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Isolation and Characterization of *DBR2* Gene Promoter from Iranian *Artemisia annua*

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ABSTRACT

Artemisia annua **is still the only commercial source of Artemisinin. To date, a number of biochemical and molecular studies about Artemisinin's biosynthetic pathway have been carried out. In metabolic engineering approach, isolation and characterization of promoters leads to an understanding of which** ***cis*****-acting elements are responsible for the regulation of gene expression.** ***DBR2*** **is a key enzyme in Artemisinin biosynthetic pathway. In order to allow chromosome walking beyond the 5'-flanking region of** ***DBR2*****, two specific primers were used in combination with 6 arbitrary primers in TAIL-PCR method. A 696bp upstream of** ***DBR2*** **start codon was isolated and cloned. The subsequent sequence analysis using bioinformatics softwares revealed that there were several** ***cis*****-acting elements such as** ***TATA*****-box,** ***CAAT*****-box, and MeJA-responsive element, and several** ***W*****-box and light-responsive elements inside the** ***DBR2*** **promoter. These results can be helpful in understanding of artemisinin biosynthesis regulation and will facilitate metabolic engineering of the compound.**

Keyword: Artemisinin, Cis-acting element, Chromosome walking, Metabolic engineering.

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INTRODUCTION

Artemisia annua (also known as sweet wormwood) is a great plant genus that belongs to the family of Asteraceae. This genus is native to China and has 300 species around the world, and about 34 species of which are grown in Iran [1]. The main importance of *Artemisia* is due to a sesquiterpene lactone isolated from it, which is called artemisinin. For the first time, artemisinin was isolated from the traditional Chinese herb *A. annua* L. by Chinese scientists in 1970s [2]. Artemisinin and its derivatives are highly effective against *Plasmodium falciparum* [3] that causes malaria disease and this is why artemisinin is widely used for the treatment of malaria [4]. Because of the resistance development to classical quinoline antimalarial compounds such as chloroquine and antifols [5], artemisinin has become an important component of artemisinin-based combination therapies [6]. Accordingly, in 2005, the World Health Organization (WHO) recommended the use of artemisinin-based combined therapy as the first-line malaria treatment [7]. Artemisinin is effective not only against malaria but it has also shown effects against many other diseases such as different types of cancer [8], other parasites like schistosomiasis, some viruses like hepatitis B [9] and some animal diseases as well [10]. Due to these facts, it is expected that the artemisinin industry will expand rapidly to meet the huge worldwide demand for treatment medication.

Artemisinin mainly comes from aerial parts of the *A. annua* L., in which the contents of artemisinin are naturally very low (0.01–1% DW) [11]. This is the main limitation of artemisinin-based treatment of

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malaria [12], which makes artemisinin an expensive drug, especially for economically disadvantaged people in developing countries where malaria frequently occurs [13].

Many attempts have been made to increase the artemisinin yield, such as chemical synthesis, plant cell cultures, hairy root cultures, and fermentation of the engineered microorganism. However, none of these methods is commercially feasible and, currently, *A. annua* is still the only commercial source of this drug [13]. In order to increase the artemisinin content in *A. annua* and to maintain a reliable and low cost supply of artemisinin, a number of biochemical and molecular studies about artemisinin biosynthesis have been carried out [14]. One of the most promising approaches to reduce the price of artemisinin-based antimalarial drugs is the metabolic engineering of the plant in an attempt to obtain higher artemisinin content plants [2]. The artemisinin biosynthetic pathway is well established [10, 13 and 15] and many of its genes are identified and cloned. An important area in metabolic engineering is identification of tissue-specific promoters. Since the biosynthesis of artemisinin occurs in specialized 10- celled biseriate glandular trichomes present on the leaves, stems, and inflorescences of *A. annua* plants, their genes have tissue-specific expression [16], which are controlled by their promoters [17]. How a gene is expressed differentially is a key to our understanding of genetic regulation. One method to study this question is to map the functional sequence domains of a gene and determine what sequences are bound by proteins (presumably *trans*-acting factors) during expression in different tissues. Isolation and characterization of promoter leads to an understanding of what *cis*-acting DNA sequences are responsible for the regulation of gene expression and how these sequences allow appropriate gene expression. A key enzyme for the artemisinin synthesis is Artemisinic Aldehyde_11 [13] Reductase (DBR2) that recently has been detected and its cDNA was cloned [18]. DBR2 is one of the key enzymes involved in artemisinin biosynthetic pathway, which catalyzes an important step in the pathway and reduces the artemisinic aldehyde to dihydroartemisinic aldehyde. Two key gene promoters in the artemisinin biosynthesis, namely, *ADS* and *CYP71AV,* already have been isolated and some *cis*-acting regulatory elements motifs in their sequence have been identified [11, 17 and 19]. Isolating the promoter and identifying *cis*-acting regulatory elements of *DBR2* gene can be important steps in understanding of the variation in *DBR2* expression between different chemotype and how it can help genetic engineering to manipulate *Artemisia annua.* The objectives of this study were to isolate sequence of *DBR2* promoter from Iranian *A. annua* and to characterize its *cis*-acting elements using bioinformatics softwares.

MATERIALS AND METHODS

Plant Materials and Genomics DNA

The *Artemisia annua* seeds were obtained from Iranian Biological Resource Centre (Accession Number: P1000060) and were grown in a growth chamber with a photoperiod of 16 hours and light intensity of 5000 1X LUX at 23ºC. After 30 days, seedlings were transplanted into plastic pots and were kept under photoperiod of 12 hours and light intensity of 7000 1X LUX at 25ºC. Samples were taken from a 3 month old plant.

Total genomics DNA was extracted using modified CTAB (Cetyl Trimethyl Ammonium Bromide) method [20]. In order to obtain high quality DNA from *A. annua*, the original protocol was changed in a way that 2% PVP was added to the extraction buffer and phenol:chloroform:isoamylalchol was used (25:24:1) instead of chloroform:isoamylalchol.

In order to allow chromosome walking beyond the known *DBR2* sequences into the unknown 5' flanking region, TAIL-PCR [21] was employed with two essential modifications: firstly, 10 mer random primers were used instead of degenerate 16 mer as the short primer according to Terauchi and Kahl method [21]; secondly, a total of two, rather than three, gene-specific primers were used in nested positions to ensure selection of the correct target fragments, as explained below.

On the basis of the cDNA sequence of *DBR2* gene from *A. annua* (AN: EU704257), two gene-specific primers in nested positions close to the 5'-end of the coding regions were designed and synthesized. The primers for *A. anuua DBR2* are shown in Table 1. Because there exists a very high similarity on nucleotide sequence between *DBR2* and *OPR3* genes (about 96%), these genes were first aligned and two points that were different on a single or multiple nucleotides were identified and selected for design of two specific primers. Later, two specific primers corresponding to these different points between the two genes were designed so that their 3'-ends were different from *OPR3* gene but matched *DBR2* sequences.

Additionally, six arbitrary degenerate (AD) primers were used in combination with the specific primers for TAIL-PCR. TAIL-PCR protocol was performed according to the method described by Liu *et al*. [22]. The thermal cycling conditions are summarized in Table 2. Two rounds of PCR were carried out on a Biorad Thermal Cycler using the product of the primary PCR as a template for the secondary one, and employing a common arbitrary primer and nested gene-specific primers in a consecutive manner. The primary PCR was conducted in a 25-μL volume containing 150 ng of genomic DNA, 0.2 μM gene-specific primer (Primer SP1), 2.0 μM 10 mer primers, 100 μM of each dNTP, 0.2U high fidelity LA Taq DNA polymerase (TaKaRa CN: RR02AG) and 1X buffer supplied with the enzyme. The secondary PCR was performed via Primer SP2 in combination with the same arbitrary primer as used in the primary PCR. The reaction solution was the same as for the primary PCR, except that 1 μL of a 1:50 dilution of the primary PCR product was used as a template.

The products of the primary and secondary PCRs were separated in adjacent lanes on Agarose gel to determine whether discrete PCR products from the two gene-specific primers show size differences corresponding to the relative positions of the nested primers or not. PCR products were excised from the Agarose gel and were cloned into PTZ57RT vector for subsequent nucleotide sequencing. DNA sequencing was performed on an ABI 373A automated sequence. Then, promoter prediction, characterization, and search for the putative *cis*-acting elements were carried out using different databases: Softberry, PlantCARE [23] and PLACE [24].

Table1. Primers used for isolation promoter region of *DBR2* gene.

Primer name	Sequence (5' to 3')
SP1	*5'-GCTGAACAAGGAATCATTTTTATGGG-3'*
SP2	*5'-CAGTATCCTCGATTGTGGTGGGAC-3'*
AP1	*5'-GGT GCT CCG T-3'*
AP2	*5'-CGA TGA GCC C-3'*
AP3	*5'-CTA TCG CCG C- 3'*
AP4	*5'-CGC AGA CCT C-3'*
AP5	*5'-GTG TGC CCC A-3'*
AP6	*5'-ACG GTG CCT G-3'*

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Table 2.The PCR programs used in TAIL-PCR reactions.

Reaction	Cycle no.	Thermal condition
	1	92 ° C(3 min); 95 ° C (1 min)
Primary PCR	5	94 ° C (30 s); 55 °C(1 min); 72 °C(2 min)
(AP/Primer	1	94 ° C (30 s); 32 ° C (2 min); ramping to 68 ° C over (2 min); then 68 ° C (2 min)
SP1)	15	94 ° C (30 s); 55° C (1 min); 72 ° C (2 min); 94 ° C (30 s); 55 ° C (1 min); 72 ° C (2 min); 94 ° C (30 s); 39 ° C (1 min); 72 ° C (2 min)
	1	72 ° C (5 min)
Secondary PCR	12	94 ° C (30 s); 58 ° C (1 min); 72 ° C (2 min); 94 ° C (30 s); 58 ° C (1 min); 72° C
(AP/Primer SP2)	1	(2 min); 94 ° C (30 s); 39 ° C (1 min); 72 ° C (2min) 72 ° C,(5 min)

RESULTS AND DISCUSSION

Isolation of the 5'-Flanking Regions of *DBR2* Gene

The *DBR2* 5'-Flanking sequence was isolated using thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR). It has been illustrated that this technique is quite suitable for the promoter and unknown isolation of the known genes [22]. Among the 6 arbitrary primers tested in combined with a set of *DBR2* gene-specific primers (SP1 and SP2), 5 primers resulted in amplification of discrete PCR products. Specificity of the products was confirmed by the size differences between the relative positions of the two nested *DBR2*-specific primers on their Agarose electrophoresis gel and more confirmation was achieved by observation of the perfect overlapping between DNA sequences of PCR products obtained using DBR2 primer and the 5'-end sequence of cDNA. Among all primary and secondary TAIL-PCR products, target PCR products ranged from 600 to 1,200 bp (Figure 1), which were cloned into PTZ 57RT vector. Finally, the 696bp upstream of the putative site codon start of *DBR2* was isolated (Figure 2). The sequence has been deposited in DDBJ, EMBL, and GenBank databases under the accession NO: JX413513.

cis-Acting Elements Analysis

Softberry database was used to determinate the putative transcription start site (TSS) of *DBR2* gene, to which the number +1 was assigned. A putative TSS of *DBR2* was predicted at 17 bp upstream of the translation initiation ATG-codon. TSS for the other Artemisinin biosynthesis genes, including *ADS* and *CYP* genes, was determined at 51 and 18 bp upstream of their start codons, respectively [11 and 19]. In order to identify *cis*-acting elements of *DBR2* promoter, the PLANTCARE and PLACE softwares were used to analyze the obtained sequence.

A putative *TATA* box sequence was found at –25th bp (-25TATAA-21) upstream before the TSS, which has also been found commonly in eukaryotic promoters and typically contains T/A-rich sequence, located about 25 to 30 bp upstream of the transcription start site [25]. This is considered to be the core promoter sequence and the binding site of either general transcription factors or histones. It is also involved in the process of transcription by RNA polymerase, which usually is located around 25 bp upstream of the TSS [19]. The important roles of the *TATA*-box in correct selection of a transcription initiation site were also reported [26]. *CAAT*-box is the binding site for proteins called *CAAT*-box binding proteins/*CAAT*-box binding factors.

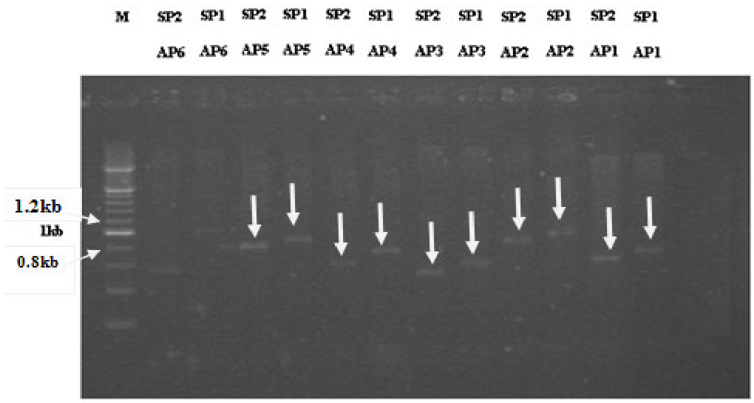

Figure 1. Analysis of TAIL-PCR products by Agarose gel electrophoresis. SP1 and SP2 are gene specific primers for, respectively, the first and second TAIL-PCR and AP1-6 are arbitrary 10-mer primers. M is molecular weight marker [200bp Plus ladder (Fermentas #SM0633)]. Results for 5 arbitrary primers show the 10 target fragments of primary and secondary TAIL PCR products, which were identified by the stepwise change in the sizes and cloned into PTZ57RT vector for subsequent nucleotide sequencing.

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                                                                    MBS
-679  AGCTACTCGGGGTAACGTTGTAGTTACTATGTACATATGATCATATGATTCTTACCTTC
-620 AGTTACGTCATTTCGAAGTTTCGTTACAAATTAATATTCTAGACACATAAATATAAGGTG
                             CAAT-box
-560 TTATGTACATAATTTATTTTATACCCGAAAGTACATTTTTGTTAGCCTCAAAGTCACATG
                                                             G-box
-500 GTCATTTGGAATTCGTTAAATAAATATCTAGGATAATATCTAAGTTAAGAAATAAGCAAT
                                                          CAAT-box
-440 GTGGTTTTCAACTTGATAGACCTTGTAAGTCTATAAGGTTGGTGACACTGTGACAGTTAT
                                             L-box           MBS
-380 TGTATCAACTTTTGAGTCTGTACATTATATGTCCTTGTATGGAGATCCACCTTACATATG

-320 AAATACGGTTTACCCGAACTTCTTTTTGAAGACTCTTTGTTTGTGCCTTGTACTTTGTAC

-260 ATGTTGACAATCAAGAAAGCTCTTATAACCTTCAATTATCACGTTTTCTCGGAGATCCGG
        CAAT-box                             G-box
-200 TGTTATTAAGTTCTTGAGCGGAAAAGACTCTTAAGTAGTAGTTTCACTTAGGTTATCTGT
                                                       MRE
-140 GATAACGGTTTATAGTTAGGTAGTGGACATGGTATATTTTTTTATAGTTTAGCAATCGTT
                                                        CAAT-box
 -80 ATTTTTGTTTCCACACAAGTGACTGTTAATTATGAAACATAATGCATAATTCGTGTATAA
                                                          TATA-box
 -20 CCTATAACCTGTAGTTTAGTACTCGTGTATGCGGTAGATGTCTGAAAAACCAACCTTGTT
                         +1
  41 TTCTGCCTACAAGATGGGCAAGTTCAATCTTTCTCACAGGGTGGTGTTAGCTCCTATGACT
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Figure 2. Nucleotides sequence of the cloned *DBR2* promoter with 84bp sequence of the first exon. Transcription start site is indicated with +1.Bold letters indicates start codon (ATG). TATA, CAAT, G, and some other boxes also are shown.

The *CAAT*-box signal is the binding site for RNA transcription factors. A common *cis*-acting element in promoter and enhancer regions, *CAAT*-box was predicted at -90bp (-90AGCAATCG-83) upstream of the TSS, which matches the traits of a prokaryotic promoter [27]. Moreover, three other CAAT-boxes based on sequence homologies were predicted. These consensus sequences have been recognized as responsible for the tissue specific promoter activity of a *pea legumin* gene and *napin* gene in tobacco and brassica, respectively [27].

One method to enhance Artemisinin through metabolic regulation is using the exogenous hormone and elicitors to treat the wild-type *A. annua* and where the transcription level of relative endogenous genes can be elevated [28]. These elicitors induced the expression of biosynthesis genes via specific motifs, which are located in gene promoters. In the *DBR2* promoter, two TGACG-like motifs were found [29] that play important roles in the methyl jasmonate (MeJA) responses [30]. Maes *et al*. [16] recently showed that expression of *DBR2* gene significantly increased in response to methyl jasmonate. Gao-Bin *et al*. [31] found that the addition of two elicitors, salicylic acid and methyl jasmonate, led to induced increases in *A. annua* growth and artemisinin level. Therefore, the increased expression may be due to the presence of the MeJA motif in *DBR2* promoters. Inserting this region into the constitutive CaMV 35S promoter caused MeJA responsive ability. Previous studies have demonstrated that the TGACG-motif is a binding site for bZIP transactivating factors, and mutagenesis of these motifs abolishes MeJA responsive expression [30]. A *TGA*-box with consensus sequence (-611TGACGTAA-618) was identified in the promoter. This *cis*-acting element is responsible for auxin and is present in many auxin inducible gene promoters [32]. Although, the effects of many elicitors and hormones on Artemisinin biosynthesis have been evaluated but there is no auxin evaluation. Two *TGTCA* motifs were also detected in the promoter sequence (-286TGTCA-290 and -252TGTCA-256) that were the characteristic *cis*-element DNA sequence of the homeodomain transcriptional factors and its relation to resistance responses in rice [33]. A modified GARE- motif (-517TAACAAA/G-523) was found in the promoter region (Table 2), which involved in Ghibelline-responds [34]. Wang *et al.* [19] have reported that ADS promoter contains a multiple motif involved in the response of *A. annua* to plant hormones. In *DBR2* promoter, a WUN-motif was also detected at the *-613TCATTTCGAA-604* position. This motif is wound-responsive element [35]. The effects of mechanical wounding on the artemisinin biosynthesis were evaluated and results indicated that there was a remarkable enhancement of the artemisinin content 2hrs after wounding treatment. The expression profile analysis showed that many important genes involved in the artemisinin biosynthesis were induced in a short time after wounding treatment [36]. It must be considered that MeJA is involved in the wounding signal transduction in plants [37] and the *CGTCA*-motif with WUN-motif may induce gene expression in artemisinin biosynthesis and increase the artemisinin content when the plants is affected by mechanical wounding .

The *G*-box (CACGTG) is a ubiquitous *cis*-acting DNA regulatory element found in plant genomes. Proteins known as *G*-box factors (GBFs) bind to *G*-boxes in a context-specific manner and mediate a wide variety of gene expression patterns [38]. It was first characterized as essential *cis*-element involved in regulation of light-responsive genes, which can interact specifically with a family of bZIP proteins. Two *G*-boxes were predicted in *DBR2* promoter (-221CACGTT-216 and -506CACATG-501, see Figure 2). In functional analysis of plant promoters, the role of *G*-box in the promoter activation has been demonstrated and reported to respond to stimuli such as light, UV light, abscisic acid, oxygen-free conditions, and several plant hormones [39]. Other elements putatively involved in light-mediated regulation are the *L*-box (TGTCACCAACC; position –394 to -404), the *TCT*-motif (TCTTAC; position –630 to –625), the rbcS-CMA7a motif (GTCGATAAGG; position –412 to –402) and the Box 4 (ATTAAT; position –591 to –586). *LTR*-box (-536CCGAAA-531), a *cis*-acting element involved in low-temperature responsiveness, was identified in promoter region of *DBR2* and it has been illustrated the motif regulates cold which motif regulates cold, drought, and also ABA-related gene expression [40].The presence of

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many light inducible elements in promoter region may be helpful in explanation of the fact that the artemisinin output of *Artemisia* varies greatly and depends on daytime [11].

ACGTT *cis*-acting DNA sequence elements have been identified in a multitude of plant genes regulated by diverse environmental and physiological cues. *In vivo* transient and transgenic plant expression studies have shown that these *ACGT* elements are necessary for maximal transcriptional activation [29]. Many plants possess a conserved family of DNA-binding proteins, which are specific for these DNA sequence motifs. Promoter region of *DBR2* contained an *ACGTT*-box at the position of -666AACGTT-661. Five putative *GATA*-boxes were found in the early promoter region of *DBR2* as shown in Table 3. *GATA*-factors are a class of transcriptional regulators present in plants that are involved in light-mediated regulation and are normally recognized in the consensus sequence *T/AGATAG/A* [41]. Two putative Skn-1 motifs with consensus sequence (GTCAT), *cis*-acting regulatory element required for endosperm expression [42], were found in *DBR2* promoter, which may implicate that this gene is expressed in seeds. A previous study revealed that there were eight motifs Skn-1 in *ADS* promoter [19]. Furthermore, a number of Amorphane sesqui terpenes including artemisinic acid, dihydroartemisinic acid, and arteannuin B have been isolated from seeds of *A. annua* showing that artemisinin biosynthesis enzymes are expressed in seeds [43]. Since there are no trichomes on seeds, presence of these Artemisinin precursors in seed may be due to the existence of Skn-1 motifs in the promoter of Artemisinin biosynthesis genes. *MRE*-box is a MYB recognition element. This element possesses a functional core that is essential for light responsiveness and is specifically recognized by two distantly related MYB-like proteins [44]. There are multiple *MRE*-boxes in *DBR2* promoter (Table 3) which are MYB binding site involved in light responsiveness [44], MYB binding site involved in drought-induction [45], MYB-related protein for expression in flower [46], and AtMYB2 (MYB) function as transcriptional activators in absecisic acid signaling [47]. Several *W*-boxes with consensus sequence (TGAC and TGACG/T) were found in the promoter sequences of *DBR2* gene (see Table 3). WRKY proteins have only recently been identified as a new family of transcription factors. WRKY proteins also seem to be involved in plant-specific processes, such as trichome development and the biosynthesis of secondary metabolites [48]. *In vitro* and *in vivo* studies have indicated that WRKY proteins specifically bind to the *W*-box and trigger the transcription of the gene [49]. The WRKY transcriptional factors can either up- or down-regulate the expression of a given gene [50]. The *A. annua* transiently over-expression of AaWRKY resulted in an increased levels of *HMGR*, *ADS*, *CYP71AV1,* and *DBR2* genes, indicating that these genes were induced by binding of AaWRKY to the *W*-boxes [2].

CONCLUSIONS

The specific regulation of a single eukaryotic gene requires a molecular machine involving the cooperation of dozens of different proteins [51].Therefore, in addition to its special importance in understanding the regulation of gene expression, identification of plant promoters may serve as an essential element in gene annotation as well as in developing computational promoter prediction approaches. Promoters responsible for specify both the timing of transcriptional induction and the amount of synthesized transcription and made these feasible by their elements [52]. In order to identify putative transcription factor binding sites and conserved plant *cis*-acting regulatory elements, the 679bp *DBR2* promoter sequence was isolated and analyzed by using PlantCARE and PLACE databases. Computer analysis of the promoter sequence identified *cis*-acting elements with significant homologies to the elicitor, transcription

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Table 3. Putative Cis-acting regulatory elements identified in the *DBR2* promoter analysis obtained sequence PlantCARE (PC) and PLACE (P) databases.

Element	Database ID	Position	Strand	Expected function
CAAT-box	PC and S000028	*-90AGCAATCG-83* *-254GACAATC-249* *-446AGCAATG-440* *-496TACAAATT-489*	+ + + +	Common Cis-acting element in promoter and enhancer regions
TGA-box	PC	*-611 TGACGTAA -618*	-	Part of an auxin-responsive element
CGTCA-motif	PC	*-615CGTCA-611* *-611TGACG-615*	+ -	Cis-acting regulatory element involved in the MeJA-responsiveness
WUN-Motif	PC	*-613TCATTTCGAA-604*	+	Wound-responsive element
rbcS-CMA7a	PC	*-412GTCTATAAGG-403*	+	Part of a light responsive element
Box-4	PC	*-591ATTAAT-586*	+	Part of a conserved DNA module involved in Light responsiveness
4cl-CMA2b	PC	*-394TGTCACCAACC-404*	-	Light responsive element
W-box	S000390	*-257TTGAC-253*	+	WRKY DNA binding proteins
W-box	S000442	*-505TGACT-509*	-	A novel WRKY transcription factor in barley
W-box	S000447 S000447 S000447	*-398TGAC-395* *-390TGAC-387* *-256TGAC-253*	+ + +	Environmental stresses responsive elements WRKY binding site
W-box	S000457	*-60TGACT-57*	+	POSSIBLE involvement of NtWRKYs and autorepression
MYB-PZM	S000167	*-397MACCWAMC-404*	-	A flower-specific Myb protein activates transcription of phenylpropanoid biosynthetic genes
MBS	PC	*-616TAACTG-621* *-382TAACTG-387*	- -	MYB binding site involved in drought-inducibility
MRE	PC	*-147AACCTAA-153*	-	MYB binding site involved in light responsiveness
MYB-1AT	S000408	*-434T/AAACCA-439*	-	Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid
GATA-BOX	S000039	*-469GATA-466* *-436GATA-433* *-140GATA-147* *-221GATA-224* *-461GATA-464*	+ + + - -	Part of a light responsive elements
GARE-box	S000439	*-517TAACAAA/G-523*	-	GA-responsive element
ACGTG	S000414	*-217ACGTG-221*	-	Function in induction by dehydration stress and dark-induced senescence
ACGTT-box	S000132	*-666AACGTT-661*	+	Necessary for maximal transcription activation
CGAACTT-motif	S000375	*-316CGAACTT-310*	+	Involved in the transcriptional expression of the nitrate reductase gene in Chlamydomonas reinhardtii
*AMY*BOX1	S000020	*-519TAACAA/GA-525*	-	Conserved sequence found in 5'-upstream region of alpha-amylase gene of rice, wheat, barley;
BIHD1OS	S000498	*-286TGTCA-290* *-252TGTCA-256*	- -	Transcriptional factor involved in disease resistance responses
LTR	PC	*-536CCGAAA-531*	+	Cis-acting element involved in low-temperature responsiveness
Skn1-motif	PC	*-614GTCAT-610* *-500GTCAT-496*	+ +	Cis-acting regulatory element required for endosperm expression
TCT-motif	PC	*-630TCTTAC-625*	+	Part of a light responsive element

factors and the hormone-responsive elements. Sequence analysis showed that *TATA* box [TATATAA) is located 25bp upstream from TSS. Many known regulatory elements such as many light-responsive elements, MeJA-responsive element, MYB binding sites, and *W*-boxes were identified in *DBR2* promoter. These results can be of a great help in understanding how artemisinin biosynthesis is regulated and will facilitate metabolic engineering. The obtained sequence has been deposited in GenBank databases under the accession number: JX413513.

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جداسازی و شناسایی پروموتر ژن *DBR2* از *آرتمیزیا آنوا* ی ایرانی

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چکیده

تنها منبع تجاری آرتمیزینین، گیاه *آرتمیزیا آنوا* می باشد. مطالعات بیوشیمیایی و مولکولی زیادی پیرامون مسیر بیوسنتزی آرتمیزینین انجام شده است. در حوضه مهندسی متابولیک، جداسازی و شناسایی پروموتر منجر به شناسایی عناصر درگیر در تنظیم بیان ژن ها و چگونگی بیان های مناسب یک ژن می شود. در مسیر بیوسنتزی آرتمیزینین، *DBR2* یک آنزیم کلیدی می باشد. برای کاوش ژنوم به طرف ناحیه مجاور '۵ ژن *DBR2*، ۲ پرایمر اختصاصی به همراه ۶ پرایمر تصادفی در روش PCR نامتقارن دمایی بکار گرفته شد. ۶۹۶ جفت باز از توالی بالا دست کدن آغاز ژن *DBR2* جداسازی و کلون شد.

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تجزیه بیوانفورماتیکی توالی بدست آمده آشکار کرد که چندین عنصر تنظیمی پروموتری مانند جعبه های TATA، CAAT، عنصر پاسخ دهنده به متیل جاسمونات، و چندین جعبه عملکردی W و عناصر پاسخ دهنده به نور در درون ناحیه پروموتری *DBR2* وجود دارد. این نتایج می تواند به فهم چگونگی تنظیم سنتز آرتمیزینین کمک کرده و مهندسی متابولیکی آن را تسهیل نماید.