / ml) was used at the time of extraction.

- SDS 10% and NaOH .2M (Ingredients for Solution No. 2). For each of the compounds, a stock was prepared and the desired values were freshly combined.
- potassium acetate 3M (solution 3).
- Isopropanol and ethanol for plasmid deposition
- Tris-HCl 8mM for storage and dissolution of precipitated plasmid.

To investigate the quality of the extracted plasmid, 3 µl of sample was run on gel. If the plasmid extraction process is well performed, two bands should be seen. The upper band corresponds to the linear plasmid and the lower band represents the circular plasmid. A spectrophotometer is also used for quantification. The wavelength number of 260 nm entered in the following formula and the amount of DNA, RNA or plasmid is measured.

Nucleic acid value in micrograms per milliliter = OD_{260}× reversedilution × 100

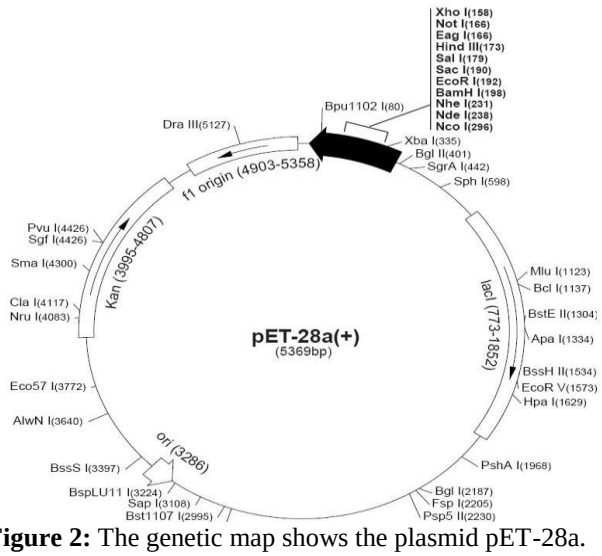

Figure 2: The genetic map shows the plasmid pET-28a. Each plasmid has three main features: the antibiotic resistance gene (hereinafter kanamycin), the ori site of

replication and the multiple site for cloning (MCS). In the MCS domain, XbaI and HindIII enzymes were

2.3.2. Preparation of competent cells

In this study, heat shock was used to perform transformations. To prepare the competent cells, a 5-liter pre-culture, was used which was shaken at 37° C for 12 h with 200 rpm. The next day, 100 µl of the suspension was transferred to the liquid LB medium. After bacterial growth, the bacteria were precipitated when the culture medium was adsorbed to the appropriate OD (Their absorption at 600 nm (OD_{600}) was in the range of 0.7 to 0.9.). The supernatant was discarded and then the competent cells were prepared using heat shock (Using cold calcium chloride)

2.3.3. PCR Product Purification

In order to prepare for the enzymatic digestion process byrestriction enzymes, first, the PCR products need to be free of any nucleotides, primers, specific salts, and et cetera. For this reason, a purification kit (Bayoner Company) was used. The PCR product purification process is a multi-step process performed by centrifugation at room temperature. This product contains three buffers (binding buffer, washing buffer and PCR product storage buffer) and columns that are completely specific to bonding the DNA strands.

2.3.4. Enzymatic digestion of PCR product and plasmid

At the two ends of the primers used, cleavage sites for the XbaI and HingIII enzymes are embedded. The plasmid and fragment amplified by these two enzymes were cut. After the digestion process, it is time to perform the process of binding the amplified gene to the plasmid. After cutting, it is necessary to perform the purification step again.

2.3.5. Insert PCR product into plasmid

The T4 DNA Ligase enzyme was used to insert the gene into the plasmid. The purified PCR product and plasmid were poured into a vial of 7 µl (50 ng plasmid and 150 ng PCR product). Then 0.4 µL of T4 DNA Ligase enzyme and 2 µL of buffer were added to enzyme. with sterile deionized water, the total volume of the reaction was brought to 25 µl and the sample was incubated at 22 ° C for 12-14 hours. Then the sample was incubated at 70 ° C for 10 minutes to deactivate the enzyme.

2.3.6. Transformation (transferring recombinant vector to host cell)

In order to performing transformation, the freeze competent bacteria were used. First, 1 µL of the plasmid that received the gene was added to 50 µL of competent bacteria and slowly mixed. Then the prepared suspension was placed on ice (temperature 4 ° C) for 20 minutes. After that, the samples were incubated at 42 ° C for 90 seconds and they were immediately put on ice. Next, 800 µl of LB culture medium was added to the vials. Finally, bacteria were incubated in the incubator for 45 minutes (37 ° C, 200 rpm).To investigate the transfer process, LB medium containing 1% agar and kanamycin (50 µg / ml) was used. This is a selective environment, and only the bacteria with the desired plasmid grow on it. After addition of about 100 µl of cell suspension to the selective culture medium, they were incubated for 14–16 h at 37 ° C. Three control samples were also used here. Plasmid culture lacking the target gene, culture medium with competent (plasmid-free) cells, and culture medium lacking bacteria.

2.3.7. Post-transformational analysis

After growing the bacterial cells on the selective medium, it can be seen that the plasmid molecule has been transferred. But it is not possible to determine whether the plasmid has the FNSI gene or not. Plasmid extraction from bacterial cells and enzymatic digestion of extracted plasmid were used to ensure this.After plasmid extraction, approximately 500 ng of plasmid extracted for PCR was used. Obviously, if the gene is inserted into the plasmid vector, it should be identified in the expected region of the gene band. The PCR program is similar to the one mentioned before.After enzymatic digestion, two bands (one digested plasmid and the other a digested PCR product) should be observed at the expected sites. If both bands were observed, the recombination and gene expression within the vector would be confirmed. The cutting and binding conditions must be repeated twice.

3. Results

3.1. Flavone synthase I gene RNA extraction

Extracted RNA quality was performed by rRNA banding on 1% agarose gel. As shown in Figure 3, the two bands corresponding to the 28 S and 18 S rRNAs show the appropriate quality of the purified and intact RNA.

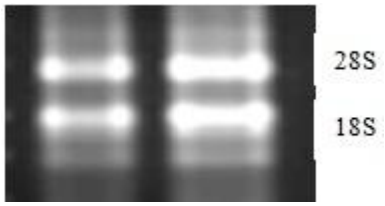

Figure 3: Extracted RNA sample

3.2. Construction of cDNA and RT-PCR

After cDNA extraction, gene amplification was performed at different binding temperatures and the best band quality was obtained at 51° C.

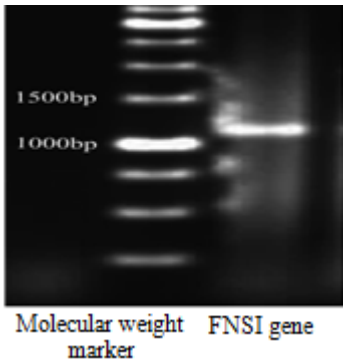

Figure 4: Agarose gel sample for the amplification of the flavone synthase I gene

3.3. Plasmid purification

Cloning of FNSI gene into pET28a first plasmid was purified. The agarose gel %1 was used to evaluate the purification quality and as shown in Figure 5, three plasmid

bands with different isoforms were observed. Also PCR products were purified before cutting and binding.

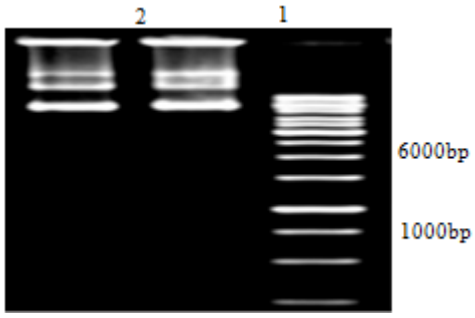

Figure 5: Extracted plasmids: Nos. 1 and 2 belong to the purified pET28a plasmid

3.4. Digestion of PCR and plasmid products

Enzymatic digestion of the gene and plasmid fragments was performed using XbaI and HindIII enzymes simultaneously using Tango buffer for 1 hour. After enzymatic digestion, the plasmid circular molecules must appear linearly (Figure 6). As can be seen in the figure, the cleavage was performed by enzymes correctly.

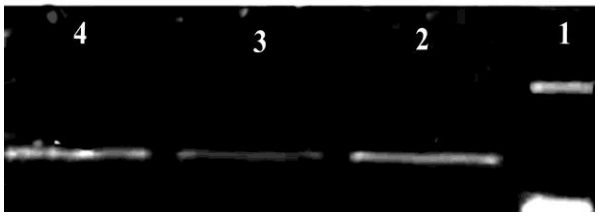

Figure 6: Displays intact plasmids (1) and digested by the enzymes XbaI (2), HindIII (3), and double digestion using both enzymes (4).

3.5 Insert PCR Products into Vector (Ligation)

After purification of PCR products and plasmid digested with kit to ligate the target gene into pET28a vector, ligation was performed by T4 ligase. After preparation of competent cells, the vector was transferred into it. After the appearance of the colonies, three different plates were used to confirm the transformation indicating that the transformation was successful. One plate lacking the kanamycin antibiotic was used to confirm viable of competent cells. As can be seen in Figure7 (C), cells grow in this medium, indicating healthy and growing cells. The second plate contains the culture medium containing the antibiotic kanamycin and non-transformed competent cells are cultured on them. The competent cells cannot grow on this medium. This plate was used for antibiotic confirmation (Figure 7 (A)). Finally, the third plate contained kanamycin medium and the transformed cells were grown on it that were used of this plate to confirm transformation(Figure 7 (B))

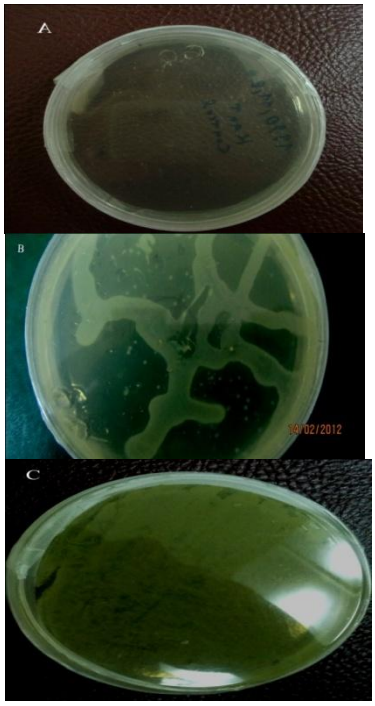

Figure 7: Confirmation of transformation using selective media (containing antibiotic medium). (A) Culture medium containing kanamycin and non-transformed competent cells. (B) Culture medium containing kanamycin and transformed cells. (C) Culture medium without kanamycin and untransformed competent cells.

3.6. Post-transformational analysis

To confirm the presence of the gene within the plasmid, a single colony was picked from the bacteria grown on the medium containing kanamycin and purified manually after growing in liquid LB medium. Then PCR and enzymatic digestion processes were used. After PCR using specific primers, a single band was observed in the 1091 bp region which could be a reason for the recombination of the extracted plasmid (Figure8).Also, after cleavage of the plasmid two bands one was observed in the 1091 bp region of the FNSI gene and a heavier band corresponding to the linearized plasmid (Figure 9)

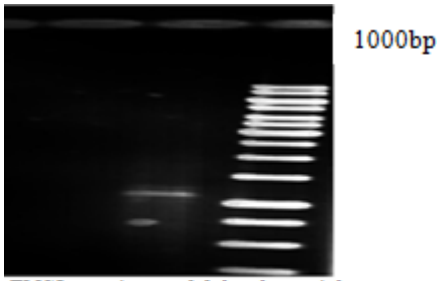

Figure 8: Transformation Analysis and Confirmation Using the Duplication Process Using Specific Primers

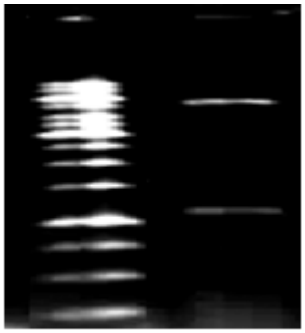

Figure 9: Confirmation of transformation using enzymatic digestion process

4. Discussion

Flavones are one of the most important secondary metabolites produced in the flavonoid biosynthetic pathway by the flavone synthase enzyme. It contains two types of FNSI and FNSII. Flavone synthase I is a soluble dioxygenase that has been reported in the umbrella family to be dependent on 2-exoglutarate and Fe ++, [14-8] Important properties of flavones are their allochemical properties and protection of the plant against UV radiation. Flavones interact with other microorganisms. Along with other roles of flavones in plant physiology, ecology and biochemistry, flavones are important constituents in human nutrition and health [17,7].The properties of flavones include antioxidant, anti-cancer, anti-tumor, anti-inflammatory, anti-bacterial, antifungal, antiviral and anti-clotting properties of the veins. It also has pharmacological and biochemical properties that are very beneficial to human health [18-22].The first FNSI enzyme was identified from leaflet of *Petroselinumcrispumcv*. Activityas says confirmed that FNSI converted 14C-radiolabeled flavanones to the corresponding flavones without forming a detectable reaction intermediate [11]Following these initial studies, FNSI enzymes were identified from a number of species of the Apiaceae family, and it was believed for many years that FNSI-type enzymes were restricted to the Apiaceae [23,24]. However, recent studies showed that the rice FNSI enzyme OsFNSI-1 converts the flavanone (2S)-naringenin into apigenin in vitro [15], indicating that 2-ODDs with FNS activity are more widely distributed than initially believed. Indeed, a FNSI enzyme (PaFNSI) was cloned from the liverwort Plagiochasma appendiculatum [25].Recombinant PaFNSI not only had FNSI activity, but was also able to catalyze the conversion of flavanones to 2-hydroxyflavanones, and therefore displayed flavanone-2-hydroxylase (F2H) activity in vitro [25].[32] Recently, FNSI enzymes have been characterized from maize (Zea mays) and Arabidopsis (Arabidopsis thaliana) [26] ZmFNSI-1 and its Arabidopsis counterpart, AtDMR6, harbor in vitro FNSI activity. While dmr6 Arabidopsis mutants show increased resistance to various pathogens including Pseudomonas syringae, dmr6 plants transgenic for ZmFNSI-1 are equally susceptible to the pathogen as wild-type plants. The dmr6 mutants accumulate higher salicylic acid levels than wild-type plants, and the increased salicylic acid levels present in dmr6 are likely responsible for the increased tolerance to Pseudomonas syringae [27].Thus, a feedback relationship between flavones (e.g., apigenin) and the salicylic acid metabolic pathways was proposed [24]. Possibly, to increase success in the plant, P.syringae (and other pathogens [28] induce flavone accumulation, which results in the decrease of salicylic acid, and therefore increased pathogen susceptibility [29].Flavones are important compounds for plant biology and human health, also specialized metabolites to better understand the chemical complexity of plants and how different enzymes have evolved to use the same substrates to produce identical products, sometimes in the same organism. Flavones pathway characterization in a number of different plant species provides a valuable source of clones and enzymes for metabolic engineering to produce organisms with advanced tolerance to biotic and abiotic stress conditions or to improve animal and human nutrition. And the identification of proteins that specifically interact with pendants provides the next frontier in determining how specialized metabolites are associated with such a variety of biological activities. And finally, Given the high importance of flavones in plants as well as the high pharmacological value of these compounds, achieving a cloned set of FNSI genes can be of therapeutic and commercial value because they can be transmitted to plants, especially plants Which are in areas with high ultraviolet radiation, can have a protective effect on the plant and its transmission to edible plants such as vegetables can give the plant very high medicinal importance.

5. Conclusion

Soluble FNS I prevailing in the Apiaceae was cloned for the first time and has become available in quantity for mechanistic studies as well as for the convenient preparative synthesis of radiolabeled flavones which enable further biosynthetic and biotechnological studies. The evolutionary context for the expression of the soluble synthase exclusively in the Apiaceae remains to be established. In addition, the recombinant enzyme may be of value for the production of flavone-nutraceuticals due to their antioxidant and anticancer potentials [7]. Due to having a cloned complex of this gene is of great importance. First, the gene can be transferred to a suitable edible plant, whereby the drug can be prepared for human consumption through food. And on the other hand, this cloned complex can be used directly to extract these secondary metabolites.

6. Acknowledgment

This research was fundedbyKerman Graduate University of Advance technology (Iran)under Contract No. 1/4036. Therefore, the presenter and her colleagues express their gratitude to the respected officials of that university.

References

[1] Stapleton, AE.Ultraviolet radiation and plants: burning questions, Plant Cell, 1999; 4, 1353.
[2] Sakihama, Y and Yamasaki, H. Lipid peroxidation induced by phenolic in conjunction with aluminum ions. Biol. Plant. 2002; 45: 249

[3] Ajith, TAand Janardhanan, K.K. Indian medicinal mushroom as a source of antioxidant and antitumor agents. J. Clin. Biochem. Nutr. 2007; 40(3): 157-62.
[4] Ramawat K G and Merillon, J. M. Biotechnology; Secondary Metabolites. Science Publisher, NH, USA. 1999
[5] Zargari, A. Medical plants, University of Tehran publication, Volum 2, 1997.
[6] Forkmann, G. Genetics of flavonoids, in The Flavonoids, Advances in Research Since, Harborne, J.B., Ed., Chapman & Hall, London, 1980; 1988: 399
[7] Harborne, J.B. and Williams, C.A. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55: 481–504.
[8] Heller W. and Forkmann, G. Biosynthesis of flavonoids, in The Flavonoids: Advances in Research, Harborne, J.B., Ed., Chapman & Hall, London,1986; 1994, 499.
[9] Middleton, E, Kandaswami Jr, C. and Theoharides, T.C. Theeffects of plant flavonoids on mammalian cells: implications forinflammation, heart disease, and cancer, Pharmacological Reviews. 2000; 6:73–75.
[10] Prabhat, K. Metabolic engineering of yeast for the production of plant secondary metabolites, Research Exercise Summary. 2002
[11] Martens S, Forkmann G, Matern, U, and Lukac, R. Cloning of parsley flavone synthase I. Phytochemistry. 2001; 58: 43–46.
[12] Brundrett M.C. Coevolution of roots and mycorrhizas of land plants. New Phytologist 2002; 154: 275–304.
[13] Kistner, C. and Parniske, M. MEvoluti on of signal transduction in intracellular symbiosis, trends in Plant Science. 2002; 7: 511–518.
[14] Rice-Evans, C.A., Miller, N.J. and Paganga, G. Antioxidant properties of phenolic compounds.Trends Plant Sci. 1997; 2: 152.
[15] Lee Y J, Ho Kim J, Gyu Kim B, Lim Y and Ahn J H. Characterization of flavone synthase I from rice, BMB reports, Seoul Korea, pp. 2007; 143-701.
[16] Chawla S H, Introduction to plant biotechnology, 2009; pages: 310-249
[17] Lowry, B, Lee, D, Henabt C. The origin of land plants: a new look at an old problem, 1980; *Taxon 29*, 183–197.
[18] Kromhout, D. Dietand cardiovascular diseases, Journal of Nutrition, Health, and Aging 5, 2001; 144–149.
[19] Tabak C, Arts I.C, Smit H.A, Heedrik D, Kromhout D.Chronic obstructive pulmonary disease and intake ofcatechins, flavonols, and flavones: the MORGAN Study American Journal Respiratory and Critical Care Medicine. 2001; 164, 61–64.
[20] ArtsI.C, Hollman P.C. Polyphenolsand disease risk in epidemiologic studies. American Journal of Clinical Nutrition 8. 2005; pp. 317–325.
[21] Ross J.A, Kansum C.M. Dietaryflavonoids: bioavailability metabolic effects, and safety. *Annual Review of Nutrition*,2002;22, pp. 19–34.
[22] Manach C, Mazur A, Scalbert A. Polyphenols and preven-tion of cardiovascular diseases. Current Opinion in Lipidology. 2003; 16, 77–84.
[23] Gebhardt, Y.H, Witte S, Steuber H, Matern U, Martens S. Evolution of flavone synthase I from parsley flavanone 3β-hydroxylase by site-directed mutagenesis. Plant Physiol. 2007; 144, 1442–1454
[24] Gebhardt Y, Witte S, Forkmann G, Lukacin R, Matern U, MartensS. Molecular evolution of flavonoid dioxygenases in the family Apiaceae. Phytochemistry 2005; 66, 1273–1284
[25] Han X.J, Wu Y.F, Gao S, Yu H.N, Xu R.X, Lou H.X, Cheng A.X. Functional characterization of a Plagiochasma appendiculatum flavone synthase I showing flavanone 2-hydroxylase activity. FEBS Lett. 2014; 588, 2307–2314
[26] Falcone Ferreyra M.L, Emiliani J, Rodriguez E.J, Campos-Bermudez V.A, Grotewold E, Casati P. The identification of maize and Arabidopsis type I flavone synthases links flavones with hormones and biotic interactions. Plant Physiol. 2015; 169, 1090–1107.
[27] Zeilmaker T, Ludwig N.R, Elberse J, Seidl M.F, Berke L, Van Doorn A, Schuurink R.C, Snel B, Van den, Ackerveken, G. DOWNY MILDEW RESISTANT 6 and DMR6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. Plant J. 2015; 81, 210–222
[28] Van Damme M, Huibers R.P, Elberse J, Van den Ackerveken, G. Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. PlantJ. 2008; 54, 785–793
[29] An C, MouZ. Salicylic acid and its function in plant immunity. J. Integr. Plant Biol. 2011;53, 412–428.