Original Research Article

# Isolation and in silico characterization of defensin genes from some indigenous plant species of Pakistan

**Abstract:**

Aimes: Damage of crop plants due to pathogenic attacks, postharvest crops spoilage and lethal effects of chemical pesticides has enforced scientists to find some potential natural alternative. Plants produce different types of antimicrobial peptides including defensins in response to biotic and abiotic stresses. Defensins are small cysteine rich, cationic peptide with 40-45 amino acid residues with a variety of biological activities. Hence defensins have pharmaceutical and agricultural significance especially wide range of antifungal activities.

Methodology: Nine plant species viz *Brassica napus, Brassica niger, Conyza bonariensis, Alhagi marorum, Sonchus arvensis, Brassica compestris, Eruca sativa, Cirsium arvensis* and *Brassica juncea* were selected for the study. Four set of primers were applied on extracted genomic DNAs and four amplified genes were isolated from different plants species by PCR. The amplified genes were cloned via pTZ57R/T in *E.coli*. The cloned fragments were sequenced and characterized by different bioinformatics tool such as coding length and peptide sequence, site of cell aggregation, peptide physico-chemical properties, antimicrobial properties and gene expression pattern.

Result: The amplified products from *B. napus, B. nigra, B. compestris, E. sativa* and *B. juncea* were about 330 bp in length and showed upto 85% nucleotide homology to the reported defensins. Deduced amino acid sequence from Bn-Def, Es-Def Bj-Def and Bc- Def showed the conserved defensin domain. These sequences were also characterized for different characteristic like charged amino acids, pI value, shelflife and stability. *Sonchus arvensis* didn't give any specific product by prescribed set of primers. However the products from *Alhagi marorum* was about 770 bp and *B. napus* was of 270 bp but both give homology below 30%.

Conclusion: this study showed that *In silico* characterization of deduced antimicrobial peptides from different plant species has depicted these as an effective alternative to communal therapeutics. However, need further work to validate reliability.

**Key words:** Damage of crops, food spoilage, chemical pesticides, antimicrobial peptides, Defensins.

## 1. Introduction

Antimicrobial resistance is a great threat to the human race as stated by UN assembly in 2015. Defensin peptides can be used to counter the antimicrobial resistance along with other antimicrobial agents against pathogens. Use of defensins as natural analog to design and synthesize

new molecules can be very optimistic approach to develop new field of applications including antimicrobial therapy and use of possible vaccine adjuvants [1]. In addition antimicrobial resistant defensins also assist in abiotic stress. By using their properties new opportunities of biomedical strategies and crop improvement can be developed with a great impact on humankind [2]. Novel antifungals are in high demand due to the challenges associated with resistant, persistent and systemic fungal infections.

Defensins are ubiquitous in plants [3], insects [7], animals [4, 5 and 3], human [6] and mediate innate nonspecific response [3]. They are classified into different categories according to their amino acid composition, size, and conformational structures [8]. Defensins are divided at least into five classes. First three classes α, β, and θ- defensins belong to birds and mammals whereas defensins from insects and plants constitute fourth and fifth classes [9].

These small cationic peptides with highly conserved signature tertiary structure [5] consists of one α-helix and a triple-stranded antiparallel β-sheet stabilized by the disulfide bridges into a compact shape named as "cysteine- stabilized α-helix β-sheet motif (CSα/β)" [4, 10 ]. Expression of defensins increases in response to abiotic stress, injury and pathogen attack [3, 11]. These properties have made this peptide an attractive effective tool (13). Many defensin and defensin like peptides have been isolated from medicinal plants (12, 14).

Herbs and plants species are endowed with a variety of clinically important compounds and have been extensively used locally against different diseases but need further exploration especially. Here we report defensin genes from Brassicacea plant species with potential antimicrobials.

## 2. Materials and Methods

### 2.1. Collection of plants

Medicinal plants used by local societies and herbalists of Cholistan desert for different diseases were collected. Morphological identification of collected medicinal plants was made with the help of Plant taxonomists, Department of Botany, University of Agriculture, Faisalabad, Pakistan. The plants were preserved as plant vouchers. The plants species with higher antimicrobial potential were subjected to defensin gene isolation and expression.

### 2.2. DNA extraction

The genomic DNA was extracted from *Cirsium arvense*, *Brassica nigra*, *Brassica compestris*, *Eruca sativa*, *Brassica juncea*, *Brassica napus*, *Sonchus arvensis*, *Alhagi marourum*, *Conyza bonariensis* by following two CTAB methods. The DNA was treated with RNase A (EN0531, Thermo Scientific). Traces of contaminants were removed following chloroform: isoamyl alcohol (24:1) extraction and ethanol precipitation. The extracted DNA samples were quantified on Picodrop$^{TM}$ Spectophotometer at 260 nm [15]

### 2.3. Amplification of defensin genes

Amplification of candidate genes was carried out by polymerase chain reaction Defensin genes specific primers were designed using the nucleotide sequences available in database of National Commission on Biotechnology Information (NCBI) in Genbank. Four sets of primers were designed (Table 1) with the help of different primer designing bioinformatics tools. PCR reaction for defensin gene was carried by using 2X Green dream *Taq* master mix (Thermo scientific) in the thermocycler (BioRad T100). PCR conditions (Table 2) and annealing temperature (Table 3) were optimized for optimal amplification of the genes. After confirmation by agarose the amplified products were purified from primer dimers and other impurities by ISOLATE ll PCR purification kit (Bioline) following user defined protocol.

**Table 1. Primers used for amplification of defensin genes from different medicinal plants species**

| Name | Sequence(5′-3′) | Primer | Tm (°C) |
|---|---|---|---|
| Ma-ast | CATGGATCCATGGCCAAAAAATTCAGTTG | Forward | 63 |
| | AATGGATCCTCAAGGTGTAACTGGCA | Reverse | 62 |
| Ma-fac | CTTGTCCTTCCTCTTCCT | Forward | 52 |
| | GCACCTGCCACTAAGTA | Reverse | 51 |
| Ma-bst | CATGGATCCCTTGAGCTTAGTTCAGTAA | Forward | 63 |
| | CATGGATCCTTTGCTACATACTCAAGGT | Reverse | 63 |
| Ma-bas | GGCTAAGTTTGCTTCCATC | Forward | 53 |
| | ATACACTTGTGAGCAGGGAA | Reverse | 54 |

**Table 2. Optimized PCR conditions for the amplification of defensin genes from Brassicaceae plant species**

| Sr. # | Components | Stock conc. (25µL reaction) | |
|---|---|---|---|
| 1 | Forward Primer | 20µM | 0.5 µL |
| 2 | Reverse Primer | 20µM | 0.5 µL |
| 3 | DNA (Template) | Variable | 2 µL |
| 4 | dNTPs | 10mM | 2.5 µL |
| 5 | *Taq* DNA polymerase Buffer | 10X | 2.5 µL |
| 6 | $MgCl_2$ | Variable | |
| 7 | *Taq* DNA polymerase | 1U/L | 0.5 µL |
| 8 | Deionized water | ------ | Up to 25 µL |

**Table 3. PCR Programming for amplification of defensin genes from Brassicaceae plant species**

| Sr. # | Cycle Step | 3 Step Protocol | | Cycles |
|---|---|---|---|---|
| | | Temp. (°C) | Time (min) | |
| 1 | Initial Denaturation | 94 | 3 | 1 |

| | | | | |
|---|---|---|---|---|
| 2 | Denaturation | 94 | 1 | 33-40* |
| 3 | Annealing | 47-50* | 30-60* | |
| 4 | Extension | 72 | 1 | |
| 5 | Final Extension | 72 | 10 | 1 |

* **Variables depending on experiment.**

### 2.4. Cloning of amplified product

The purified PCR product was ligated into TA-cloning vector pTZ57R/T. Transformation of *E. coli* competent cells was performed by heat shock method [32].

The colonies were screened by Blue /White screening and colony PCR. Plasmid DNA was extracted by Plasmid DNA extraction kit (ThermoScientific#k0512) following manufacturer's instructions. To confirm the recombinant pTZ57R/T, restriction digestion analysis was done by using *Pvu*II and product sizes were analyzed according to the vector maps.

### 2.5. Sequencing of cloned defensin genes.

The clone of defensin genes from *Cirsium arvense*, *Brassica nigra*, *Brassica compestris*, *Eruca sativa*, *Brassica juncea*, *Brassica napus* and *Alhagi marourum* were sequenced using DNA Sanger Sequencing Technology on ABI Prism 3730XL (Applied Biosystems/Sanger) according to the dideoxy chain-termination method [34] from Center for Applied Molecular Biology (CAMB), Lahore, Pakistan.

### 2.6. Sequence analysis

The nucleotide sequence of all cloned products was analyzed using different bioinformatics tools. The sequences were trimmed and checked for ambiguous nucleotides. Contiges were obtained for defensin gene from each plant species and built consensus sequence by using the Bio Editv7.2.5 (https://www.mbio.ncsu.edu/bioedit/bioedit.html/). Sequence editing was made by SnapGene Viewer (3.2.0)(https://www.snapgene.com/products/snapgene_viewer). Consensus sequence was exported from BioEdit for further analysis.

To find similarity in regions, all the nucleotide sequences obtained from BioEdit were queried in BLAST for the sequence Database available on the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UniProt Data base (http://www.uniprot.org/blast/) separately.

Amino acid sequence deduced from nucleotide sequence by online available bioinformatics tool JustBio.com (http: http://www.justbio.com/index.php?page=translator) and ExPASY tool ((http://www.expasy.org/translate/). The predicted protein sequence was again BLAST to determine similarity using two database NCBI GenBank database and UniProt database. This analysis also predicted the function of protein to be expressed. Characterization of deduced amino acid sequence (amino acid composition, ratio of positively charged amino acids to negatively charged amino acids, pI value, shelflife and stability) was made by ProtParam tools of ExPASY Proteomics server (http://www.expasy.org/cgi-bin/protparam/protparam/).

The conserved regions of plant defensins were located among deduced amino acid sequence by the conserved domain finder tool of NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/).
Signal sequence was determined by predisi (http://www.predisi.de/predisi) and SignalP 4.0 [16]. The cleavage site of the deduced amino acid sequences were also predicted in signal peptide.

**2.7. Multiple sequence alignment**

To findout the comparison between species, these nucleotide sequences of different defensins were aligned individually with defensins sequences from other species through Clustal Omega tool (http://www.ebi.ac.uk/Tools/msa/clustalo/).

**3. Results and Discussion**

**3.1. Isolation of defensin genes**

Genomic DNA was successfully isolated by following the modified CTAB method from medicinal plant speiecs. Four sets of primers (Table 1) were designed from the sequences that showed highest homology. Selectivity and specificity of these primers was confirmed by aligning these primers sequences to different reported defensin sequences. Recommended parameters for primer designing like GC%, melting temperature, GC clamp, primer length, absence of hairpin and self/cross dimerization and annealing temperature were calculated using different online available bioinformatics tools e.g; primer 3.0 and hosted tools of justbio.com. Seven out of these nine plants species yielded positive amplification of the defensin genes.

Ampliconse from Brassica *napus, Brassica compestris, Eruca sativa, Brassica nigra* and *Brassica juncea* were of the size 330 bp (Fig. 1). Amplification of single band for all plants indicated there was no isoform of these defensins genes. Defensin gene sequence from *Brassica juncea* with 300 bp is analogous to that reported by Oguro *et al.* (2014).

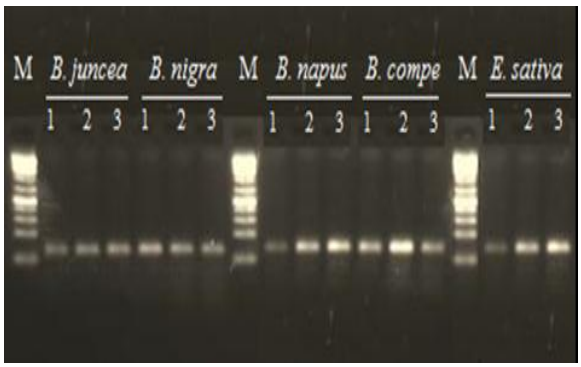


**Fig. 1. PCR amplifications of defensin gene (330 bp) from. *Brassica nigra, Brassica compestris, Eruca sativa, Brassica juncea, Brassica napus*.**


**Fig. 2. Defensin gene amplification from genomic DNA of *Alhagi marorum, Cirsium arvensis* and *Sonchus arvensis.* M: 1 kb DNA ladder, PCR reaction results in amplification of 782 bp fragment, 300 bp fragment from *Circiumarvensin* and no-amplification from *Sonchus arvensi***

As no defensin has been reported until now from *Eruca sativa* besides its diversely reported activities against microbes, this is the first reported sequence of the defensin gene from this species. Similarly, no defensin sequence has been reported from genomic DNA/ complete coding sequence of mRNA of *Brassica rapa* with subspecies *compestris* and *Brassica nigra.*

TBLASTN analysis of amplified fragment from *Alhagi marorum* showed only 10% homology to antifungal genes from fabaceae family. However, its deduced amino acid sequence analysis TBLASTP did not show considerable identity to reported defensin on NCBI databank. So this sequence was not used for further proceedings. Amplified products exhibited DNA band of about 300 bp approximately for *Cirsium arvensis* and no specific product was obtained for *S. arvensis*

though tried with varied PCR conditions. It might be due to the absence of that specific defensin in that particular plant species.

Up till now no defensin has been reported form *Cirsium arvensis* however other plant species of astereraceae have been used for defensin gene isolation. Defensin genes from fabaceae and asteraceae family showed efficient amplification at 1 mM of $MgCl_2$ with 10 ng/µL of genomic DNA. When PCR reaction mixtures for defensin amplification from fabaceae and brassicaceae were analyzed on 1% agarose gel, showed clear single bands without any smear. The amplified products were pure but *Cirsium arvensis* showed primer dimer very close to its product size in reaction mixture. Hence this PCR product was purified using Bioline PCR purification kit following the instructions of manufacturer.

Confirmation of the purified product was made on 1.2 % agarose gel. Rest of the two plants species did not result in amplification although tried with different primer sets at different annealing conditions and reagent concentration. Absence of expression of some defensin gene fragments in some species could be explained by the differential presence of defensin genes in the defensin multigene family in different species or tissue specific expression [17].

**3.2. Cloning of amplified defensin genes**

The defensin genes form all three families corresponding to six plant species were amplified in thermocycler using heat stable *Taq* DNA polymerase. The PCR amplified gene fragments of Brassicaceae and Fabaceae were ligated into pTZ57R/T vector whereas PCR purified product of *Cirsium arvensis* was used.

The plasmids were screened for recombinant plasmid by restriction analysis. Although blue white screening is efficient method for screening of recombinant plasmid but presence of false positives in blue white screening has minimized its efficiency [18].

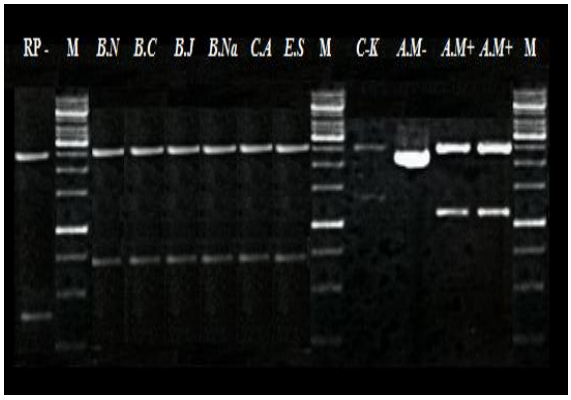


**Fig.3. Agarose gel electrophoresis of restriction digestion analysis of recombinant cloning vectors for confirmation of inserts. Fractions of plasmids DNA were digested with *puv11*. C.A: *Cirsium arvense*, B.N: *Brassica nigra*, B.C: *Brassica compestris*, E.S: *Eruca sativa*, B.J: *Brassica juncea*, B.N: *Brassica napus*, A.M+: Digested recombinant plasmid of *Alhagi marourum*, *A.M-:* undigested recombinant plasmid of *Alhagi marourum*, RP-*:* Non recombinant plasmid restriction digestion, C-K: positive control from ligation kit. That carries both circular and linearized plasmid.**

**Amplified products**


**Fig. 4. Agarose gel electrophoresis of Colony PCR for defensin genes for confirmation of recombinant pTZ57 plasmid. M: 1kb DNA ladder, C.A: *Cirsium arvense*, B.N: *Brassica nigra*, B.C: *Brassica compestris*, E.S: *Eruca sativa*, B.J: *Brassica juncea*, B.N: *Brassica napus*, A.M: *Alhagi marourum*.**

*Brassica nigra, Brassica compestris, Brassica juncea, Eruca sativa, Brassica napus and Cirsium Arvensis* had fragment sizes of about 707 bp. Fragment size of *Alhagi marorum was* about 1177 bp.

**3.4. Sequence analysis of cloned defensin genes**

Positive clones after confirmation by restriction digestion and colony PCR were sequenced from CAMB, Lahore, Pakistan (http://www.camb.edu.pk/dnacf.asp). Bioedit and SnapGene viewer softwares [33] were used to remove unusual sequences/plasmid portion and developed corresponding contigs. The sequences thus obtained were used to make nucleotide and amino acid based studies.

PCR based screening of genomic DNA of *Brassica juncea* allowed isolation of putative defensin sequence of 331 bp with an open reading frame of 192 bp and 39.9% of GC count. Nucleotide sequence analysis of this amplicon in TBLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi/) showed highest homology to super family of plant antimicrobial peptides named plant defensins so the isolated gene sequence was named *Brassica juncea* defensin (*Bj-Def*). Blast analysis in Uniport (http://www.uniprot.org/blast/) with threshold value-10 showed 97% identity to the defensin from *Brassica Juncea* (E3WAM6), defensin from Orych0phragmus violaceus (C9WGT1) and antifungal peptide (*rafp1*) from *Raphanus sativus* (Q00313). The amino acid sequence was deduced using translator tool of JustBio (http://www.justbio.com/index.php?page=translator) and Expasy (http://www.expasy.org/translate/). Sequence Analysis of *Bj-Def* encoded amino acid using ProtParam tools of ExPASY Proteomics server depicted 111 amino acid peptide with Molecular weight:12960.20 Da. Amino acid composition analysis showed 14 cysteine residues and higher content of arginine and lysine as compared to negatively charged amino acids and might be responsible for cationic property of peptide. Cysteine residues have highest (12%) ratio of all amino acid residues in peptide. Amino acid sequence was compared against the protein in NCBI data base using blastP (https://blast.ncbi.nlm.nih.gov/Blastp/). It gave highest homology of 89% with 97% identity to defensin protein from *Brassica rapa (*XP009128139.1*)* and 97 % identity to defensin from *Brassica juncea* (ABB59548.*1*) over 31% of query sequence.

Nucleotide sequence amplified fragment from *Brassica napus* 275 bp and 780 bp from *Alhagi marorum* did not show significant similarity to defensin gene sequence. *Alhagi marorum* fragment showed only 17 bp identity to antifungal gene clone from *glycine max.*

Nucleotide sequence of *B. nupus* amplicone yielded homology upto 30% to defensin sequences. Presence of signal peptide of 22 amino acids was also predicted but its deduced amino acid sequence showed absence of conserved domain for defensin. So the amplicone was not complete coding sequence of defensin gene.

BLAST results revealed that nucleotide sequence of *Brassica juncea, Brassica compestris, Brassica nigra* and *Eruca sativa* had more than 65% homology to the reported defensin sequences. Mostly reported defensin peptide sequences are mRNA based and the number for DNA based sequences is extremely low. Among all the plant species used in this study only *B. juncea* had two reported deoxyribonucleotide sequences for defensin and revealed highest homology(>85%) to our said plant deoxyribonucleotide sequences. However similarity index of nucleotide sequence for defensins of *Brassica campestris, Brassica nigra, Eruca sativa,* to other brassicaceae species was quite distinct. BLAST tool of both databases NCBI and UniProt to screen the biological sequences for local similarity to their database sequences revealed statistical significance [19]. Use of two databases to find out the nucleotide similarity was made to ensure our work output as recommended by Silverstein and colleagues (2005).

The deduced amino acid sequences of these gene products were analyzed by TBLASTp of NCBI and BLAST of UniProt. Mature peptide coding sequence revealed highest similarity to defensin and antifungal peptides with different species of brassicaceae. Bn-Def and Bj-Def amino acid sequences had significant similarity to defensin and antifungal peptides from model plant

*Arabidopsis thaliana* that itself hosts 306 defensin like peptides [20]. Bn-Def also indicated homology to defensin from *Arabidpsis hellari* involved in its zinc tolerance ability. Schutte *et al.*, (2002) discovered five conserved β-defensin gene clusters using computational tools whom products were confirmed by following this strategy.

Weerden and Anderson in (2013) depicted that the plant defensins had very limited conservation in amino acid sequences except 6-8 cysteine, one glutamic acid, one histidine residue and two glycine residues at specific positions. This specific position of cysteine residues signature a conserved γ-core motif. This γ-core motif has significant effect in antimicrobial activities of cysteine rich peptides [23, 24] and consists of almost 14 residues (roughly) in length [4]. All sequences containing BLAST hits for defensins were studied further with NCBI conserved domain finder. For the functionality of defensin peptides, conserved domain γ-core motif ($GXCX_{3–9}C$) is crucial. Studies on plant defensins reveal that γ-core domain is primarily located at C-terminal of active peptide. The only genes Bc-Def, Bn-Def, Es-Def and Bj-Def indicated the presence of Gamma-thionin conserved domain. Plant defensins were originally called γ-thionin because of their size and cysteine content (6-8) similar to the thionin [25]. Later identification of two antifungal peptides Rs-AFP1 and Rs-AFP2 by Terras and his colleagues (1995) which were structurally and functionally related to defensins from insects and mammals, hence this family was renamed as plant defensin.

Deduced amino acid alignment results showed that γ-core motif ($GXCX_{3–9}C$) (14 amino acid) had 100% homology in their amino acid sequence for Bn-Def, Es-Def and Bj-Def but Bc-Def had 4 different amino acid in this region. Among these 4, two have cationic replacement to cationic ones as Bj-Def have lysine and Bc-Def had arginine in replacement at position 2 and in second position the Histidine in Bc-Def is replaced by arginine, and in remaining two, one hydrophobic amino acids alanine in Bj-Def is replaced to hydrophilic serine residue and one hydrophobic asparagine in Bj-Def is replaced to hydrophilic glutamine in Bc-Def. Difference in amino acid sequence contributes to difference in antifungal activity and their morphogenecity. [26]. Even a single amino acid variation in γ-core motif can change the target and mode of action of defensins [11]. This 24 amino conserved region "LCERPSGTWSGVCGNNNACKNQCI" of Bj-Def, Bn-Def, and Es-Def had 100% homology to antimicrobial protein (27 amino acids only) with fungicidal characteristic from *brassica 'rapa* (UniProtKB/Swiss-Prot: P30227.2), antifungal protein (25 amino acids) from *Sinapis alba* (AAB25088.1) antifungal protein (27 amino acid) from *Arabidopsis thaliana* (AAB25092.1) and antifungal protein from *Raphanus sativa* (AAB22710.1) predicted for its fungicidal characteristic.

The deduced amino acid sequences peptide gives 100% homology to peptide sequence which were isolated in functional form. Antifungal activity of antifungal proteins mainly depends on the net charge of peptide of this amino acid in this region [26]. Mostly plant defensins are positively charged peptides as increase in positive charge increase their binding towards negatively charged pathogens. However, anionic defensins have been reported with increased pore formation ability as compared to cationic peptides [27, 28]. Another semi-conserved region of plant defensins reported by Picart *et al.,* in 2012 is α-core $GXC(X_{3–5})C$. This region is not hallmark structure of plant antimicrobial peptides like γ-core and similarly have no contribution to their characteristic function/activity. This region had shown 100% homology for Bc-Def and Bj-Def but among four only a few residues are common. Moreover, Wu and colleagues (2017) has explained the multitasking characteristics of defensins lies in its different regions.

As γ-core is responsible for antimicrobial activity similarly their ability to effect $K^+$, $Na^+$ and $Cl^-$ channels is due to $CysX_3Cys/CysX_1Cys$ motif [26]. This motif was founded in Bj-Def only

and can propose its biological activity in addition to antimicrobial activity. Amplicone sequences from *Brassica napus* and *Cirsium arvensis* missed the conserved domain. Genes with less homology can show activity but absence of conserved domain lose their function and results in pseudogenes [29]. Besides γ-thionin domain, Bj-Def showed an additional domain for Knot1 and NCBI conserved domain finder has classified this peptide to Knot1 domain containing peptide under pfam00304. *Knot1* domain proteins are restricted to eukaryotes and are called Knottins or cysteine-knot1 peptides. Such proteins have protease and insecticidal activity like scorpion toxin along with antibacterial/antifungal activity [30]. pfam database has collection of γ -thionin conserved domain sequences from about 70 plant species and our amplicon showed significant homology to their database sequences.

The subcellular location of these protein sequences was predicted by PrediSI and SignalP. According to PrediSI, almost all transcripts including *Brassica napus* were predicted to be secretory except *Cirsium Arvensis*. However, the SignalP has predicted Bc-Def in contrast to PrediSI and amplicon from *Cirsium arvensis as* non-secretory peptides. All transcripts with reliable predictions were gone along with localization of proteins to secretory pathway. Secretory pathway means the proteins will be located in apoplast of the cells. Custers; (2015) used this strategy to verify peroxidase products.

**Conclusion**

Defensins are natural antibiotics and have a great potential for therapeutic use in agriculture and healthcare. These are good alternative to their chemical counterparts to protect plants, animals and human from disease. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Besides antimicrobial resistance (AMR) to some existing antibiotics an increasingly serious threat to global public health requires action across all government sectors and society. So to protect human, animals and plants there is a need to develop inexpensive antimicrobials. In this study, with the use of EST databases, the defensin gene sequences were identified followed by, isolation and sequence of amplified genes from different plant species. The genes were characterized by various bioinformatics tools but further work is needed to verify at protein level. Conclusively the study showed that the defensin genes of plant species share functional characteristics with other reported defensins, Hence have a great antimicrobial potential.

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Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.