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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and cloning of WRKY 7 transcription factor from *Brassica juncea* and its molecular characterization through *In silico* analysis

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ABSTRACT
Mustard, an economic oilseed crop in India, is one of the largest sources of vegetable oil and protein. It is affected by both biotic and abiotic diseases, out of which Alternaria leaf blight caused by Alternaria brassica is the most serious biotic stresses, which cause a reduction in yield and heavy losses. Mitogen-activated protein kinases (MAPK), an important plant signaling pathway of the specific serine/ threonine kinases, respond to plant defense mechanics and activate defense genes regulated by various transcription factors. One of the important transcription factors is WRKY, which plays a major role in biotic stress by interacting with MAPK, leading to the activation of various downstream genes. It has been reported that during defense mechanism, MPK3 interacts with various transcription factors and activates various plant defense-related genes like WRKY7, which is induced by wound, and SA in Brassica Rapa. According to the present study, MAPK3 gene improves resistance against fungal pathogens. Further, due to the function of WRKY7 of Brassicas Juncea against blight and its interaction with MAPK3, Brassica Juncea WRKY7 was considered for molecular cloning in this study. For this, WRKY7 was isolated and cloned in PGEM-T easy vector and subjected to BLASTP and conserved domain search insilico study, which confirmed it to be WRKY7 gene, but Phyre2 , Swiss model and ClusPro insilico study confirmed the interaction between Brassica Juncea WRKY7 and MAPK3.
Keywords**:** *Alternaria brassica,* WRKY7, *Brassica Juncea,* MAPK3, BLASTP

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INTRODUCTION

The family *Brassicaceae* consists of 51 genera, of which *Brassica* is an economically important genus containing 37 different species. Mustard seed is the 3rd biggest source of vegetable oil in the world after soybean and palm. Mustard oil meal is the second biggest source of protein in the world after soybean meal. Mitogen-activated protein kinases (MAPK) are known to be one of the important mediators in signal transmission, connecting the perception of external stimuli to cellular responses [1]. MAPK cascades are not only involved in signaling to several biotic and abiotic stresses like wounding and pathogen infection, temperature stress and drought but also in plant hormones mediated signals such as ethylene and auxin. Moreover, MAPKs have also been involved in the regulation of cell cycle and developmental processes [2]. MAPK cascades, the signaling kinase modules found in all eukaryotes. They function downstream of sensors/receptors by transmitting extra cellular stimuli into intracellular responses and amplifying the transducing signal [3]. The three components of MAPK, including MAPKKK (MAPKK kinase), MAPKK (MAPK kinase), and MAPK (MAP kinase), sequentially get phosphorylated during transmission of a signal from receptor to downstream targets. WRKY transcription factors are considered as one of the largest families of transcriptional regulators in plants. The WRKY gene family, involved in several diverse pathways, contains one or two highly conserved DNA binding domains called WRKY domain that gets interrupted by an intron. In this study, the WRKY domain binds specifically to various W-box elements, each containing a TGAC core sequence [4].The promoters for various plant defense genes, including *PR* genes containing W-box sequences, are recognized by WRKY proteins and necessary for the inducible expression of these defense genes. WRKY transcription factors are important regulators of SA-dependent defense responses [5, 6]. In *Arabidopsis*, 74 WRKY transcription factors have been reported [7], and most of them play a role in plant defense responses. Forty-nine of 72 WRKY tested genes were differentially regulated in *Arabidopsis* plants treated with SA [8]. WRKY proteins also play an

important function in gene expression in response to SA treatment [9, 10]. It has been observed that in *Arabidopsis,* WRKY7 is induced by SA and *Pseudomonas syringa* [8, 11]. For example, it has shown that MAPK6 mutant comprise disease resistants where as MAPK4 mutant showed increase resistance to avirulent strain of *Perensospora parasitca* and virulent strain of *Pseudomonas syringae* [12]. WRKY7 gene is coordinately induced under various SAR-inducing conditions [5]. These observations suggest that WRKY7 plays a potential role in SA-regulated plant defense responses. Although passive or preformed defense mechanisms could prevent infection, more frequently plants show active responses to pathogenic infection including gene transcription and the synthesis of defensive products leading to delayed pathogen development or plant cell death [13]. The significantly elevated SA levels in WRKY7 over-expressed plants after *P. syringae* infection that resulted from the reduced negative feedback regulation of SA biosynthesis by the compromised SA signaling [14].

MATERIAL AND METHODS

Brassica juncea L. cv Varuna (Indian Mustard) was used in this study. For this, the soil was cleaned by removing unwanted wastes like plant debris, pebbles, small stones, and rotten wood pieces, etc. Then, it was mixed with the required amount of manures and filled into autoclave bags followed by autoclaving at 121 °C at 15 psi for 15-20 minutes. After autoclaving, about ½ kg of soil was taken and filled in the pots with proper aeration for watering the pots. The seeds of both the plant varieties were sown in separate pots and maintained in the transgenic chamber.

Isolation of the *Brassica rapa* WRKY7 Gene

The specific primers of WRKY7 were designed according to the published sequences. The retrieved sequences were fed into primer blast (the online primer designing tool) where all the parameters were adjusted according to the need of the experiment to design both forward primers 5′-TACGGTGGCGTTAGAGTCAA-3' and 5′-TACGGTGGCGTTAGAGTCAA-3′ as well as reverse primers 5′-GCCGCAACGACCAAAGTTA-3′. The amplicom of primers PCR product were purified, and cloned in to **pGEM-T easy** vector which was further used for sequencing.

RNA Extraction, Reverse Transcription RT-PCR and cloning in pGEM-T easy vector

Total RNA was extracted from fresh or frozen samples of *B. juncea L. cv Varuna* using TRIzol reagent (Invitrogen) according to the standard protocol. RNA integrity was analyzed on a 2% agarose gel. RNA quantity was determined using a NanoDrop 1000C Spectrophotometer (Thermo Scientific, USA). First-strand cDNA was synthesized with the extracted RNA using sterile tube, and 2000 ng of RNA template was added to it at 42 °C for 1 hour, which was used as a template for PCR[15]. Amplification was performed using the following cycling parameters: 94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute (denaturation), 55 °C for 45 seconds (annealing), and 72 °C for 1 minutes and 40 seconds (elongation). The PCR products of size 1080 bp, which is the specific size of our gene, were obtained and then analyzed using 1.5% (w/v) agarose gels. The expected size band (1080 bp) was gel eluted and quantified spectrophotometrically, and was later cloned in pGEM-T easy vector (3015 bp). The ligated products were transformed into *Escherichia coli* (DH5α) cells, and the recombinant plasmids were used as a sequencing template.

In-silico analysis of wrky7 gene sequence

The nucleotide sequences and amino acids of WRKY 7 genes were obtained from the Arabidopsis Information Resource (TAIR; http://www.Arabidopsis.org/). Nucleotide sequence of the WRKY7 gene of *B. juncea L. cv Varuna* was translated into amino acid sequence using expasy translation tool (http://web.expasy.org/translate/). Protein sequence homology of translated amino acid was searched with BLASTP **(**http://blast.ncbi.nlm.nih.gov/**).**

Multiple sequence alignment, phylogenetic analysis, and domain detection

A multiple sequence alignment was carried out using the software Clustal Omega. The WRKY7 sequences of *B. juncea, Brassica rapa, and Arabidopsis thaliana* were taken for sequence alignment (http://www.ebi.ac.uk/Tools/msa/clustalo/)**.** The functionally conserved domains in the translated WRKY7 amino acids sequence were depicted by using NCBI online software conserved domain search, and the functional domains of WRKY7 protein were compared with related species (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The translated amino acid sequence of WRKY7, along with the sequences showing its homology according to BLAST result, were used to construct phylogenetic tree by using the online tool MEGA6**.**

Analysis of phosphorylation site

The amino acid sequences of the WRKY7 were analyzed for putative phosphorylation site by utilizing Netphos 2.0 site for this study (**http://www.cbs.dtu.dk/services/NetPhos/**).

Protein-protein interaction of WRKY7 and MAPK3 of *B. juncea*

The 3-dimensional structures of both WRKY7 and MAPK3 were predicted by Phyre2 and Swiss model, respectively, and subjected to docking or analyzing the protein-protein interaction of the two protein molecules by using the ClusPro software (http://cluspro.bu.edu).

RESULTS

Molecular cloning of WRKY7 gene

Several studies showed the role of WRKY transcription factor in plant defense signaling, and SA-mediated signaling pathway regulated by WRKY7. MAPK6 and MAPK10 interact and phosphorylate WRKY7 protein. Thus, WRKY7 protein and WRKY7 gene are targeted for further investigation in defense against *Alternaria* blight disease and for cloning, respectively.

Isolation of total RNA & c-DNA preparation

RNA isolated from *B. juncea* was quantified in order to synthesize cDNA. The $OD_{260/280}$ value was found to be around 2.01. cDNA was prepared and checked with amplifying the actin gene, which is a house-keeping gene. After checking c-DNA, it was further used for amplification **(Fig1).** The desired WRKY7 gene from *B. juncea L. cv Varuna* of 1080 bp band length was observed when the amplified product was checked along with DNA ladder of 100 bp and around 1 kb specific desired band was obtained in the gel (**Fig2**).

Ligation of PCR product

The eluted product WRKY7 was cloned into pGEM-T Easy vector (progema), which further confirmed the successful transformation of the ligated product into the *E.coli* strain DH5α.

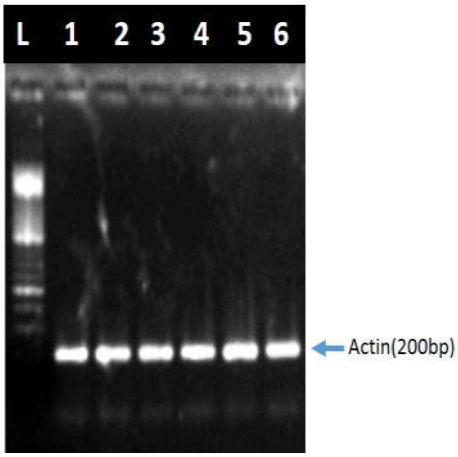


Fig 1 -DNA checking by WRKY7 gene of *Brassica juncea*

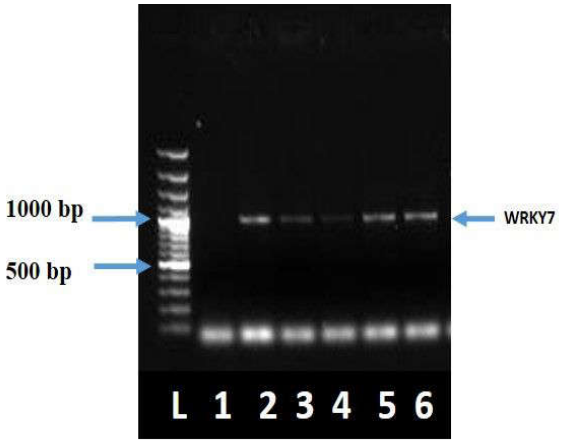


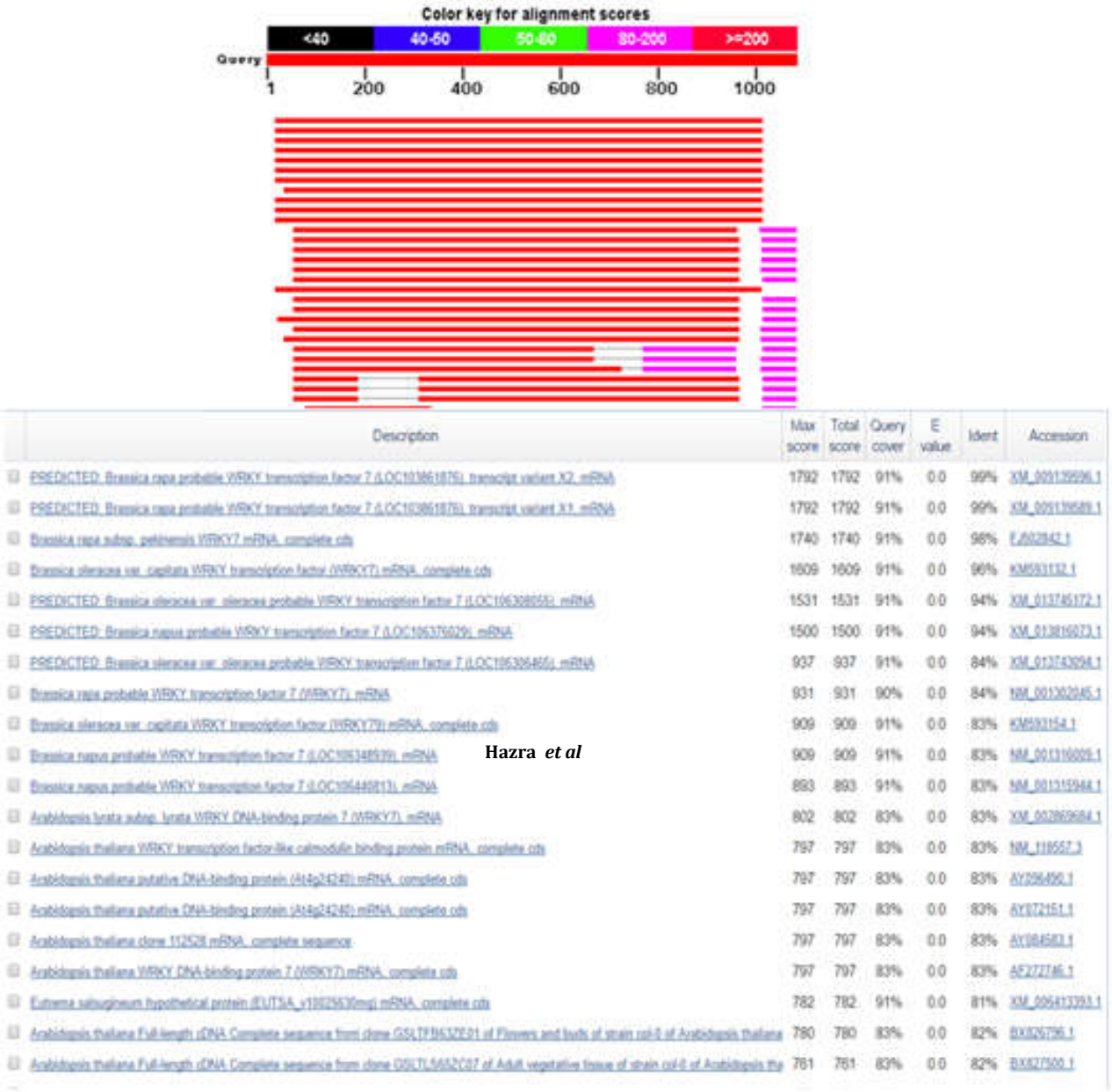
Fig 2. PCR amplification of amplifying Actin gene

Sequencing of the cloned gene

The confirmed clones were sent to the Department of Biochemistry, Delhi University South Campus, New Delhi for sequencing. The sequence of the *B. juncea* WRKY7 gene is given as:

CCGCGAATTCACTAGTGATTGCCGCAACGACCAAAGTTAAAGAGTTTTGTCATGATGCGTCTCGAGCACCAAAGC
ATGGTTGTGGTCTCCTTCGTAAGTCACGATAAGCATCATCGCATCGTCGAGTGCACGCTCCACGTGCTTACGCGCC

GGACAACCTCTTACACTGCTGCATTTGTAATATCCCCGAGGATGAGGAGAGCCTTTGATCGGTTTTTGACCATAT
TTTCTCCATGAATATTCGTCAGATGGTATATCAGCCATCTTGCTGCTTACGGCAGGAACCCTAATCACTCTCTTC
ACTCTAGATTTCCTTTTCTTGGTACAATGGCAACGACTTGAGGGAGATGAGTCACACCTTCTCTTCAACGAAACT
GATGATAAAGGAGGCTTACCAATCGAACCCGAGATTTGGGATGATGGGTTAGTGAACTCGAATCCTGAAGACAT
CTGGTTACTCTCGGTGTCACATCTATGAGAAGACATGAACGAGTTCGCCGCGGAGACAGGTGGTGACGGCGCGAA
GTTCATCGTTGATGGCTCTTGTGGTCTTCCGTTGTTAGCCGAAGTAGACGAACCGTTCTTTGTTGTCAGACTGTT
GTTGTGTGATAAAGGAGGACGTCGATGGATTGGTGTCGGACAATAAACTCTAAACGCAGATCCACCGTTCTGCTC
CGTTTCCTGTCTGTTTAACACGACGGCGCTTGTTGCTGGCTTCTCCTCCGTCTTCCAATCTCCTCCTTCTTTGGTT
TTTGTCGTAACCGGTGCTCGTCTGAATCTAGCGTGTCCGGTTCTAGATCTACCGAGAAGTGATATCACCTTCTTG
GAACTGTTAACGGCGACGTCAGTCACCGCCGTTACCTCTTCTGTCGGTTGACTTTTTTGATTAATTAGTTTAAGA
AACTCCTTAACGCCGTGAATCCCAGCAGACGCAGCTTCTCTCAGGGCGGTGTCTTCCATATTTGCAGAAAAGCCG
CCGTCGTCTTCTCCTTTGACTCTAACGCCACCGTAAATCGAATTCCCGCGGCCGCCATGGCGGCCGGGAGCATGCG
ACGTCGGGCCCAATTCGCCCTATAGTGA



Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: Brassica rapa probable WRKY transcription factor 7 (LOC103861875), transcript variant X2, mRNA	1792	1792	91%	0.0	99%	XM_009139996.1
PREDICTED: Brassica rapa probable WRKY transcription factor 7 (LOC103861875), transcript variant X1, mRNA	1792	1792	91%	0.0	99%	XM_009139989.1
Brassica rapa subsp. pekinensis WRKY7 mRNA, complete cds	1740	1740	91%	0.0	98%	FJ932842.1
Brassica oleracea var. capitata WRKY transcription factor (WRKY7) mRNA, complete cds	1609	1609	91%	0.0	96%	KM593132.1
PREDICTED: Brassica oleracea var. oleracea probable WRKY transcription factor 7 (LOC106308055), mRNA	1531	1531	91%	0.0	94%	XM_013745172.1
PREDICTED: Brassica napus probable WRKY transcription factor 7 (LOC106376029), mRNA	1500	1500	91%	0.0	94%	XM_013816073.1
PREDICTED: Brassica oleracea var. oleracea probable WRKY transcription factor 7 (LOC106306465), mRNA	937	937	91%	0.0	84%	XM_013743094.1
Brassica rapa probable WRKY transcription factor 7 (WRKY7), mRNA	931	931	90%	0.0	84%	NM_001302045.1
Brassica oleracea var. capitata WRKY transcription factor (WRKY79) mRNA, complete cds	909	909	91%	0.0	83%	KM593154.1
Brassica napus probable WRKY transcription factor 7 (LOC106348539), mRNA	909	909	91%	0.0	83%	NM_001316009.1
Brassica napus probable WRKY transcription factor 7 (LOC106446813), mRNA	893	893	91%	0.0	83%	NM_001315944.1
Arabidopsis lyrata subsp. lyrata WRKY DNA-binding protein 7 (WRKY7), mRNA	802	802	83%	0.0	83%	XM_002869684.1
Arabidopsis thaliana WRKY transcription factor-like calmodulin binding protein mRNA, complete cds	797	797	83%	0.0	83%	NM_118557.3
Arabidopsis thaliana putative DNA-binding protein (At4g24240) mRNA, complete cds	797	797	83%	0.0	83%	AY096490.1
Arabidopsis thaliana putative DNA-binding protein (At4g24240) mRNA, complete cds	797	797	83%	0.0	83%	AY072151.1
Arabidopsis thaliana clone 112528 mRNA, complete sequence	797	797	83%	0.0	83%	AY084583.1
Arabidopsis thaliana WRKY DNA-binding protein 7 (WRKY7) mRNA, complete cds	797	797	83%	0.0	83%	AF277746.1
Eutrema salsugineum hypothetical protein (EUTSA_v10025639mg) mRNA, complete cds	782	782	91%	0.0	81%	XM_006413393.1
Arabidopsis thaliana Full-length cDNA Complete sequence from clone GSLTF95325E01 of Flowers and buds of strain col-0 of Arabidopsis thaliana	780	780	83%	0.0	82%	BX825796.1
Arabidopsis thaliana Full-length cDNA Complete sequence from clone GSLTL56527C07 of Adult vegetative tissue of strain col-0 of Arabidopsis tha	761	761	83%	0.0	82%	BX827500.1

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Fig 3: Homology search for WEKY7 gene of *Brassica juncea* by BLASTn program

In-silico analysis of WRKY7 nucleotide sequence

Different bioinformatics tool was used to analyze the full-length WRKY7 sequence. It contained an ORF of 921 bp encoding 307 amino acids followed by a stop codon. This protein is expected to have a molecular

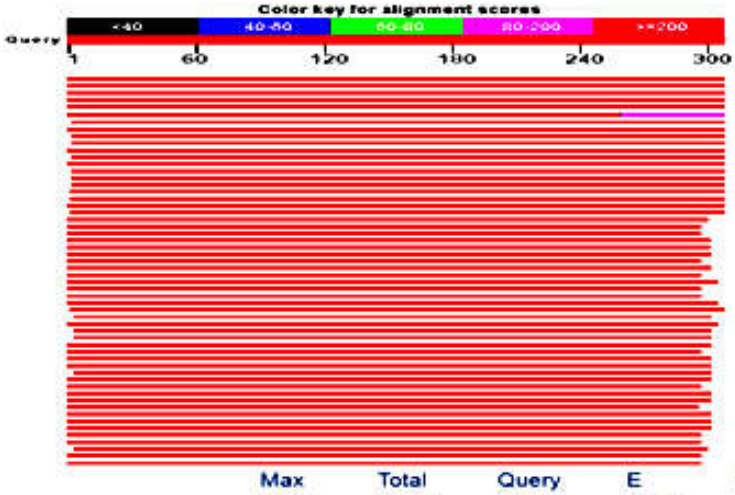
weight of 33728 Da and a theoretical pI of 9.83. The full-length sequence of the WRKY7 obtained was then translated by using the ExPASy translate tool (http://ca.expasy.org/tools/dna.html) to obtain the amino acid sequence. In total, 28 negatively charged residues (Asp+Glu) and 47 positively charged residues (Arg+Lys) were found in the sequence. *In silico* studies of WRKY7 sequence included homology searching, phylogenetic tree preparation, translation into potential amino acid sequence, and functional domain identification in the deduced amino-acid sequence. These studies were completed with the help of different online bioinformatics tools like BLAST, ExPAsy, Swiss-model, Phyre2, Netphos 2.0, Clustalw, MEGA6, *etc*.

Nucleotide Sequence Homology Search

Nucleotide sequence homology search was performed by using nucleotide BLAST for available nucleotide sequences of WRKY7 gene, which showed a high identity with WRKY7 gene sequence of other *Brassica* species. The homology percentage with other known WRKY7 sequences varied from 83% to 99% **(Fig 3).** The nucleotide sequence of *B. juncea* WRKY7 shows identity with WRKY7 sequence of *B. rapa (99%), Brassica oleracea (96%), Brassica napus (94%),* and *A. thaliana* (83%). Thus, the relation between the homology in the sequence with the species suggests that it belongs to the WRKY7 gene.

Amino Acid Sequence Homology Search

Translated amino acid sequence of *B. juncea* WRKY7 gene showed a significantly high identity with its other species like *B. rapa (98%), B. oleracea (93%), and A. thaliana (78%)*. *Brassica* species WRKY7 amino acid sequence was found to vary between 78% and 98% **(Fig 4).**



Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: probable WRKY transcription factor 7 [Brassica rapa]	628	628	100%	0.0	98%	XP_009137837.1
WRKY7 [Brassica rapa subsp. pekinensis]	617	617	100%	0.0	97%	ACQ99198.1
WRKY transcription factor [Brassica oleracea var. capitata]	594	594	100%	0.0	93%	AJA32869.1
PREDICTED: probable WRKY transcription factor 7 [Brassica oleracea var. oleracea]	580	580	100%	0.0	91%	XP_013600626.1
PREDICTED: probable WRKY transcription factor 7 [Brassica napus]	576	576	100%	0.0	90%	XP_013671527.1
BnaA01g13720D [Brassica napus]	519	612	100%	0.0	97%	CDY34269.1
probable WRKY transcription factor 7 [Brassica rapa]	483	483	99%	9e-170	81%	NP_001288974.1
WRKY transcription factor-like calmodulin binding protein [Arabidopsis thaliana]	483	483	100%	1e-169	78%	NP_194155.1
BnaA03g46550D [Brassica napus]	476	476	99%	5e-168	81%	CDY10996.1
PREDICTED: probable WRKY transcription factor 7 [Brassica oleracea var. oleracea]	476	476	99%	6e-168	80%	XP_013598548.1
WRKY DNA-binding protein 7 [Arabidopsis lyrata subsp. lyrata]	476	476	100%	1e-167	79%	XP_002869730.1
WRKY transcription factor [Brassica oleracea var. capitata]	477	477	99%	1e-167	80%	AJA32891.1
hypothetical protein CARUB_v10005147mg [Capsella rubella]	474	474	100%	4e-166	79%	XP_006284025.1
probable WRKY transcription factor 7 [Brassica napus]	473	473	99%	4e-166	79%	NP_001302873.1
probable WRKY transcription factor 7 [Brassica napus]	473	473	99%	8e-166	79%	NP_001302938.1
BnaC07g38840D [Brassica napus]	473	473	99%	9e-166	79%	CDX92626.1
PREDICTED: probable WRKY transcription factor 7 [Camelina sativa]	454	454	99%	4e-158	75%	XP_010432796.1
PREDICTED: probable WRKY transcription factor 7 [Camelina sativa]	452	452	99%	9e-158	76%	XP_010439068.1

Fig 4: Amino acid sequence BLAST of WRKY7 Protein *Brassica juncea*

Multiple Sequence alignment

Multiple sequence alignment of WRKY7 species, including of *B. juncea, B. rapa* and *A. thaliana*, was carried out for sequence alignment using Clustal Omega. The result of this multiple sequence alignment shows that the maximum sequence similarity of *B. juncea* WRKY7 was shown with the *B. rapa* WRKY7 gene compared to that of *A. thaliana*. This result, obtained in the phylogenetic analysis, suggests the close relation between *B. juncea* and *B. rapa.* **(Fig 5).**

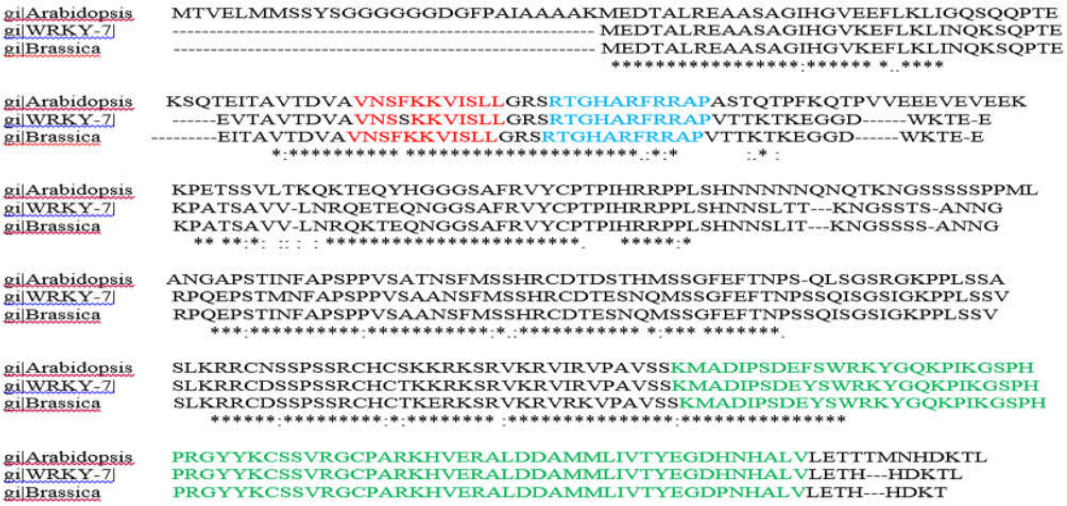


Fig 5: Sequence comparison of WRKY7 of *Brassica juncea* for the conserved domain sequence.

Phylogenetic analysis and analysis of conserved motifs

The conserved domain or WRKY7 domain was found by using conserved domain search tool. It is a DNA binding domain involved in various plant-specific physiological functions including plant defense. The domain specifically binds to (T)(T)TGAC(C/T) DNA sequence motif, which is known as W-box. By this analysis, it was found that the domain resides between 237-295 amino acids of the protein sequence (**Fig 6**). The horizontal dimension of the phylogenetic tree, constructed by Neighbor-Joining method using MEGA6 software, gives the amount of amino acid changes in the WRKY7 protein sequence of different plant species. The horizontal lines are branches and represent evolutionary lineages that change over time. The units of branch length are the amino acid substitutions per site that represent the number of amino acid changes or 'substitutions' divided by the length of the sequence**.** According to phylogenetic tree analysis, WRKY7 amino acid sequence in *B. juncea L. cv Varuna* is closely related to *B. rapa* amino acid sequence because the distance between them is the least, i.e., 0.0 units, compared to other plant species. Through phylogenetic studies, it could be interpreted that the WRKY7 gene, which plays an important role in defense and other physiological process, are conserved throughout the evolutionary process.

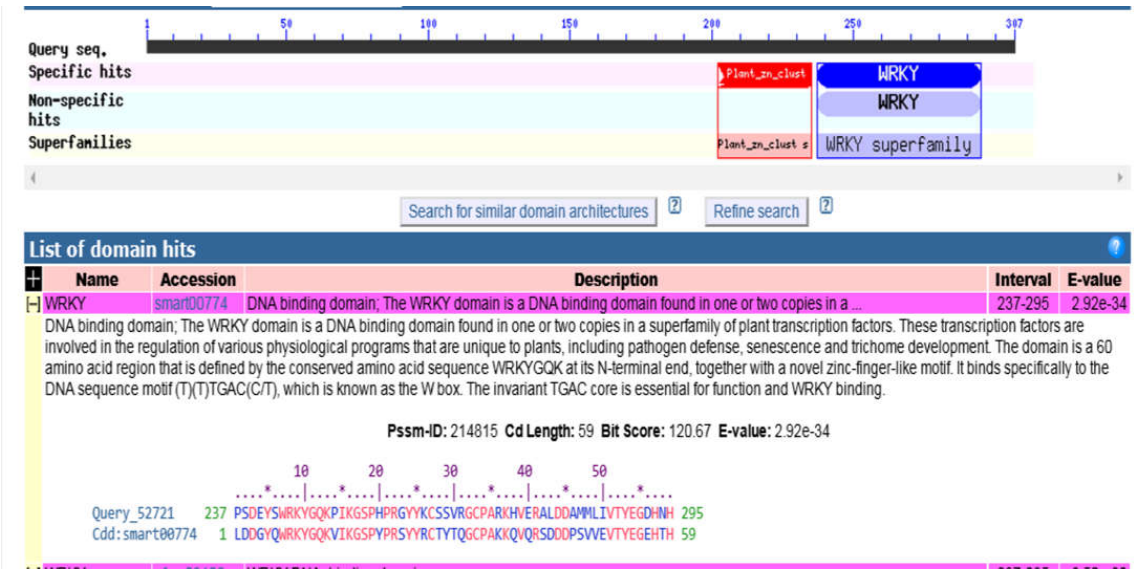


Fig6: Conserved WRKY domain present in *Brassica juncea* WRKY7

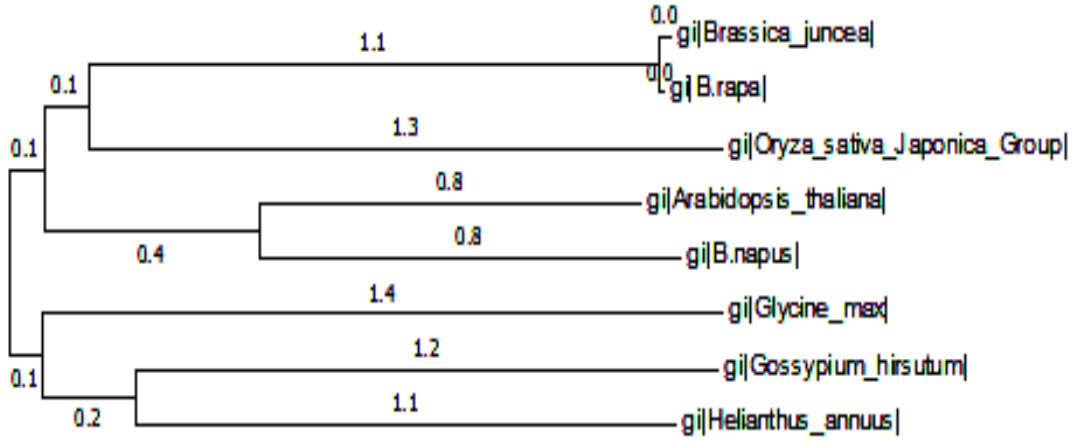


Fig 7: Phylogenetic tree of WRKY7 of *Brassica juncea*

Phosphorylation site prediction

Phosphorylation of protein plays a central role in regulating cellular functions, particularly signal transduction cascades. MAPK cascades have a significant role in plant innate immunity. Phosphorylation of the WRKY transcription is vital for effective DNA binding, which leads to the activation of several downstream signaling like plant defense response. On analyzing the full length protein of *B. juncea* WRKY7 for putative phosphorylation sites using **Netphos 2.0,** it was found that BjWRKY7 possess 24 serine 6 threonine 2 tyrosine residues as putative phosphorylation site (**Fig 8**).

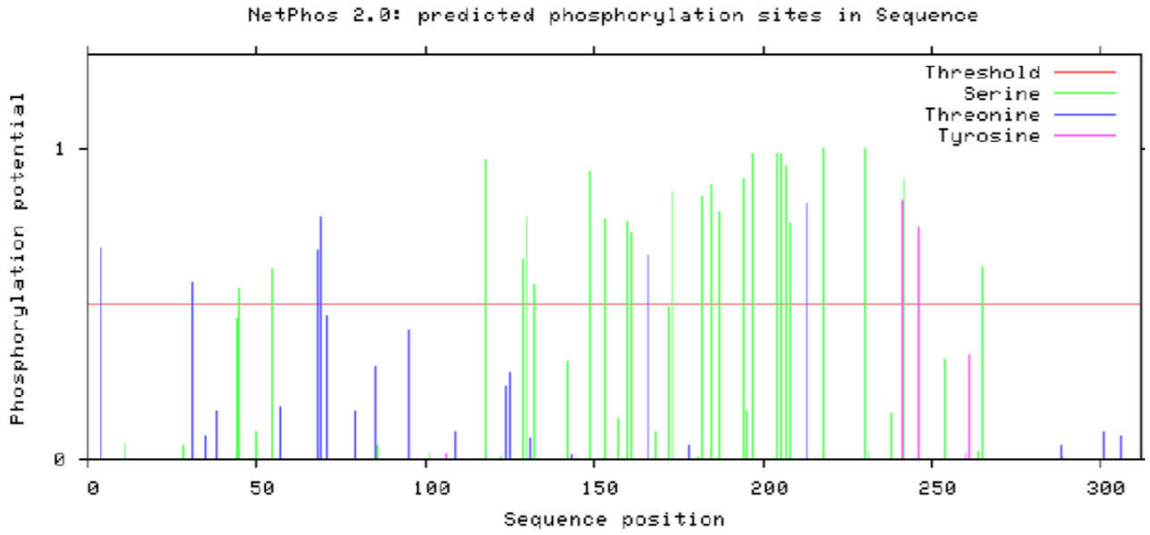


Fig 8: Phosphorylation site prediction on WRKY7 protein of Brassica juncea

Protein-protein interaction of MAPK3 and WRKY7 of *B. juncea*

The sequence of MAPK3 gene of *B. juncea* was taken in FASTA format from the National Centre for Biotechnology Information database, and the WRKY sequence gene of *B. juncea* were isolated in vitro. The three-dimensional structures of MAPK3 gene and WRKY gene were constructed using Swiss Model and Phyre2, respectively. Phyre2 (Protein Homology/Analogy Recognition Engine), a web-based service for protein structure prediction, is one of the most popular method for protein structure prediction. On the other hand, the Swiss Model is a fully automated protein structure homology-modeling server, which is used to for Protein Modeling by making it accessible to all biochemists and molecular biologists (**Fig 9)**.

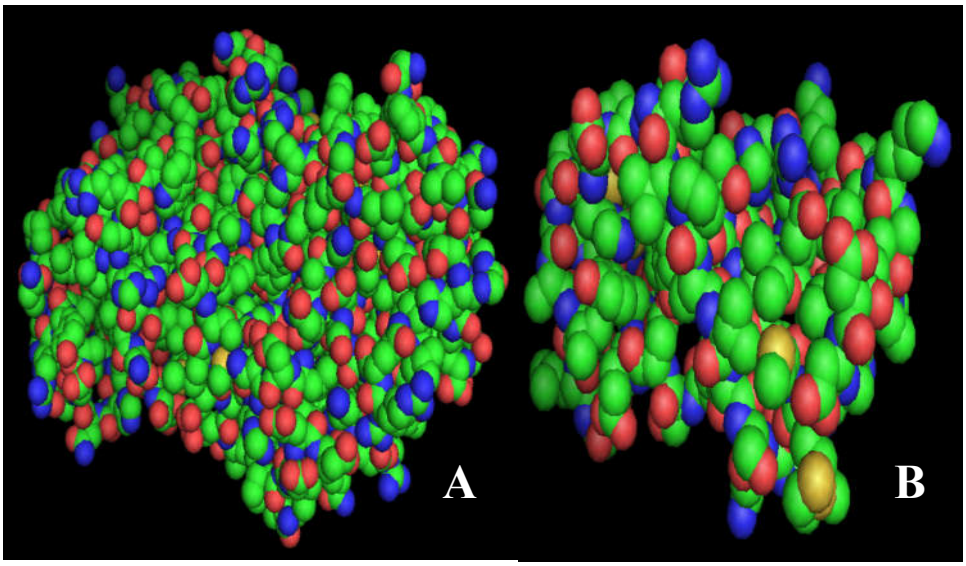


Fig 9: 3-d structure of (A) MAPK3 and (B)WRKY7

After model prediction docking was done by Cluspro software using Cluspro algorithm, there was a need to select between DOT (19, 20) and ZDOCK (21, 22) to perform rigid body docking. Both of these programs are based on the fast Fourier transform (FFT) correlation techniques. Cluspro is the fully automated web server for the prediction of protein–protein interaction, and it allows the generation of the putative structures so that filtering and clustering methods can be applied. After docking, we get five models with different binding energies. The model having binding energy **-803.7 Kcal/mol** was selected as it showed the lowest and the most favorable energy status (**Fig 10)**.

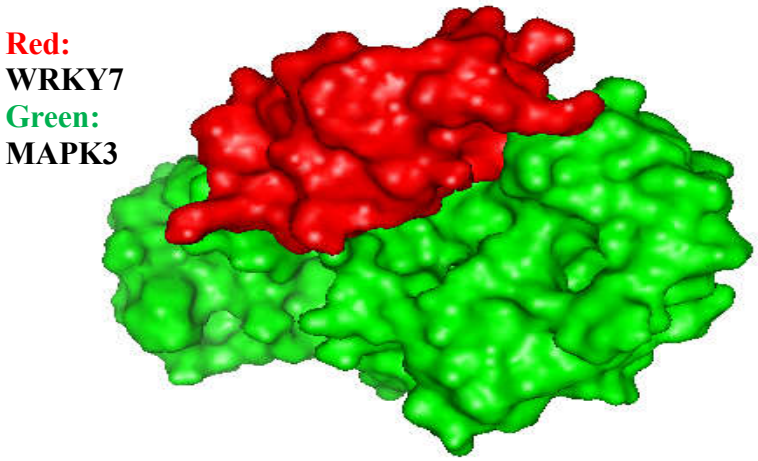


Fig 10: Molecular Docking of MAPK3 and WRKY7 of *Brassica juncea* by using Cluspro software.

As per the present study findings, both MAPK3 and WRKY7 play important roles in plant defense mechanism against pathogens. The study by[16] showed that *B. rapa* and *B. napus* WRKY7 interact with MAPK3.

DISCUSSION

Several studies showed the role of WRKY transcription factor in plant defense signaling, and SA-mediated signaling pathway regulated by WRKY7. According to [17], RNA isolated from *B. juncea* was quantified in order to synthesize cDNA was prepared and checked with amplifying the actin gene, which is a house-keeping gene However, up to now, in the NCBI database only one WRKY nucleotide sequence from *T. dicoccoides* was reported through the isolation of differentially expressed cDNA [18]. The homology percentage with other known WRKY7 sequences varied from 83% to 99% [19]. The full-length sequence of the WRKY7 obtained was then translated by using the ExPASy translate tool. Nucleotide sequence homology search was performed by using nucleotide BLAST for available nucleotide sequences of WRKY7 gene, which showed a high identity with WRKY7 gene sequence of other *Brassica* species. The result of

this multiple sequence alignment shows that the maximum sequence similarity of *B. juncea* WRKY7 was shown with the *B. rapa* WRKY7 gene compared to that of *A. thaliana* [20]. The domain specifically binds to (T)(T)TGAC(C/T) DNA sequence motif, which is known as W-box. By this analysis, it was found that the domain resides between 237-295 amino acids of the protein sequence [21]. The three-dimensional structures of MAPK3 gene and WRKY gene were constructed using Swiss Model and Phyre2, respectively. After model prediction docking was done by Cluspro software using the Cluspro algorithm, there was a need to select between DOT (19, 20) and ZDOCK [21] to perform rigid body docking. The study by [16] showed that *B. rapa* and *B. napus* WRKY7 interact with MAPK3.

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