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Isolation of Main Genes Involved in Flowering of Saffron (*Crocus sativus* L.) in Order to Study Effective Flowering Protein

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Abstract: Saffron (*Crocus sativus* L.) always is grown for using its flowers in nutrient industry, color industry and healthy compounds due to its flowers and specially stigmas. Because of its expensive flowers, surveying and recognizing on effective genes for flowering is very important and its results can help us to control rate and timing of flowering at an early stage of flowering. The gene and gene state meant Pistillata like MADS box (PIC2) were surveyed for recognizing its molecular mechanism. The molecular sequence at the genes has high similarity to members of family MADS that is a factor for controls of protein at flowering stage. PIC2 gene was studied by bioinformatics resources. Primers were designed for replicating the gene and DNA and RNA were extracted from saffron's leaves. The gene's cDNA was built by recopying enzyme and used such a pattern for replicating gene PIC2 at polymerase chain reactions (PCR). Segments were replicated such 900 cDNA pair-nucleotides and a segment such 2,100 of DNA's pair-nucleotides. The gene codes a protein that was composed of 210 amino acids that has MADS sequence box. Analysis of protein's molecular structure and homological modeling of the protein indicated that it has a regular structure.

Key words: Saffron, PIC2 gene, MADS sequence box, gene state, homological modeling.

1. Introduction

However, saffron's effective matter is very important but the least researches were done for recognizing flowering pathways and its genes and most researches focused on improving yield by good-cultivate methods. At flowering plants, flower supports plant's existance because flowers produce seeds. Plants must complete their vegetative phase and then enter to productive phase and next, receiving signals and several pathways can be entered to the phase [1]. Based on importance of saffron's flower, recognizing of effective genes on flowering, can help to manage rate, severity and regulatory and timing at flowering [2]. Genes that control to induce flower's organs are box genes. Most of box genes have MADS box. Mutation at the genes will change inducing organs at their neighbor that the compounds control inducing flowering organs [2]. At eukaryote cells, MADS box's genes have recopying factors [3]. Genes of MADS box were set at a conserved domain and are divided to types 1 and 2. The types were found and showed at plants and animals and fungi [4]. In this work, all of MADS box's genes were colonized and the results indicated that the genes have important effects on growth and development and physiological process and shifting to flowering and building saffron's corms [5]. MADS box is replicated sequenced that is conserved around family of recopying MADS proteins. The box's sequence lengths are different at different projects from 168 until 180 pair-nucleotides. MADS box's proteins have a linked domain to DNA's terminal N-tail. The proteins usually need to link to dimmer unit that affects to control cellular activities. Most metabolites are produced at stage flower's development. Related to genes to develop of flowers classified A, B, C, E rate. These genes are named to

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APETALA1/FRUITFULL (AP1/FUL) and SQUAMOSA (SQUA) at class A, APETALA3 (AP3) and PISTILLATA (PI) and DEFICIENS (DEF) and GLOBOSA [6] at class B and AGAMOUS at C, D class and AGAMOUS-LIKE 6 (AGL6) and SEPALLATA (SEP) at E class [7]. Above genes influence on some characters of flowering. Other genes influence on starting flowering and developing organs. All of genes were recognizing but not AP2. Recent reports do not explain how it starts flowering and developing saffron's flowers but there are reports on the production of metabolites around development stage. So we tried to state a comprehensive pattern for aggregating crossing at flower's different parts and growth's stigmas development. The study surveyed [8], five factors recopying from five genetically families are selected [9]. The results indicated that ULTRAPETALA (ULT) gene is found very high quantity at stigma tissues. In fact, the gene codes a rich protein from the system and at *Arabidiopsis* plant is effective on primitive cells of flowering [5]. PIC2 gene is responsible for opening second and third rings of saffron's flowers and for opening flower's sepals [7]. Based on previous results, influenced genes produce secondary metabolites and genes flowering process have significant relationship for aggregating and producing secondary metabolites so the project was done to study flowering process. Consideration of structure nucleotides gene and its appearing protein will be caused to deep understanding for saffron's flowering.

2. Materials and Methods

Firstly, native clone of saffron's corms Torbat Heydarieh was gathered and they were planted at Agriculture College's greenhouse of Torbat Heydarieh University. At next step, newborn flower's stigmas were picked and used for DNA and RNA extraction. Extraction of DNA and RNA depends on flower's timing and genes flowering timing for aim proteins. Extractions of total RNA and cDNA's synthesis and extractions of DNA were done by specific kits. Total RNA was extracted by Vivantis's kit. cDNA was synthesized by cDNA syntheses kit of SIGMA company and based on oligo dT-primmer. The primers were designed below:

3'CGGCACTCAGATTCTCATGG5'

3'GAACTCAACCAACAGGCAGG5'

Primers and promoters were designed by software such primer three and informed bank is named NSBI for replicating genes pick two and sequences genes flowering that PCR was done by master mix PCR and cDNA were pattern segment. The process was done to extract genes of native materials of Torbat Heydarieh. Informed banks proteins were used to genetically character structure and analyze 3-D structure proteins from its appearing phylogeny studies of sequence proteins of PISTILATA-LIKE MADS BOX at saffron from colon Torbat Heydarieh and similar protein based on information GENEBEE. Calculation of theoretical iso-electric and molecular weight was done based on data of EXPASSY. Acid amino was surveyed on the proteins structure.

3. Results and Discussion

In this study, PIC2 gene was extracted and studied and below results were gathered. Specific primers for gene PIC2 with a segment around 1,300 pair-nucleotides from genomic DNA and a segment at length of 900 pair-nucleotides from cDNA were made from extraction RNA from saffron's stigmas at PCR (Fig. 1). Fig. 1 indicated that the gene was set on chromosome 3 number of saffron and has three zones

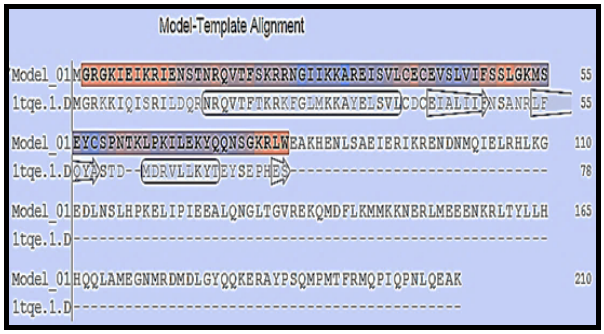

Fig. 1 Gel electrophoresis and PCR, 1,300 and 900 bp fragment of genomic DNA and cDNA.

of introns and four zones of exons.

Amino acids sequencing for PISTILLATA-LIKE MADS BOX protein showed that there is high similarity between member's families MADS BOX that is recopying factors. So the improvement of PIC2 gene state will be caused to improve saffron's flowering rate. That significant improvement indicated that improvement of gene state of members of family MADS BOX is influenced by growth and development and flowering timing of saffron. Studies on protein structure of the gene and bioinformatic analysis indicated that protein which became the gene has 210 acid amines. Iso-electric point and protein's molecular weight based on software EXPASSY were respectively 9.26 and 24,760.68 kDa. The structure of amino acids units of the protein was showed in Table 1. The atomic formula for the protein is C1071H1764N318O322S16. Protein's functional aspects were influenced by their 3D structure that based on homological modeling was designed. Protein's spatial figures state using and spatial functions for per protein. For change at the structure can change its functions, the project made similarity protein's 3D structure PISTILLATA-LIKE MADS BOX. Natural situations of the similarities showed that a mixture of water and thiaphai helper resolver organic aggregate around the protein and take a matrix those distributed water poles. Blasting of similar sequencing causes to do protein's 3D structure. And based on homological modeling, its functions were understood better. Protein PIC2's molecular structure of model in Fig. 2 designed amino acids 1-80. Diagram of sequential blasting of acids amines proteins PISTILLATA-LIKE MADS BOX has high similarity to homologues of proteins projectors myocyte 2B that is histone proteins and showed that they have 47.44% semi-designed similarity. Space between acids amines 1 until 80 at recopying protein of PIC2 gene, has regular proteins that are composed of two structure alpha helix and two structure beta plates and four structure rendomcoili (Figs. 2 and 3). The secondary structure such as alpha helix and beta plates belongs to the second regular structure. Other parts of the secondary structure, such as random coils, belong to the irregular section. Regulative ability of the protein's structure due to the existing amino acids such as thyrosin, tryptophan, phenylalanine, lusin, isolusin, valin, cystein, aspragine, other factors such as hydrophilic and flexibility and molecular weight and iso-electric point are effective to regular the structure based on the fact that economic value of saffron is due to its flowers so it needs to recognize genes and translate and recopy proteins by molecular and genetically methods. And it should attend to flowering's time and sense because recognizing the genes and pathways of genes state is very important.

Table 1 The amino acids of the proteins expressed from genes PIC2.

Amino Acid	Composition	%	Amino Acid	Composition	%
Ala (A)	**7**	**3.3**	**Leu (L)**	**22**	**10.5**
Arg (R)	**16**	**7.6**	**Lys (K)**	**21**	**10**
Asn (N)	**14**	**6.7**	**Met (M)**	**13**	**6.2**
Asp (D)	**5**	**2.4**	**Phe (F)**	**4**	**1.9**
Cys (C)	**3**	**1.4**	**Pro (P)**	**8**	**3.8**
Gln (Q)	**14**	**6.7**	**Ser (S)**	**12**	**5.7**
Glu (E)	**25**	**11.9**	**Thr (T)**	**6**	**2.9**
Gly (G)	**10**	**4.8**	**Trp (W)**	**1**	**0.5**
His (H)	**5**	**2.4**	**Tyr (Y)**	**5**	**2.4**
Ile (I)	**14**	**6.7**	**Val (V)**	**5**	**2.4**
Total number of negatively charged residues (Asp + Gln): 30					
Total number of positively charged residues (Arg + Lys): 37					

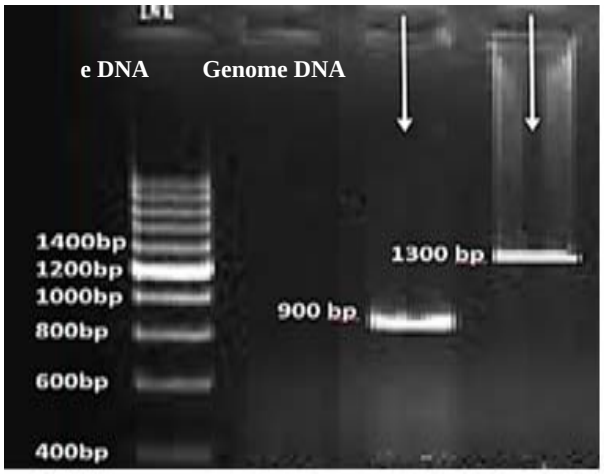

Fig. 2 Spatial and three-dimensional structure of the protein expressed by the part of PIC2 gene.

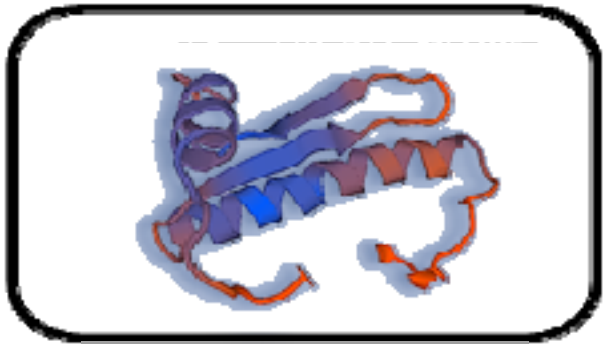

Fig. 3 The alignment sequence of amino acids forming part of the protein expressed by the gene PIC2 with Myocyte 2B protein amplification factor.

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