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Original Article

Analysis of miRNAs and Genes Related to Cardiovascular and Neurological Diseases in *Cicer arietinum* L.

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ABSTRACT

Chickpea (*Cicer arietinum*) is one of the most important economic legumes in the world, with 16 chromosomes, and belongs to the *Leguminosae* or *Fabaceae* family. This plant is sensitive to cold and is subjected to many environmental stresses every year. In general, the stress of cold, drought, and salinity, in addition to causing severe damage and yield loss, also leads to changes in physiological processes and gene expression. MicroRNAs are a group of conserved RNAs in plants and animals that play an important role in regulating post-transcriptional factors. Chickpea also plays an effective role in promoting resistance to various diseases such as cardiovascular diseases, diabetes, obesity, and cancer. Therefore, in this study, we investigated micro RNAs effective in resistance to environmental stresses, as well as clinical and bioinformatics investigations in the *MTHFR* and *FOLR1* genes against cardiovascular and neurological diseases. The results of this research showed that various miRNAs such as miR159, miR160, miR166, miR167, miR169, miR171, miR172, miR319, miR393, miR394, and miR396 are effective in creating stress resistance in chickpeas through activating different genes. This research showed that micro RNAs act as inhibitory and modulating factors against various stresses. The studies conducted from the analysis of antibodies in *C. arietinum* L showed that the *FOLR1* gene is more active in the extracellular part and the plasma membrane and the *MTHFR* gene in the cytosol compared to other cells in other organs. Also, the analysis of the expression of these genes showed that the *FOLR1* gene is less expressed than the *MTHFR* gene in heart but it is more in the brain. On the other hand, the *MTHFR* gene was more expressed in male tissues, muscle tissues, bone marrow, and lymphoid tissue. Also, GMQE in *FOLR1* (0.79) and *MTHFR* (0.87) genes showed that the three-dimensional structure provides an accurate estimation of these genes activities. Finally, it can be concluded that by conducting studies in the field of tracking miRNAs and effective genes, as well as accurate diagnosis with the help of molecular markers, an effective step can be taken to increase resistance to diseases and stresses. Also, by relying on these techniques, it is possible to significantly improve the performance of the product.

Keywords: Abiotic stress, Clinical analysis, Disease resistance, FOLR1 gene, Gene expression, MicroRNAs, *MTHFR* gene

INTRODUCTION

Today, chickpea (*Cicer arietinum*) is considered the third-most important legume in the world in terms of consumption [1]. This plant is self-pollinating and belongs to temperate regions. Research has shown that the origin of this plant is related to the southeastern regions of Turkey [2]. In general, and according to the morphological classification, chickpea seeds can be divided into two general types. The first is the desi type, with a thick coating that has small seeds and is usually brown. The second type of cable has a thin cover that has large grains and is usually seen in a cream or beige color [3, 4].

The legume chickpea is said to be nutrient-dense and contains a range of rich and beneficial ingredients, including carbohydrates, proteins, unsaturated fatty acids, minerals, vitamins, dietary fibers, and various isoflavones [3, 5]. A rich source of both carbs and protein, chickpeas are regarded as having higher-quality protein than other pulses [3]. There are 18 different kinds of amino acids in it, and 8 of them are necessary [5]. Scientific evidence supports the biological activity of chickpeas and their positive impact on human health as a vital source of nutritious components [6].

Pulses are renowned for their high nutritional content, capacity to improve health, and sustainability—they fix nitrogen in the soil, hence lowering the need for fertilizer. Pulses have a low glycemic index and an excellent nutritional content. Their drinks are also a rich source of fiber, minerals, and protein [7].

According to recent research, some legume seed protein extracts, especially the albumins from Lupinus albus, can prevent colon cancer cell migration and the metalloproteinase MMP-9, which is linked to inflammation and cancer [8, 9](Supplementary file (Table 1, Figure 1)). Additionally, it has been demonstrated that heat-resistant Pis in soy is more efficient and selective against MMP-9 than non-protein substances (such as isoflavones and saponins) [10]. MMP-9 is a significant biomarker and clinical target for inflammatory and cancerous diseases, and its inhibitors are currently thought to be promising treatment options for a range of human conditions, including cancer, osteoporosis, inflammatory bowel disease (IBD), cardiovascular disease, and even neurological disorders [11, 12]. According to some research, several little investigated legume seeds, such chickpea and lupin, have greater bioactivities than soybean and are highly beneficial in decreasing colitis and cancer spreading in vivo [8, 13].

Chickpea, soybean, pea, and lentil are among the legume sources that include dietary Bowman-Birk inhibitors (BBIs), which have the ability to inhibit or prevent inflammatory and carcinogenic processes in the gastrointestinal system [14, 15]. Additional in vitro and in vivo research demonstrated that phytic acid's antioxidant qualities are helpful in preventing cancer and positively impact the growth suppression of a number of cancer types [16].

Chickpea has a very high nutritional value and contains a huge source of vitamins and minerals such as calcium, magnesium, phosphorus, and potassium, as well as amino acids including lysine, methionine, threonine, valine, and leucine, along with beta-carotene. As we know, legumes are a rich source of folate. Folate, or vitamin B9, is very necessary in the body for the synthesis and reconstruction of DNA, cell division, and the maturation of red blood cells. Therefore, this plant has an effective effect on improving cardiovascular diseases, diabetes, neurological diseases, obesity, and cancer [17-20].

Among the several isoflavones present in chickpea are Biochanin A (BCA), calycosin, formononetin, genistein, trifolirhizin, ononin, and sissotrin. Numerous studies have shown that these compounds have unique biological properties and are essential for treating the clinical problems connected to a variety of diseases, including cancer, hyperlipidemia, osteoporosis, the development of the nervous system, and cardiovascular disorders [21, 22]. Isoflavones are the primary bioactive ingredient found in sprouted chickpea seeds among these constituents. Their wide range of biological actions, which include antioxidative, estrogenic, insecticidal, piscicidal, antifungal, antibacterial, and contraceptive qualities, have helped them to become extremely important [6]. Chickpea products have demonstrated their ability to significantly lower blood lipoprotein levels and cholesterol accumulation. This has a significant hypolipidemic effect and plays a crucial role in the prevention or treatment of a number of diseases, including cardiovascular disease and atherosclerosis, which are caused by hyperlipidemia [6]. These results suggest that BCA may be a therapeutic option for inflammatory cardiovascular disease [6]. Isoflavones are the primary bioactive elements of sprouted chickpea seeds among these elements. Due to their extensive and varied biological actions, which include antioxidant, estrogenic, insecticidal, piscicidal, antifungal, antibacterial, and contraceptive qualities, they have grown to be quite important [6].

Consuming chickpea products has been demonstrated to help prevent or treat a variety of diseases, including atherosclerosis and cardiovascular disease, which are brought on by high blood lipid levels, as well as cholesterol accumulation and blood lipoprotein levels [23]. A diet high in chickpeas reduces the risk of cardiovascular disease in older people by lowering their levels of total cholesterol (TC) and LDL-C [24].

It has been shown that WCL (wild chickpea lectin) protects DNA in a dose-dependent way. Against a variety of fungal infections, the lectin has demonstrated antifungal action. At a dosage of 10 µg/ml, WCL increased the

mitogenic response of mouse spleen cells and inhibited HIV-1 reverse transcriptase at an IC50 of 200 µM. Lectin's anticancer potential has been established against human cancer cell lines. IC50 values of 61.8, 54.4, 37.5, and 44.2 µg/ml have been established for the cell viability assay in HepG2, Ishikawa, MCF-7, and MDA-MB-231 cell lines, respectively [25].

Additionally, a variety of proteins and peptides with antibacterial, antioxidant, tumor growth inhibitory, hypoglycemic, and hypolipidemic activities may be present in raw chickpeas and chickpea processed extracts. Chickpea has been demonstrated to have several biological advantages in addition to having strong hepatoprotective, antihypertensive, antiallergic, anticonvulsant, and positive hemagglutination capabilities [6].

Every year, environmental stress causes severe damage to products, which leads to a significant drop in performance. In addition to causing severe damage and yield loss, cold, drought, and salinity stress also lead to changes in biochemical physiological processes and gene expression. Plants also deal with these stresses through several mechanisms. Factors regulating gene expression and transmission of signals, micro RNAs are among the mechanisms that ultimately lead to regulating metabolic processes and creating resistance in plants [26-28].

Research has demonstrated that consuming chickpea products can significantly lower blood lipoprotein levels and cholesterol accumulation. This has a hypolipidemic effect and plays a major role in the prevention or treatment of a number of diseases, including cardiovascular disease and atherosclerosis, which are caused by hyperlipidemia [29]. After being produced via the one-carbon-donating metabolism of methionine, the thiol-containing amino acid homocysteine is remethylated to methionine, with folates serving as methyl donors.[30]. Reduced cell division, the generation of inflammatory cytokines [31], changed nitric oxide metabolism [32], increased oxidative stress [33], enhanced apoptosis [34], and disrupted methylation processes [35] are among the potential negative consequences of homocysteine buildup and folate shortage.

Genes related to folate-mediated one-carbon metabolism and absorption have been shown to contain many variants. These polymorphisms may affect the flow of folate cofactors between DNA synthesis and methylation processes, which might modify the beneficial effects of folates and other B vitamins involved in the metabolism of methyl groups [29]. The folate methylation cycle, which turns homocysteine into methionine, involves the MTHFR gene. DNA, proteins, and lipids are methylated by the usage of S-adenosylmethionine (SAM), a methyl group donor that is derived from methionine [36]. An amino acid alteration at codon Ala222Val causes the MTHFR 677C/T variant, resulting in an unstable enzyme with decreased activity [37]. The buildup of homocysteine [38] and compromised methylation reactions are the outcomes of this mutation. One of the most prevalent repressor mechanisms of tissue-specific genes is DNA methylation. Therefore, this polymorphism's poor methylation may have an impact on the regulation of genes. Through receptors that bind folate and those that are dispersed in a way particular to certain cells and tissues, folate enters the cell. The FR-α and its mouse ortholog Folr1 are examples of such receptors. In developing embryos, Folr1 is expressed in the neural folds, NT, and yolk sac [39].

There are two categories for the roles that miRNAs from medicinal plants play: intra- and cross-kingdom. The primary focus of cross-kingdom studies on therapeutic plant miRNAs is on treating important human diseases [40, 41]. It was discovered that six Ocimum basilicum miRNAs, including miR160, 414, and 869.1, modulated 26 target genes relevant to human cancer [42]. Both Homo sapiens miRNA-378 and Aba-miRNA-9497 in Atropa belladonna targeted the 3′-untranslated region (3′-UTR) of the mRNA encoding the zinc-finger transcription factor, which is crucial to nervous systems. 691 ZNF [43]. In Bacopa monnieri, the TRAF2 gene is a putative target of miRNAs and the upstream signaling factor in the cancer pathway [44]. Within the kingdom, the primary emphasis of miRNA research on medicinal plants is on the metabolic and synthesis pathways of secondary metabolites. miR-4995, miR-5532, and miR-5368 involved in terpenoid production and culture growth conditions in *Picrorhiza kurroa*, an endangered medicinal plant [45]. Target genes for prenylflavonoid production, growth, and development were found in hop (*Humulus lupulus*) miRNAs responding to viral infection [46]. 45 Aquilegia coerulea miRNAs that target genes involved in metabolism and stress responses were found using the comparative genomics method [47]. There isn't currently a well-established study system on miRNAs in medicinal plants. With the support of this evidence, the present research was conducted to investigate micro RNAs of resistance to environmental stresses and also to investigate MTHFR (methylenetetrahydrofolate

reductase) and FOLR1 (folate receptor-α) genes effective in creating resistance against cardiovascular and neurological diseases.

Table 1 Names of miRNAs from medicinal plants that regulate secondary metabolism and cross-kingdom [41].

	Aim Pathway
Cross-kingdom	
• MIR2911	COVID-19, influenza A virus, and tumor proliferation
• Gas-miR01, and 02	Anti-inflammatory
• MiR414	Alzheimer's diseases, diabetes, hypoganglionosis, and inflammatory bowel diseases
• Oba-miR156f, and 156t	Bile duct carcinoma, lung cancer, and osteoarthritis
• Oba-miR160g	Lung cancer, nephronophthisis, and retinitis pigmentosa
• Oba-miR482a	Breast cancer, gastric cancer, and ovarian cancer
• MiR869.1	Alzheimer's diseases, cataracts, and diabetes mellitus
• Bmn-miR156, 167h, 172d, and 396g	Immune responses
• MiR166	Glioblastoma, papillary thyroid carcinoma, and secretory breast carcinomas
• Cac-miR-29c-5p	Breast cancer, and ovarian cancer
• Cac-miR-4723-3p	Prostate cancer, and renal cancer
• Cac-miR-548d-3p, 5653, 5780d, and 7009-3p	Tumor proliferation, ovarian clear cell adenocarcinoma, breast cancer, and lung cancer
• MiR10206, 5059, 5073, 5272, 6135, oba-miR531, and aba-miRNA-9497	Tumor proliferation, psoriasis, Alzheimer's disease, epilepsy syndromes, immune responses, retinitis pigmentosa, and central nervous system toxicity
Secondary metabolism	
• MiR5298b, and 8154	Phenylpropanoid
• Smi-miR396b, and miR408	Salvianolic acid
• MiR5298b, and 8154	Taxol
• MiR160b, ath-MIR160b and smi-miR396b	Tanshinone
• MiR156	Sesquiterpene
• MiR035, 1168.2, 1438, 156b, 170, 172i, 1858,1873, 2275, 2673a, 2910, 2919, 396b, 408, 5015, 5021, 5658, 828b, 829.1, 8291, f10132-akr, ain-miR1533c, ain-miR156, ain-miR157, cro-miR397a, cro-miR828a, Cs-miR156, mko-miR159b-3p, mko-miR167c-5p, mko-miR168b, mko-miR5082, mko-miR858, mko-miR8610.1, smi-miR12112, smi-miR397, smi-miR396b, and smi-miR408	Phenolic compounds
• MiR_116, _1194, _1276, _15, _1508, _1900, _2141, _2596, _334, _853, 1134, 1533, 160, 164, 167a, 167b, 171, 172, 172d-3p, 2919, 396a, 398f/g, -4995, 5563-x, 5021, 5658, 6435, 838, ain-miR1525, dfr-miR156b, dfr-miR160a, mko-miR156, mko-miR167a, mko-miR396c, mko-miR396g-5p, mko-miR5082, mko-miR827b, mko-miR858, novel-m0022-5p, pmi-miR6300, pmi-miR6173, pmi-miR530, and pmi-Nov_13	Terpenoid

• MiR159, 159a, 166, 171, 172, 2673a, 390, 396, 858, cro-miR160, EY064998, EY082442, EY107691, EY57163, leaf-miR-477, leaf-miR530, root-miR159, root-miR5140, mko-miR159b-3p, mko-miR5082, mko-miR858, mko-miR8610.1, novel miR_218, novel miR_2432, novel miR2642, novel miR_2924, novel miR_457, and novel miR_853	Esters
• MiR2673a, 396, cro-miR160, pso-miR13, pso-miR2161, and pso-miR408	Alkaloids
• MiR156, 5298b, 8154, and novel_miR_47	Saponins
• MiR5072, MIR1446-x, and MIR394-y	Quinone
• MiR156, 414, 5015b, and 5021	Essential oil
• NovelmiRNA-191, novelmiRNA-23, and novelmiRNA-58	Triacylglycerols
• Pmi-miR396b and pmi-Nov_12	Green leaf volatile
• MIR845-y	Steroid
• MiR5021	Strictosidine

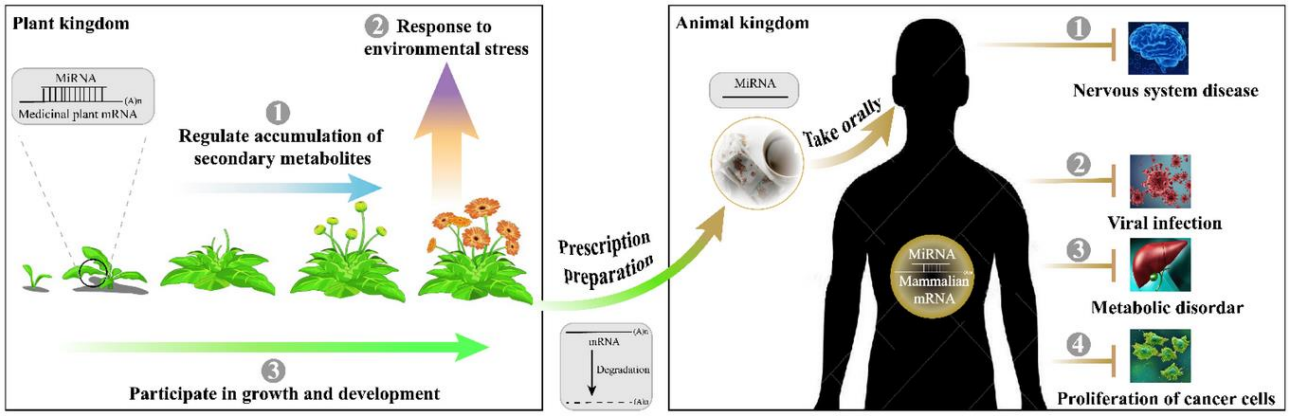

Fig. 1 miRNAs' multipurpose function in therapeutic plants [41]. This table is distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

MATERIAL AND METHODS

First, the NCBI database was used to retrieve the sequences for the *FOLR1* (NM_016729.3) and *MTHFR* (NM_005957.5) genes. These proteins were 656 amino acids long and 257 amino acids long, respectively. The UCSC database was then used to pinpoint where these genes were exactly located. The MBC database was used to calculate the 3D structure of proteins and the Ramachandran plot, while the ProtScale database was used to calculate the molecular weight and isoelectric point of proteins. Cell comparison of *MTHFR* and *FOLR1* genes and the expression of these genes were analyzed by Human Protein Atlas and OMIM database.

RESULTS

Effects of miRNAs

MiRNAs (microRNAs) are small sequences of 22 to 24 nucleotides that lead to many metabolic processes in plants and animals. MiRNAs are involved in the processes of transcription and regulation of post-transcriptional factors; they are also effective in the processes of gene silencing, methylation, translation, and regulation of gene expression [48-50]. In addition, the effect of miRNAs on key regulatory processes such as cellular regulation, biological regulation, and biotic and abiotic stresses such as drought, salinity, heat, cold, oxidative stress, and stress caused by heavy metals in plants and animals has been proven [51-53]. A group of miRNAs is highly protected in plants, especially in medicago legumes and soybeans. This group includes miR159, miR160, miR167, miR169, miR171, miR172, miR393, and miR396. In chickpeas, this group, together with miR166, miR319, and miR394, plays an effective role in dealing with environmental stresses. It has been shwon the protected and effective miRNAs against drought, salt, and cold stresses, along with the mechanisms and genes effective in inducing resistance in chickpea plants (Table 2 and 3) [54-58].

Table 2 Targets of miRNAs predicted and patterns of their expression in response to cold, drought, and salt stressors

miRNAs	Stresses	Target gene	Functions
miR159	Cold, drought, and salt stresses	GAMYB-like-TCP4	ABA response, NaCl stress response, floral asymmetry and leaf development. Transcription factor TCP4. Transcription factor EREBP-like protein. Transcription factor MYB80.
miR160	Drought and salt stresses	ARF16	phases of postgermination and seed germination
miR166	Drought and salt stresses	ATHB-15	Development of the vascular system, leaves, and axillary meristem
miR167	Drought and salt stresses	ABI5	Growth of the gynoecium and the stamen
miR169	Drought and salt stresses	NF-YA	Plant growth, timing of flowering, and reaction to various biotic stressors
miR171	Drought and salt stresses	NSP2	abiotic stress response and floral growth
miR172	Drought and salt stresses	RAP2-7	blooming period, floral organ classification, and cold stress reaction
miR319	Cold stress	GAMYB-like	F-box protein PP2. Transcription factor GAMYB-like. Transcription factor TCP2.
miR393	Cold, drought, and salt stresses	AFB2- AP2	susceptibility to virulent bacteria AP2-like ethylene-responsive transcription factor protein AUXIN SIGNALING F-BOX 2-like glycine-rich cell wall structural protein 2-like
miR394	Cold stress	MYB98	Transcription factor MYB98. glycine-rich cell wall structural protein
miR396	Drought and salt stresses	CP29	leaf and cotyledon development

As seen in Table 2, a group of miRNAs makes various regulatory chemicals available to the plant through the roots. A group also modulates the level of plant hormones and indirectly helps to control plant pathogens. MiRNAs also stimulate plant growth through ABA signaling and auxin production and are effective in creating resistance to drought, salinity, and cold [59-62].

MTHFR Gene

The *MTHFR* gene with accession number NM_005957.5 is located on chromosome 1 (1p36.22). This gene belongs to the flavoproteins group, which includes 121 genes. Flavoproteins are mostly found in mitochondria and include a nucleic acid derivative of riboflavin. A large number of biological processes include this category of proteins. These proteins frequently perform photosynthesis, DNA repair, and the removal of radicals that cause oxidative stress. The protein produced by this gene converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Also, this protein catalyzes a common substrate for the remethylation of homocysteine to methionine. Deficiency of this gene causes susceptibility to obstructive cardiovascular diseases, neural tube defects, colon cancer, and acute leukemia. In general, a mutation in this gene is associated with methylene tetrahydrofolate reductase deficiency. The number of amino acids, molecular weight, isoelectric point, number of exons, and other characteristics of the *MTHFR* gene are shown in Table 3. Figure 2A shows the location of this gene on the chromosome.

FOLR1 Gene

The *FOLR1* gene, with accession number NM_016729.3, is located on chromosome 11 (11q13.4). The protein encoded by the FOLR1 gene has a high affinity for folic acid and is attached to the membrane through a solution or a glycosyl-phosphatidylinositol bond. The mutation in this gene is directly related to the destruction of the nerve network and the lack of brain folate transfer. This gene has two promoters that contain several transcriptions start sites and mediate the transfer of 5-methyltetrahydrofolate into cells. The number of amino acids, molecular weight, isoelectric point, number of exons, and other characteristics of the *FOLR1* gene are shown in Table 3. Figure 2B shows the location of this gene on the chromosome.

Table 3 Genes sequence results of *MTHFR*, and *FOLR1*

Name	*MTHFR*	*FOLR1*
Organism	Homo sapiens	Homo sapiens
Accession number nucleotide	NM_005957.5	NM_016729.3
Accession number protein	NP_005948.3	NP_057941.1
Compartment	Cytosol	Golgi membrane
Gene ID	4524	2348
Chromosome	1	11
Cytogenetic location	1p36.22	11q13.4
Chromosome location bp	11785723-11805964	72189709-72196323
nucleotide length	7018 bp	930 bp
protein length	656 aa	257 aa
Molecular weight (Da)	74596.57	29819.13
Isoelectric point	5.22	8.30
Total exon	12	4

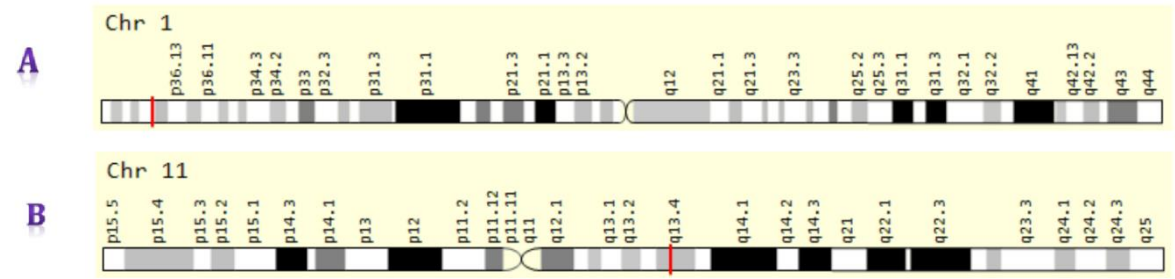

Fig. 2 A) Chromosome 1, the red area is where the *MTHFR* gene is located (1p36.22). B) Chromosome 11, the red area is where the *FOLR1*gene is located (11q13.4).

D Structure of *MTHFR* and *FOLR1* Proteins

First, the sequences of *MTHFR* and *FOLR1*proteins were determined. Then molecular homology modeling using the Expasy SWISS-MODEL server led to the three-dimensional structure of *MTHFR* and *FOLR1* proteins based on the most similar 1a02 sample (Figure 3). The estimation of protein quality was determined based on the GMQE scale. The value of GMQE for the *MTHFR* gene was estimated at 0.87 and for the *FOLR1*gene as 0.79. The GMQE values indicate that the 3D structure is an accurate estimate of the *MTHFR* and *FOLR1* genes.

Then the Ramachandran diagram related to *MTHFR* and *FOLR1* proteins was determined to determine the energy level and stability in terms of two angles φ and ψ in the proteins. According to Ramachandaran's diagram, Ramachandaran's energy level based on amino acids in *MTHFR* protein was estimated to be 97.18%, and in *FOLR1* protein 94.12%. Therefore, the proposed model was in a stable state in terms of energy in the three-dimensional structure of these proteins Figure 4.

Biological Process in *MTHFR* Gene

The molecular function of the *MTHFR* gene includes catalytic activities, methylenetetrahydrofolate reductase activity, oxidoreductase activity, macromolecular complex binding, and flavin adenine dinucleotide binding. Also, the *MTHFR* gene is effective in various biological processes such as response to hypoxia, cellular amino acid metabolic process, methionine metabolic process, blood circulation, regulation of histone methylation, response to vitamin B2, tetrahydrofolate interconversion, response to drugs, response to amino acids, S-Adenosylmethionine metabolic process, tetrahydrofolate metabolic process, folic acid metabolic process, homocysteine metabolic process, oxidation-reduction process, response to interleukin-1, and heterochromatin maintenance. In general, this gene is one of the cellular components of the synapse and cytosol, and for this reason, it is very effective in the neural network. The important paralog of this gene is *MTR*.

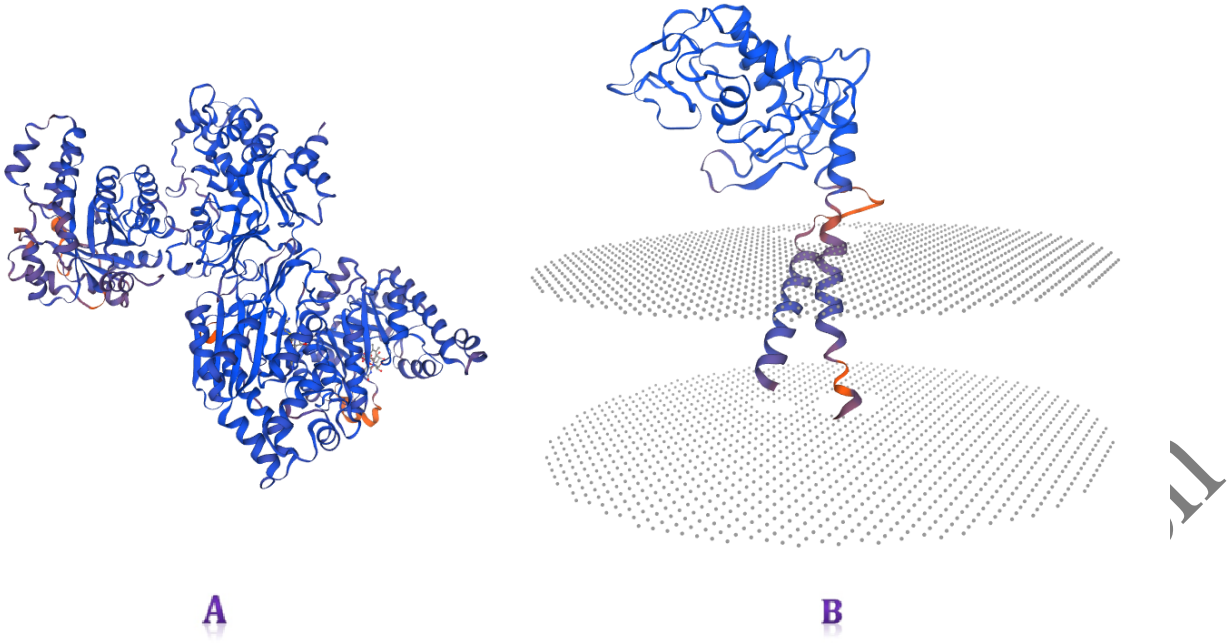

Fig. 3 A) Three-dimensional structure of *MTHFR* protein. B) Three-dimensional structure of *FOLR1* protein.

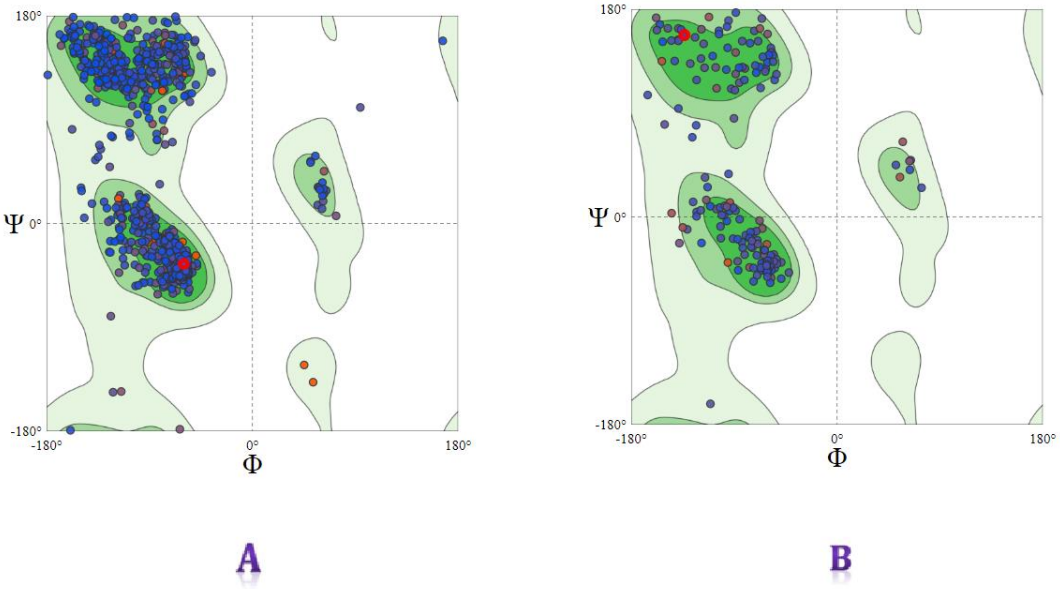

Fig. 4 The right side of the *MTHFR* protein Ramachandran diagram left side of the *FOLR1* protein Ramachandran diagram.

Biological Process in *FOLR1* Gene

The molecular function of the *FOLR1* gene includes folic acid binding, drug binding, methotrexate binding, signaling receptor activity, and folic acid receptor activity. Also, the FOLR1 gene is effective in various biological processes such as heart looping, entanglement of signals and folic acid, ER to Golgi vesicle-mediated transport, axon regeneration, and response to axon injury, pharyngeal arch artery morphogenesis and anterior neural tube closure. The important paralog of this gene is *FOLR2*.

MTHFR and *FOLR1* Gene Expression Analysis

Gene expression analysis showed that *MTHFR* gene expression is relatively lower than that of the *FOLR1* gene in the body. This gene has the highest level of expression in the brain and a low level of expression in other organs. The *FOLR1* gene also has a balanced expression in the whole body; however, it shows more expression in male tissues, muscle tissue, bone marrow, and lymphoid tissue (Figure 5 and 6).

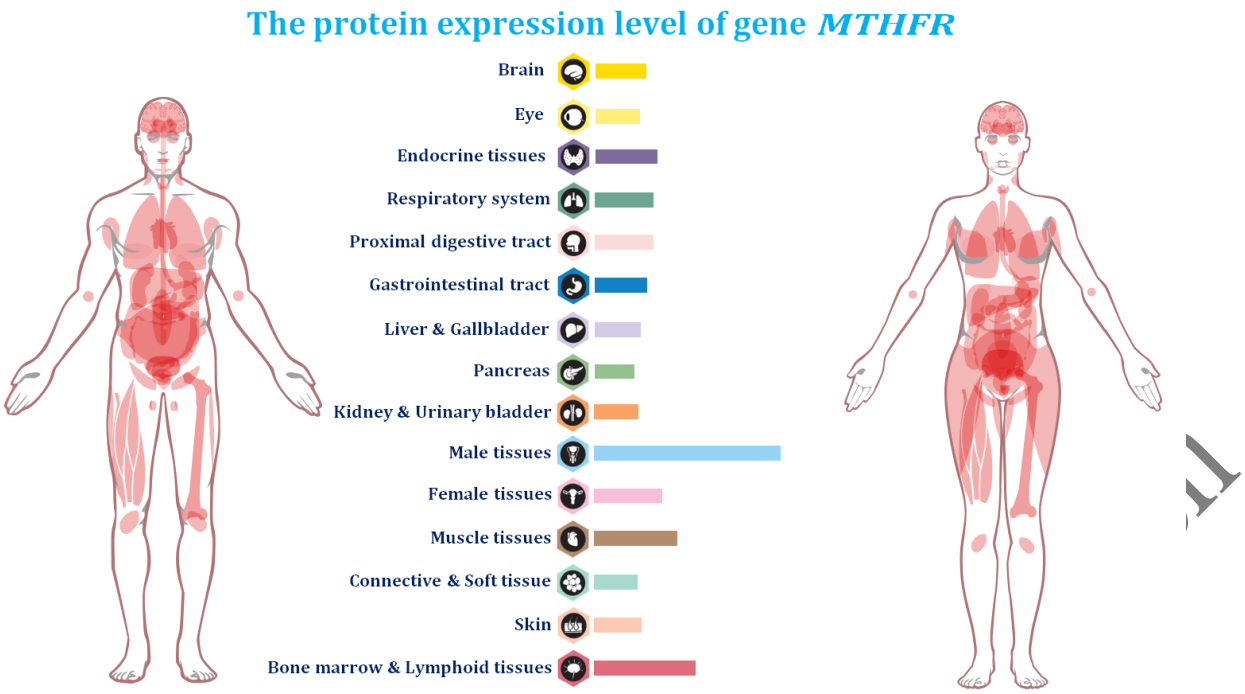

Fig. 5 Results of the study of *MTHFR* gene expression

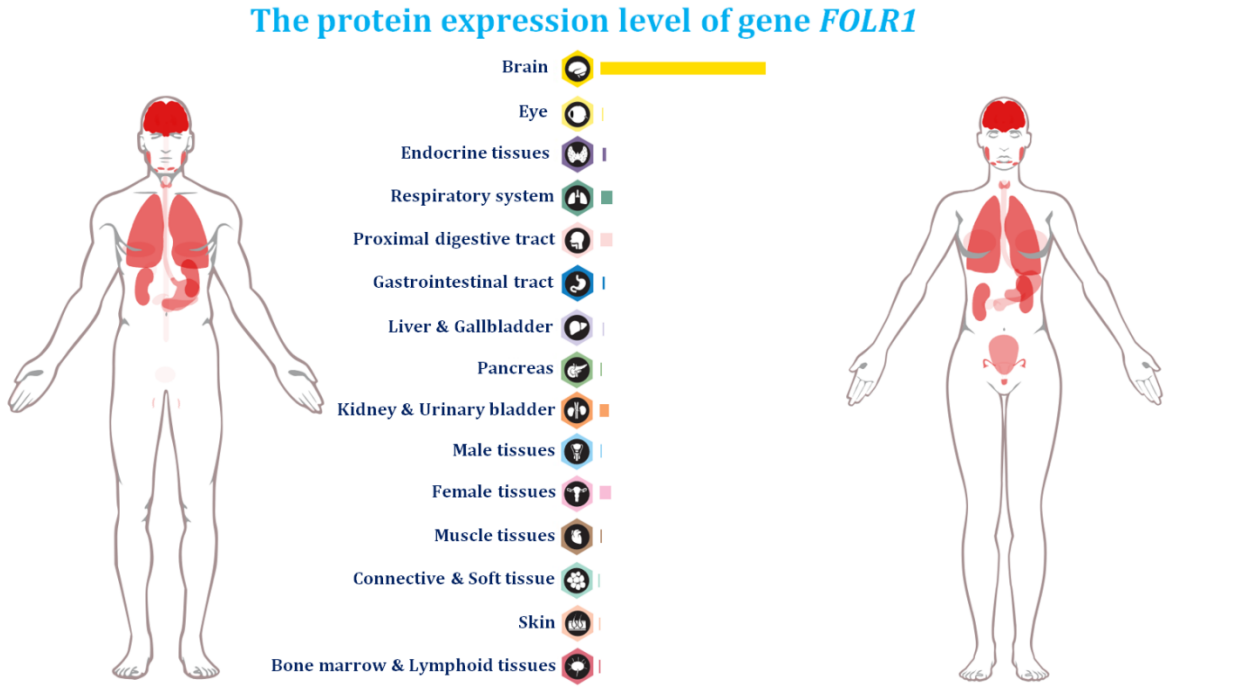

Fig. 6 Results of the study of *FOLR1* gene expression

Localization of the *MTHFR* and *FOLR1* Genes

Gene localization is based on the UniProtKB and COMPARTMENTS localization databases, as well as ontologies of cellular components visualized by the Gene Ontology Consortium. These data were obtained based on high-performance microscopy and primary sequence predictions (Figure 7, Table 4).

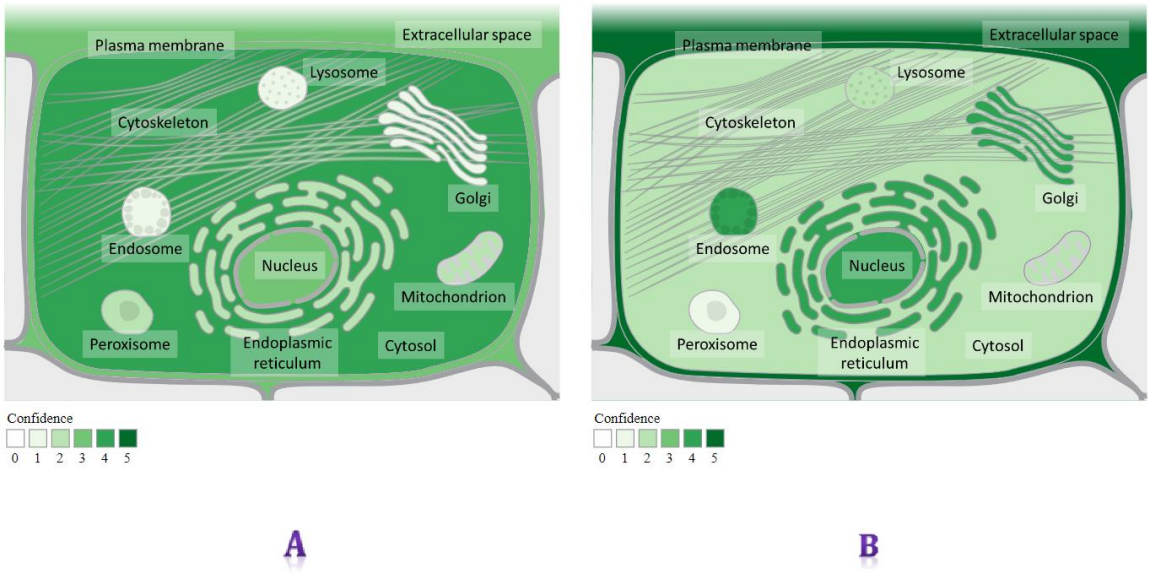

Fig. 7 A) Localization of the *MTHFR* gene. B) Localization of the *FOLR1* gene.

Table 4 Localization of the *MTHFR* and *FOLR1* genes

MTHFR		FOLR1	
Compartment	Confidence	Compartment	Confidence
Cytosol	4	Extracellular	5
Nucleus	3	Plasma membrane	5
Extracellular	3	Golgi apparatus	4
Plasma membrane	3	Endosome	4
Endoplasmic reticulum	2	Endoplasmic reticulum	4
Peroxisome	2	Nucleus	4
Mitochondrion	2	Lysosome	2
Cytoskeleton	2	Cytosol	2
Golgi apparatus	1	Mitochondrion	2
Lysosome	1	Cytoskeleton	2
Endosome	1	Peroxisome	1

DISCUSSION

MiRNAs generally cause resistance to salt and drought stress through the activation of resistance genes in the abscisic acid pathway. This type of resistance has been reported in plants such as Arabidopsis rice, poplar, cassava, Chinese plum, tomato, etc. [63-66]. Since creating resistance to cold is considered an important goal in chickpeas, and every year cold causes a lot of damage to this plant, it is possible to use miRNAs related to creating cold resistance in this plant. miRNAs such as miR159, miR319, miR393, and miR394, which are activated through the response to abscisic acid and whose target gene is MYB, are very effective in creating resistance to drought and cold.

It has investigated the effect of plant growth-promoting rhizobacteria (PGPR) on chickpea miRNAs in desi and kabuli cultivars. Their results showed that PGPR, through a direct effect on miRNAs ultimately leads to the development of resistance, especially in dealing with cold stress [67]. On the other hand, microRNAs affect metabolic and regulatory processes not only directly but also indirectly by affecting other target genes [68]. In general, according to the investigation of miRNAs and resistance genes, we conclude that miRNAs are activated during flowering and seed germination stages against drought and salt stress and through hormonal responses and transcription factors leading to resistance in the plant.

As the results of this research showed, the *MTHFR* gene is related to cardiovascular diseases, and its deficiency causes Homocystinuria. Defects related to the neural network and susceptibility to thromboembolism is also related to the *MTHFR* gene [69-71]. A deficiency of the *MTHFR* gene causes a disturbance in folate metabolism, which ultimately leads to a deficiency of methylene tetrahydrofolate reductase. Severe neurological deterioration

in adults that leads to death is caused by this phenotypic disorder [72]. Diseases related to *MTHFR* include homocystinuria caused by a lack of N-methylene tetrahydrofolate reductase activity, schizophrenia, Alzheimer's, colon cancer, and male infertility caused by misplaced promoter hypermethylation of this gene. The *FOLR1* gene is involved in migrating neural crest cells during heart formation. Also, this gene is involved in the migration of cardiac neural crest cells during the morphogenesis of the outflow tract. One of the most important things to pay attention to in the *FOLR1* gene is the active presence, diversity, and extent of this gene in different cellular components. These cellular components include the Golgi membrane, endoplasmic reticulum membrane, plasma membrane, an integral part of the plasma membrane, brush border, cell surface, ER to Golgi transport vesicle membrane, basolateral plasma membrane, apical plasma membrane, transport vesicle, clathrin-coated vesicle, an anchored component of the membrane, anchored part of the external side of the plasma membrane, cytoplasmic vesicle, brush border membrane, endoplasmic reticulum-Golgi intermediate compartment membrane, anchored part of the plasma membrane, extracellular exosome, extracellular region, nucleus, and endosome [73-78]. On the other hand, as seen, the FOLR1 gene is expressed in the brain, and FOLR1 gene deficiency causes neurodegeneration caused by a lack of cerebral folate transport. As we know, NCFTD is an autosomal recessive disorder caused by brain-specific folate deficiency, which is manifested by movement disorders, epilepsy, and leukodystrophy. Recognition and diagnosis of this disorder through the FOLR1 gene and treatment with folinic acid can reverse the clinical symptoms and improve brain abnormalities and function [79]. The MTHFR gene, located on chromosome 1 (1p36.22), is essential for folate metabolism and the remethylation of homocysteine to methionine, processes critical for DNA synthesis and repair. Variants in this gene, particularly the C677T and A1298C polymorphisms, have been linked to various health risks, including cardiovascular diseases and neural tube defects, due to their impact on enzyme activity and homocysteine levels [80, 81]. The enzyme encoded by MTHFR plays a significant role in reducing oxidative stress, which is vital for maintaining cellular integrity. Furthermore, the gene's expression and polymorphisms highlight its relevance in personalized medicine, as understanding these variations can guide therapeutic interventions in affected individuals[82]. Continued research on MTHFR is crucial for elucidating its broader implications in metabolic disorders and disease susceptibility.

CONCLUSION

Finally, it can be concluded that by carrying out studies in the field of tracking microRNAs and effector genes as well as accurate examination and diagnosis with the help of molecular markers, an effective step can be taken to increase resistance to diseases and stresses and improve crop yield loss.

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