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GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF *PBS3* PLANT-SPECIFIC TRANSCRIPTION FACTOR GENE FAMILY IN CARROT SPECIES (*DAUCUS CAROTA* L.)

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Abstract *Plant growth, maturation, biotic and abiotic stress tolerance or resistance and signalling are all significantly influenced by the PBS3 gene. Commonly referred to as Gretchen Hegan 3 (GH3) A zinc finger protein, which belongs to the C2H2-type and also anticipated to have a role in transcription of PBS3,Salicylic acid (SA) production, which regulates plant ageing. This gene involves in its final two stages. Total Salicylic acid accumulation, SA-dependent gene expression and plant defence are all compromised in Arabidopsis thaliana by PBS3 mutants. Also the most important enzyme in the route leading to SA biosynthesis has also been exhibit to be the PBS3 gene. The gene is linked to disease resistance and also have the ability to respond to particular stimuli or infections in carrots, according to research done on the vegetable. The presence and function of the PBS3 gene family in carrots have not been fully explored, despite the fact that it has been widely examined in many plant species. To understand the phenotypic and genetic traits of the PBS3 gene family in carrots, we carried out a thoroughly genome-wide investigation of the gene family in this work. Using in silico techniques, PBS3 gene family members were identified inside the carrot genome. The PBS3 gene family's reaction to particular stimuli or infections was then examined using gene expression analysis. The study also look round to investigate the evolutionary linkages and possible regulatory mechanisms of the PBS3 gene family in carrots. Our study used a multifaced strategy to investigate the genetic, evolutionary, and regulatory features of the PBS3 gene family in carrots. We did this by exploiting methods like genome mining, gene expression analysis and phylogenetic investigations. The results of this work advance our knowledge of the genetic and phenotypic characteristics of the PBS3 gene family in carrots, which may have ramifications for the creation of better cultivar types and approaches to disease prevention.*

Keywords*:* *Gene expression pattern; genome-wide analysis; Carrot plant; transcription factor; PBS3 gene family*

Introduction

Gretchen Hegan 3 (GH3) also known as PBS3 (Mackelprang 2017), is a zinc finger family protein of the C2H2-type that is essential for plant disease resistance, signalling, growth, and maturity. It is the enzyme that limits the stride at which the final two stages of the production of salicylic acid (SA) cause plants to age (Poór 2020). After much research in Arabidopsis thaliana, the PBS3 gene was shown to be the most important enzyme in the SA production course (Torrens-Spence et al. 2019). PBS3 mutations diminish SA accumulation overall, SA-dependent gene expression, and plant defence in Arabidopsis thaliana (Nobuta et al. 2007). PBS3 is involved in more than SA biosynthesis; it serves purposes other than resistance to illness (Seguel et al. 2018). It may convert amino acids into 4-substituted benzoates, which can then be used to produce pABA-Glu, 4HBA-Glu, or other similar chemical compounds (Okrent et al. 2009). In addition to its involvement in disease-resistance signalling, PBS3 is necessary for the build-up of salicylic acid and the induction of defence mechanisms (Tyagi, et al. 2022). The PBS3 gene is linked to disease resistance and plant ability to respond to particular stimuli or infections (Pottinger and Innes 2020). It has been found in several plant species, including Theobroma, cacao, and carrots. PBS3 is an all-around adaptable participant in plant biology, serving purposes other than disease resistance and SA biosynthesis.

Carrots have been shown to manifest genetic variety and perhaps health-promoting qualities through genome-wide research, which has highlighted their importance in agriculture and human nutrition (Surbhi et al. 2018). Improved carrot varieties have been developed as a result of the genomic research on carrots, which has shown the genetic basis of characteristics including flavour, colour, and disease resistance (Que et al. 2019). Additionally, international cooperation and breeding efforts to improve the nutritional quality, yield, and adaptability of carrots have been made easier by the genetic

variations discovered through genome-wide studies and the global distribution of carrot germplasm, which addresses the challenges posed by a changing climate as well as the diverse needs of consumers.

Nematodes, carrot weevils, and aphids are just a few of the insect pests that can harm carrot crops, causing damage to the leaves and roots that will obstruct growth and result in decreased yield (Kunjwal and Srivastava 2018). Furthermore, carrots are susceptible to damage from viral infections, bacterial blight, and fungal leaf spots which can cause wilting, tissue necrosis, and reduced plant vigour (du Toit et al. 2019). Carrot growth and development can also be adversely affected by severe temperatures such as heat or frost as well as by drought or abnormally high precipitation (Pathak, Barik et al. 2021). Toxins and resin flow are produced by the plant as a defence mechanism against pests and diseases, which can be triggered by biotic and abiotic stimuli (Fürstenberg-Hägg et al. 2013). Carrots have a complex defence mechanism to shield against both biotic and abiotic stresses (Selvakumar and Kalia 2022). Physical defences like cuticles, cell walls, and trichomes act as a first line of defence, keeping pathogens out and insects from feeding on them (Chaudhary et al. 2018). Carrots use inducible defence mechanisms like Induced Systemic Resistance (ISR) to boost resistance to infections by producing signalling molecules like salicylic acid (SA) and jasmonic acid (JA) in response to biotic stress signals (Arnason and Bernards 2010). Additionally, carrots include pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) and activate PAMP-triggered immunity (PTI) in response to the detection of conserved microbial compounds (Nürnberger and Kemmerling 2009).

In response to biotic stresses like an infection through pathogen or herbivore assault, PBS3 gene expression is frequently upregulated (Barah et al. 2013). When carrots detect chemicals originating from pathogens or damage-associated molecular patterns (DAMPs) from herbivores, they trigger signalling pathways that cause PBS3 expression to increase (Tom et al.). The PBS3 protein may then influence the amounts of phytohormones like salicylic acid (SA) and jasmonic acid (JA) to take part in defence signalling cascades (Pokotylo et al. 2019). These phytohormones play a crucial role in regulating plant immunity by coordinating defence mechanisms against herbivores and pathogens (Checker, Kushwaha et al. 2018). Furthermore, the PBS3 gene could contribute to tolerance to abiotic stressors like drought, high temperatures, and salt in the soil (Berens, Wolinska et al. 2019). Carrots stress response systems may cause them to upregulate PBS3 expression under unfavourable environmental conditions (Salopek-Sondi et al. 2017). To control the production of genes that respond to stress and lessen the negative impacts of external stresses on plant development and growth, the PBS3 protein may play a role in stress signalling pathways (Khan et al. 2023). PBS3's capacity to control phytohormone levels is one of its primary roles in stress reactions (Li et al. 2023). PBS3 proteins, for instance, can conjugate amino acids, such as SA (salicylic acid)and JA(jasmonic acid), to other molecules through their enzymatic activity (Okrent, Brooks et al. 2009). These phytohormone's stability and activity are changed throughout the conjugation process, which affects downstream signalling pathways that are engaged in defence reactions (Ma and Ma 2016). PBS3 maintains the proper balance between SA and JA signalling, which helps plants adapt their immune systems to various stresses (Li et al. 2023).

Materials and Method

Database and retrieval sequencing

The PBS3 domain (NP_001330075.1) was found in the peptide sequences that were located using the NCBI database (https://www.ncbi.nlm.nih.gov/) (Nobuta et al., 2007). Using the BLAST-P (Ewens and Grant 2005) represent protein-basic local alignment search tool, this domain (NP_001330075.1) was utilized to further identify PBS3 genes in the genome database of Daucus carota v2.0 at (https://phytozome-next.jgi.doe.gov) Phytozome. After verifying amino acid obtained sequences using motif finder (https://www.genome.jp/tools/motif/), conserved domains were searched using NCBI CDD (Conserved Domain Database)(http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The domain sequences of the PBS3 protein domain do not contain GH3 (Nobuta et al., 2007).

Identification of physical and chemical properties of the protein in PBS3

In the (https://web.expasy.org/protparam/) online program the weight, length, and pI-value (acidity level) of proteins were estimated using Protparam (Bhattacharya, Hota et al. 2018). Obtained details on the genes from the Phytozome v13 database, including their sequences, IDs, and locations on chromosomes (Chen, Wang et al. 2022). Then the TcPBS3 genes have new names according to their chromosomal locations. Then made use of the WoLF PSORT tool (https://wolfpsort.hgc.jp/) to predict the potential subcellular localization of PBS3 protein (Abdala, Rivero et al. 2023).

Intron-Exon gene structure and Motif Analysis

The coding and genomic sequences (CDS) for the PBS3 genes were obtained from Phytozome v13 to determine the exon/intro n organization. Additionally, the Phytozome genome database (https://phytozome-next.jgi.doe.gov) provided the Daucus carota genome's gff3 file (Sreekumar, Muhammed Sadiq et al. 2022). Gene Structure Display Server (GSDS v2.0)

visualization provided by these sequences (http://gsds.cbi.pku.edu.cn/) (Liu, Zhang et al. 2022).

Comparative Phylogenetic Analysis

Using MEGA11's MUSCLE feature, the peptide sequences of many species including PBS3 were recovered from the alignment of the carrot genome. PBS3 protein sequences from *Daucus carota, Oryza sativa L., Zea mays L., Sorghum bicolor L., Gossypium hirsutum L., Helianthus annuus, Glycine max L., beta vulgaris L., Campestris sinensis, Arachis hypogaea L., Triticum aestivum L., and Brassica oleracea L.* were utilized in a neighbour joining (NJ) approach bootstrapping set of 1000 replications to create a phylogenetic tree using MEGA 11. The phylogenetic tree was displayed using the iTOL website (https://itol.embl.de/upload.cgi) (Letunic and Bork 2021).

Cis-Regulatory Elements Analysis and MEME Analysis

1000-base pair upstream promoter sequences were obtained from the PBS3 domain's BLAST findings in Phytozome V.13 to explore cis-regulatory elements. Then, cis-regulatory elements in these extracted sequences were found using the plant CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). By using the MEME suite of programs, motifs with a significant value of 13 were found (Bailey et al. 2009). The PBS3 gene protein sequences were then analyzed using TBtools to visualize these motifs (http://meme.nbcr.net/meme/) (Zou et al. 2022).

Gene Expression Analysis

After MEME analysis we have to examine the reaction of the PBS3 gene family to different stress situations in carrot plants, two different carrot cultivars were chosen for the inquiry (Pandey 2017). The NCBI (https://www.ncbi.nlm.nih.gov/geo/) Gene Expression Omnibus (GEO) database (PMID: 30739243) made it easier to obtain the data. In particular, carrot leaf samples subjected to various stress treatments such as pathogen injection and abiotic stressors were used to collect the RNA-seq data (Jia et al. 2024). Four distinct time intervals were represented by the 16 subcategories that arose from the PBS3 gene family expression patterns in carrot genotypes: 0 hours, 6 hours, 24 hours, and 72 hours post-treatment. There were two duplicates of each time for the cultivar variants, for a total of 16 experimental situations (Dorman et al. 2003). It is interesting to note that leaf samples were obtained for RNA extraction and subsequent gene expression analysis at separate times even though the treatments were administered concurrently (Vranová et al. 2002). A pairwise comparison strategy was used to clarify how stress treatments affected the expression of the PBS3 gene (Okrent and Wildermuth 2011). The goal of this research was to pinpoint the genes that showed differential regulation that is up or down-regulation across the various treatments and time intervals (AshaRani et al. 2012). Utilizing statistical tools like Statistix 8.1, the acquired gene expression data were further examined to gauge the importance of the observed expression patterns and pinpoint putative regulatory mechanisms connected to PBS3 gene expression in carrots.

Chromosomal Mapping, Duplication of Genes and Synteny Analysis

Using TBtools software, MUSCLE protein sequence alignment, and computed Ka/Ks values, the temporal divergence of PBS3 genes was as certain (Xu et al. 2023). The molecular evolution rate was determined using the Ka/Ks ratios of paralogous genes (Wang, Liu et al. 2011). By entering the Ks value into the formula T=Ks/2 λ—where λ is comparable to 6.5 × 10−9 × 10−6—the divergence time (T) was calculated (Wu, Guo et al. 2022). TBtools is used to create syntenic maps connecting paralogous genes in carrots and orthologous genes in Arabidopsis, tomato, and rice and MCScanX to analyze gene duplication occurrences, the study was able to uncover Synteny links and produce both syntenic and dual syntenic relationships (Sun et al. 2018).

Enrichment analysis of Gene ontology

The PBS3 genes' subsequent activities use gene ontology annotation enrichment analysis (Raza, Khan et al. 2022). Database online (https://www.uniprot.org/) Uniprot is used to look for molecular functions and biological processes (Consortium 2019). On the other hand, Shiny Go v0.75, an online resource, is useful for comprehending the functions of PBS3 genes (http://bioinformatics.sdstate.edu/go/) in carrots.

Analyzing miRNAs

Using CDS sequences for every PBS3 gene, psRNATarget was utilised to identify micro-RNA (miRNA) sequences associated with PBS3 genes in carrots. The identified miRNAs' putative functions were inferred from earlier Insilco studies (Hanif, Farooq et al. 2018).

Results

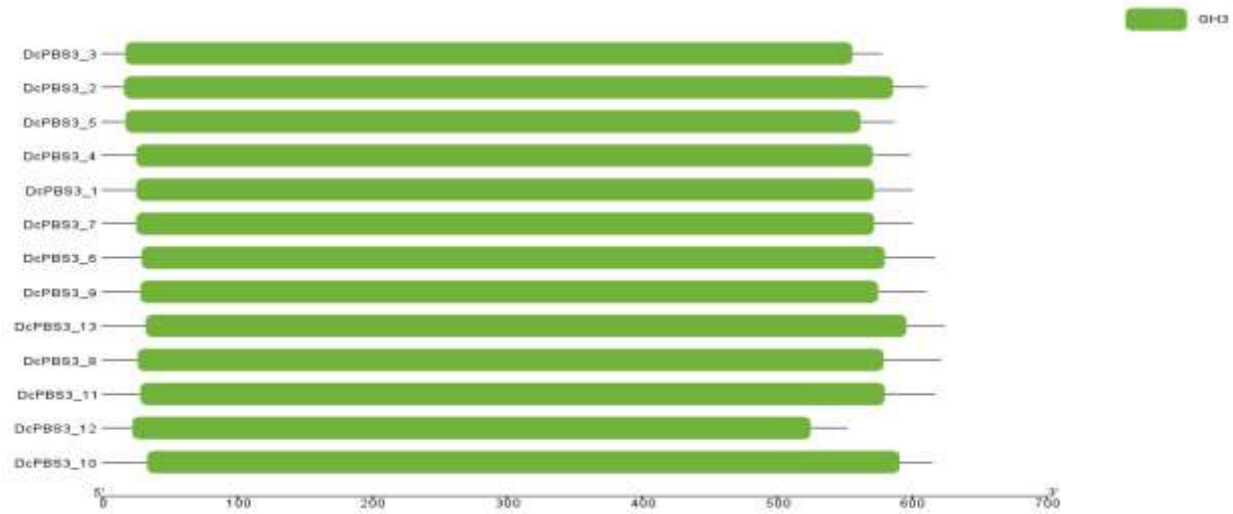
Gene Identification in Daucus carota: The PBS3 domain sequence was compared to the whole genome sequences of Daucus carota v2.0 from Phytozome using BLAST analysis to identify DcPBS3 genes. All the Thirteen DcPBS3 genes were discovered during the BLAST-p analysis and were all stored for additional investigation. DcPBS3_12 had the lowest amino acid residue count and DcPBS3_13 had the highest with a total length of 552–624 amino acid residues. The PBS3 domain sequences in Daucus carota v2.0 have a molecular weight ranging from 62096.12 to 70053.97 KD. The discovered proteins' isoelectric point (pI) values varied from 5.36 to 7.82, and the GRAVY values attested to each protein's hydrophilic nature.

Table 1. Information about the discovered thirteen PBS3 genes in carrot genome

Transcript id		Gene name	Chromosome number	Location		Strand	Size AA		Mol. Weight	no. of Amino Acid	pi	Gravy	No. of intron & exons	Sub Cellular localization	description
Tbtool ID	Phytozome ID			Start position	End position		mRNA (CDS)	Protien Length							
DCAR_003611|DCAR_003611	DCAR_003611			40617661	40619733		1800	600							
DCAR_002811|DCAR_002811	DCAR_002811	DcPBS3_1	1	33095824	33098454	reverse	1830	610	68026.82	599	5.97	-0.301	2:03	Nuclear	hypothetical protein
DCAR_025240|DCAR_025240	DCAR_025240	DcPBS3_2	1	25091856	25094589	reverse	1734	578	68999.49	609	6.04	-0.206	3:04	Nuclear	hypothetical protein
DCAR_025768|DCAR_025768	DCAR_025768	DcPBS3_3	7	30435660	30437673	forward	1797	599	64817.37	577	5.8	-0.132	3:04	cyto and p. membrane	hypothetical protein
DCAR_006967|DCAR_006967	DCAR_006967	DcPBS3_4	7	30394572	30397983	forward	1761	587	67691.39	598	5.36	-0.218	2:03	p. membrane	hypothetical protein
DCAR_010005|DCAR_010005	DCAR_010005	DcPBS3_5	2	13706929	13708962	forward	1851	617	65847.3	586	5.74	-0.197	2:03	cyto and p. membrane	hypothetical protein
DCAR_010846|DCAR_010846	DCAR_010846	DcPBS3_6	3	29459649	29461656	forward	1800	600	69654.94	616	7.82	-0.254	2:03	nucl and p. membrane	hypothetical protein
DCAR_008684|DCAR_008684	DCAR_008684	DcPBS3_7	3	158712	160800	reverse	1863	621	67723.02	599	5.52	-0.31	2:03	nucl and p. membrane	hypothetical protein
DCAR_019202|DCAR_019202	DCAR_019202	DcPBS3_8	3	37061722	37063789	reverse	1830	610	70053.97	620	5.88	-0.276	2:03	nucl and p. membrane	hypothetical protein
DCAR_012993|DCAR_012993	DCAR_012993	DcPBS3_9	5	33710913	33713837	forward	1845	615	69167.93	609	5.67	-0.28	2:03	nuclear	hypothetical protein
DCAR_022500|DCAR_022500	DCAR_022500	DcPBS3_10	4	11504548	11506665	forward	1851	617	68876.23	614	5.89	-0.279	3:04	extracellular	hypothetical protein
DCAR_023148|DCAR_023148	DCAR_023148	DcPBS3_11	6	1214940	1217477	reverse	1656	552	69443.41	616	5.65	-0.241	2:03	nuclear	hypothetical protein
DCAR_023147|DCAR_023147	DCAR_023147	DcPBS3_12	6	1219031	1221099	forward	1872	624	62096.12	551	5.69	-0.198	3:04	p. membrane	hypothetical protein

Gene Structure and Conserved Motifs

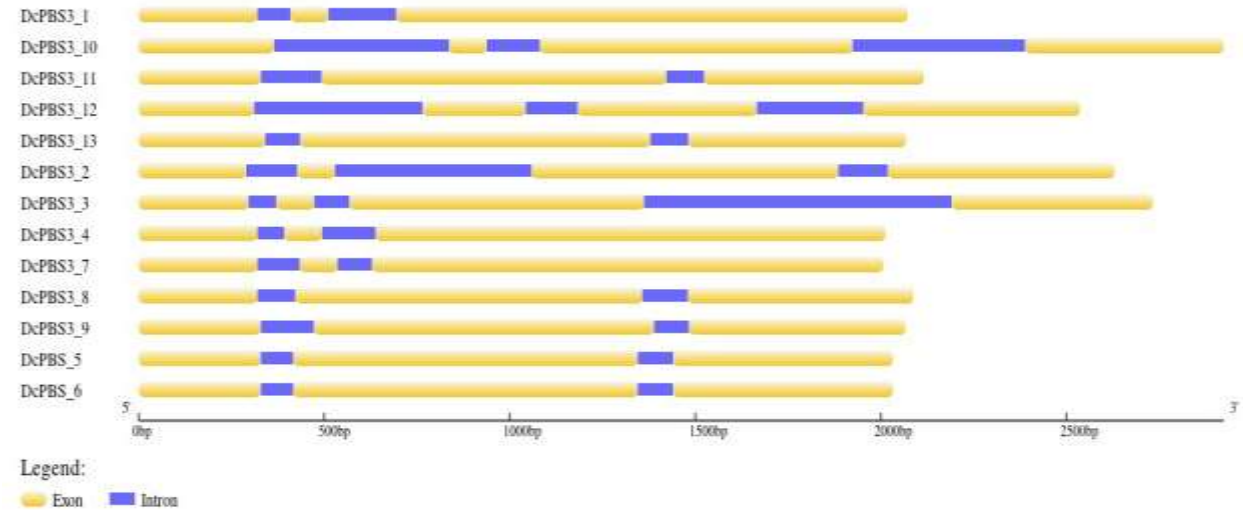
The number of introns varies between Daucus carota DcPBS3 genes, according to an analysis of intron-exon architectures. There were two to three introns, with the most common number being four (3.04%)(Zhang 2000). The phylogenetic analysis was in line with the intron and exon distribution (Donnelly et al. 1999). Daucus carota PBS3 protein sequences were found to include 13 motifs by MEME software, with each DcPBS3 gene having the same PBS3 encoding domain. The results of motif analysis showed that the same set of genes had the same motifs, highlighting the significance of conserved motifs in certain roles within a group or subgroup (Du et al. 2012). As seen in Figure 1, all 13 motifs identified in the protein sequences of the Daucus carota PBS3 genes were evaluated using the MEME technique in TBtools. All of the DcPBS3 proteins were found to contain the PBS3 domain sequence. Interestingly, DcPBS3 genes from the same group showed similar patterns, indicating that these conserved motifs are important for particular behaviors within a group or subgroup. All 13 DcPBS3 genes were found to have the same PBS3 encoding domain.


GH3
DcPBS3_3
DcPBS3_2
DcPBS3_5
DcPBS3_4
DcPBS3_1
DcPBS3_7
DcPBS3_6
DcPBS3_9
DcPBS3_13
DcPBS3_8
DcPBS3_11
DcPBS3_12
DcPBS3_10
5'
0
100
200
300
400
500
600
700
3'

A.


DcPBS3_1
DcPBS3_2
DcPBS3_4
DcPBS3_6
DcPBS3_7
DcPBS3_8
DcPBS3_9
DcPBS3_11
DcPBS3_12
DcPBS3_13
DcPBS3_3
DcPBS3_5
DcPBS3_10
5'
0
100
200
300
400
500
600
700
3'
Motif 9
Motif 5
Motif 14
Motif 6
Motif 3
Motif 15
Motif 4
Motif 8
Motif 10
Motif 11
Motif 1
Motif 7
Motif 13
Motif 2
Motif 12

B.


DcPBS3_1
DcPBS3_10
DcPBS3_11
DcPBS3_12
DcPBS3_13
DcPBS3_2
DcPBS3_3
DcPBS3_4
DcPBS3_7
DcPBS3_8
DcPBS3_9
DcPBS_5
DcPBS_6
5'
0bp
500bp
1000bp
1500bp
2000bp
2500bp
3'
Legend:
Exon
Intron

C.

D.

Figure 1 (a) intron and exon analysis showing (b) cis-regulatory elements (c) meme analysis (d) NCBI CDD analysis

Phylogenetic Analysis for genes family of PBS3

To investigate the evolutionary connections between *D. carota*, *O. sativa*, *A. thaliana*, *Z. mays*, *S. bicolor*, *H. annuus*, *G. hirsutum*, *G. max*, *C. sinensis*, *A. hypogaea*, *T. aestivum*, and *B. oleracea*, a Neighbor-joining (NJ) phylogenetic tree was created (Figure 2) (Hancock 2012). Eighty PBS3 protein sequences were included in three subgroups (PBS3_1, PBS3_2, and PBS3_3) as shown by the tree (Okrent, Brooks et al. 2009). Four DcPBS3 genes were found in the clade PBS3_1, three in PBS3_2, and six in PBS3_3. Proteins from the same phylogeny were shown to have likely functional similarities when grouped into groups.

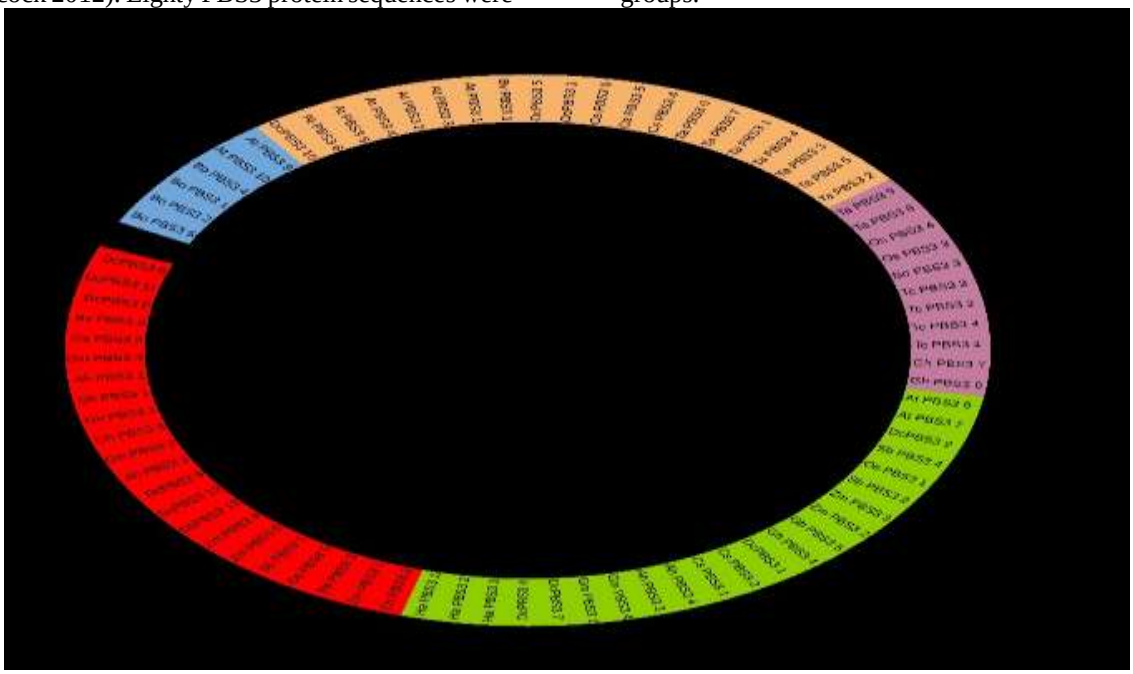


Figure 2. Phylogenetic tree analysis

Sub Cellular Localization Analysis

Daucus carota's PBS3 genes were analyzed, and the results revealed that DcPBS3 is highly expressed in the plasma membrane and cytoplasm. Other genes, DcPBS3_1, DcPBS3_2, DcPBS3_9, and

DcPBS3_10, expressed themselves in the nucleus and extracellular (Figure 3).

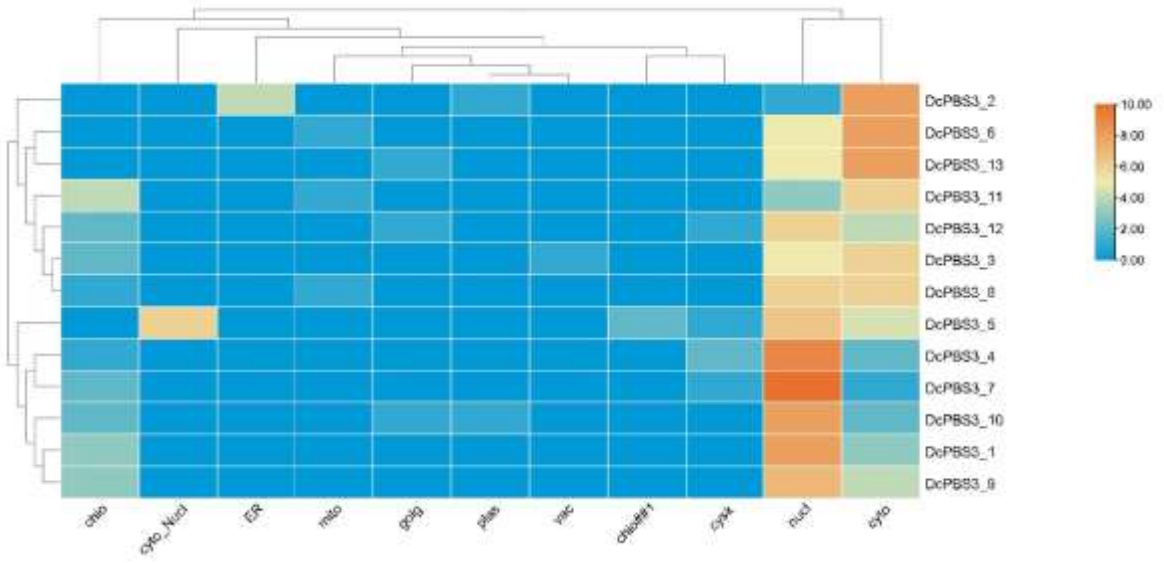


Figure 3 Subcellular analysis

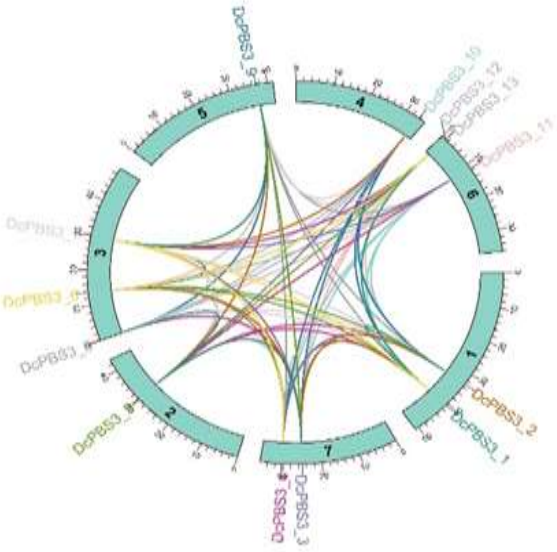
Cis-regulatory Elements (CREs) Analysis

The PLANT CARE database revealed several cis-regulatory elements inside the promoter regions of PBS3 genes that are essential for the initiation of gene transcription (Zou et al. 2022). A total of 535 cis-elements were discovered during an analysis of the promoter regions of TcPBS3 genes. These elements excluded both unknown functional elements and common elements like the TATA- and CAAT-box (Dolfini et al. 2009). These distinct cis-regulatory components are connected to stress induction, phytohormone responses, growth, and development (Saidi and Hajibarat 2019). For example, a crucial promoter element required for the start of transcription is the TATA-box (Wiley et al. 1992). The jasmonic acid signalling pathway and genes involved in defensive responses are regulated by the TGACG and CGTCA motifs (Baruah et al. 2020). While the CAAT-box enhances transcription efficiency, they ARE directs genes linked to growth and development through its auxin-responsive element (Tungngoen et al. 2011). Gene regulation is influenced by transcription factors, such as MYC and MYB motifs (Martin and Paz-Ares 1997). G-box controls light and stress responses, whereas AE-box links auxin signalling to growth. As-1 contributes to the protection that is mediated by jasmine and salicylic acid. Whereas ERE and ethylene are associated with fruit ripening and senescence, ABRE stands for ABA-responsive components (Pilati et al. 2017). The TCA element plays a crucial role in salicylic acid-mediated pathogen defence (Saleem et al. 2021). Genes connected to gibberellin-related growth are regulated by the TGA-element and GARE-motif; genes responding to light and stress are regulated by Box II, Box 4, and ACE (Akram, Siddique et al. 2023). W box and WUN-motif are involved in salicylic signalling. These patterns play a crucial role in how different genes express themselves in plants in response to specific hormonal cues (Yang, Ma et al. 2021). Figure 1 displays cis-regulatory elements linked to important physiological processes, such as stress responses, circadian control, light responsiveness, proliferative gene expression, and plant growth (Covington, Maloof et al. 2008). 254 cis-elements in PBS3 genes were linked to the light response, 20 to gibberellin responsiveness, 7 to stress response, 7 MYB binding sites linked to drought-inducibility, 25 to anaerobic induction, plant development, and 9 to salicylic acid response, according to a cis-regulatory analysis. Furthermore, 53 were connected to the reactions to auxin and MeJA, 77 to the ABA response, circadian control, and regulation of zein metabolism, respectively, and the remaining components were related to the responses to low temperatures, endosperm expression, and cis-regulation (Zhuang et al. 2024).

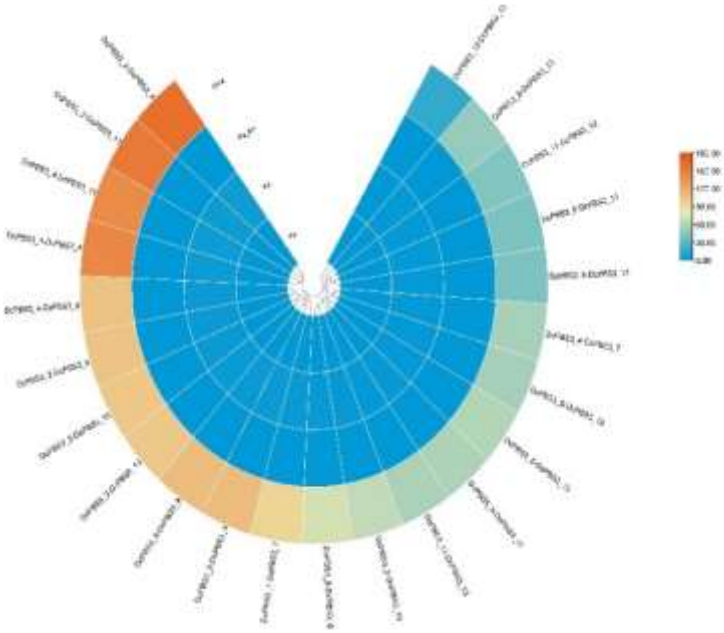
Chromosomal Location and Evolutionary Background

PBS3 gene synteny analysis, gene duplication, and chromosomal locations were examined (Figure 4ABC). It was shown that chromosomes 1, 2, 3, 4, 5, 6, and 7 have PBS3 genes (Okrent, Brooks et al. 2009). The largest number is found on chromosomes 6 and 5, which correspond to DcPBS3_11 to DcPBS3_13 and DcPBS3_6 to DcPBS3_8. The ratio of Ka to Ks represents the equilibrium between advantageous and harmful mutations inside a gene (Lu, Tang et al. 2006). This is calculated by dividing the total number of synonymous substitutions (Ks) by the total number of nonsynonymous substitutions (Ka) per nonsynonymous site (Comeron 1995). A

score greater than one indicates that certain adjustments are advantageous (Brown et al. 2014). The ratio of Ka/Ks indicates the level of evolutionary constraint when favorable mutations are rare (Wang et al. 2011). Both segmental and tandem duplications of PBS3 gene pairs had Ka/Ks ratios that were above 1, indicating evolutionary pressure from purifying selection (Song, Wang et al. 2016). Chromosomes 1 and 7 share two genes, according to an analysis of gene duplication patterns in carrots but chromosomes 3 and 6 show many instances of gene duplication (Wang, Liu et al. 2011). PBS3 gene pairs arising from duplications in carrots appear to be under purifying selection. According to the Ka/Ks ratio, a measure of evolutionary pressures on genes, with Ka/Ks values continuously above 1. Extensive analysis reveals intriguing results on the synteny links among PBS3 genes in carrots (Okrent, Brooks et al. 2009). Synteny analysis, for example reveals evidence of tandem duplication events with PBS3 genes on chromosome 6 and segmental duplication with PBS3 genes on chromosome 3 (Okrent, Brooks et al. 2009). These findings demonstrate how the PBS3 gene family in carrots has been shaped by intricate genetic interactions and an intricate evolutionary history (Okrent, Brooks et al. 2009). Research on comparative synteny using model organisms such as Arabidopsis thaliana, Solanum lycopersicum (tomato) and Oryza sativa (rice) has shown that carrot and these crops share both orthologous and paralogous gene pairs (Iorizzo, Ellison et al. 2016). The evolutionary relevance and functional conservation of PBS3 genes in carrots are highlighted by the discovery of conserved gene connections across species (Szczepaniak, Książkiewicz et al. 2018).



A.



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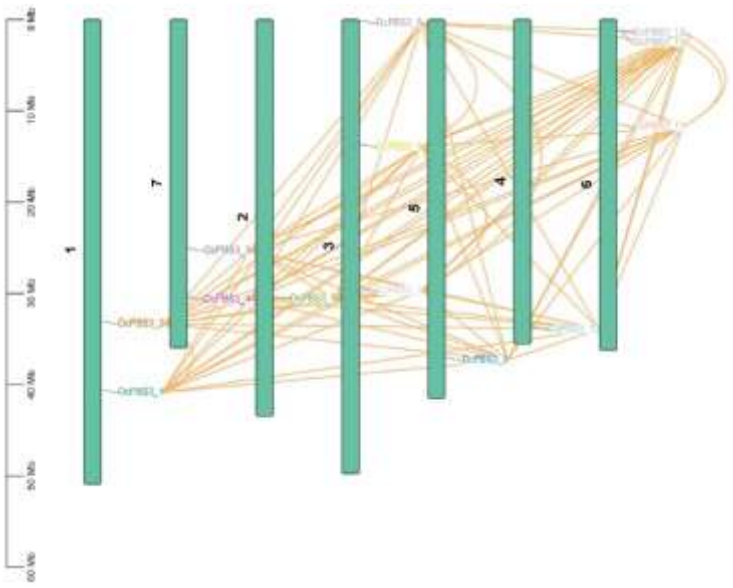

C.

Figure 4ABC. Chromosome location mapping

GO annotation

We determined the involvement of each PBS3 protein across biological processes, molecular activities and cellular components using Gene Ontology (GO) categories (Figure 5) (Henegar, Tordjman et al. 2008). The great majority of proteins in biological processes were associated with processes involving response to stimuli (GO: 0050896) (Huang et al. 2015). In terms of molecular activities, the enriched phrases were: forming carbon-nitrogen bonds (GO: 0016879), catalytic activity (GO: 0003824), acid-amino acid ligase activity (GO:0016881), and ligase activity (Din et al. 2018). Within the category of cellular components, the most often enriched category was

found to be cytoplasm (GO: 0005737) (Watson et al. 2022). These results demonstrate the many functions of PBS3 proteins in the metabolism of cells (Li et al. 2021).

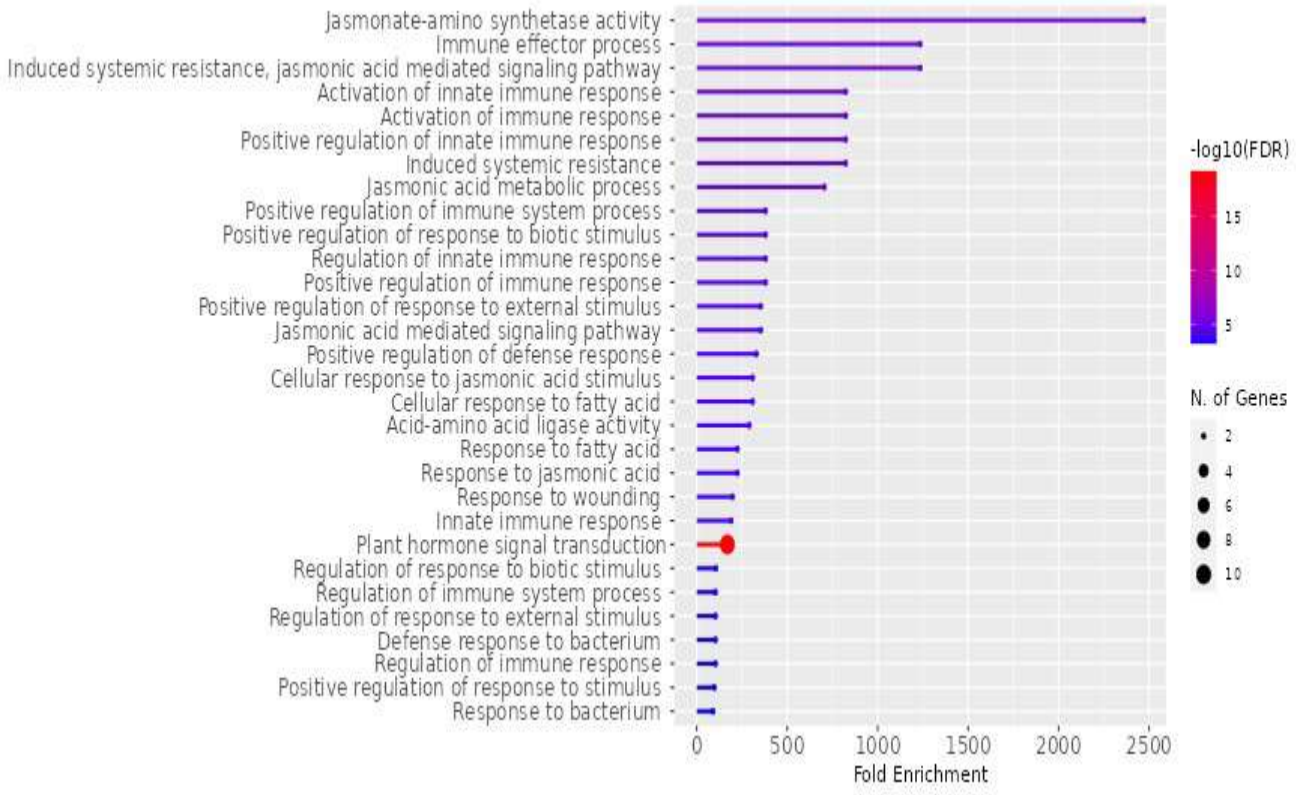

Figure 5 Gene ontology analysis

miRNA targets in Daucus carota

Mature miRNA sequences that target the DcPBS3 gene were obtained via the web tool psRNATarget (https://plantgrn.noble.org/psRNATarget/analysis). The DcPBS3 genes' CDS sequences were utilized to identify the miRNA targets.

Discussion

Research on the DcPBS3 gene family within Daucus carota has been noticeably restricted in comparison to other organisms, although the PBS3 gene family has seen substantial bioinformatics analysis in a modest number of species in recent years. Our ability to fully comprehend this gene family in the unique setting of this plant species is challenged by the information gap around PBS3 in D. carrot (Pandey 2017). Research that focused on thirteen genes from the Daucus carota genome examined the physicochemical characteristics of those genes to find differences across proteins in the same clade (Li et al. 2021). Every single one of the discovered DcPBS3 proteins was hydrophilic, as shown by negative GRAVY values, which indicated a preference for interacting with water and having net electrical charges at different pH levels. Subcellular localization studies revealed that DcPBS3 proteins are distributed across many organelles, such as the mitochondria, cytoplasm, nucleus, plasma membrane, and extracellular space. Interestingly, more than 80.5% of these proteins were found in the nucleus and plasma membrane, indicating that DcPBS3 proteins may play important roles in the operation of these organelles.

We created a phylogenetic tree comprising PBS3 genes from *D. carota, O. sativa, A. thaliana, Z. mays, S. bicolor, H. annuus, G. hirsutum, G. max, C. sinensis, A. hypogaea, T. aestivum, B. vulgaris and B. oleracea* to gain a thorough knowledge of the connection between DcPBS3 genes found in Daucus carota. Based on the DcPBS3 genes that are present in the tree, we separated all of the genes into three groups, designated PBS3_1, PBS3_2, and PBS3_3. We argue that Daucus carota genes may have the same roles as their homologs in Arabidopsis since genes in the same group typically exhibit comparable structures and functions (Liu et al. 2020). After doing a structural examination of the DcPBS3 genes, it was discovered that whereas DcPBS3_2, DcPBS3_3, DcPBS3_10, and DcPBS3_12 have three introns and four exons while DcPBS3_1, DcPBS3_4, DcPBS3_5, DcPBS3_6, DcPBS3_7, DcPBS3_8 and DcPBS3_9 have two introns and three exons. Sequencing analysis was conducted to uncover conserved regions shared by all DcPBS3 genes to delve more into this finding. Thirteen tissue-specific amino acids (DcPBS3) have been found in carrots, a crop of economic significance

that supports 40–50 million farmers. In plant growth, function control, and response to biotic stressors, the PBS3 family is essential (Li et al. 2021). Thirteen PBS3 genes are found in the study and they are unevenly spread across seven chromosomes (Nicholson et al. 2015). The PBS3 and GH3-family domains are conserved in the low molecular weight PBS3 proteins (Nobuta, Okrent et al. 2007). The study looks at how PBS3 genes affect the growth of carrots and how they defend against biotic stressors (Prajapati et al. 2015). Based on their structure and regulatory mechanisms, DcPBS3 are varied, according to the research, suggesting that they are involved in a variety of cellular processes linked to stress responses and development.

The research might lead to the development of fungus-resistant cultivars that increase carrot yield (Baranski et al. 2007). Using GO-annotations, we were able to decipher the function of the DcPBS3 genes and discover that they operate similarly to their orthologous in *A. thaliana*. However, we have performed cis-element analysis on the corresponding genes to learn more about the gene structure and function (Wittkopp and Kalay 2012). Cis-regulatory elements play a crucial role in the binding of transcription factors to their specific target sites, which aids in the control of gene expression (Biłas et al. 2016). Since light responsiveness, plant growth, circadian regulation, proliferative gene expression, and stress-related phenomena were all detected, we discovered that all DcPBS3 genes had cis regions associated with light responsiveness. One may argue that photosynthesis and the expression of these genes are related (Pfannschmidt 2003). Nevertheless, further research is required to precisely determine the specific purpose of these cis components (Hernandez-Garcia and Finer 2014). According to structural analysis, homologous groups inferred from phylogeny could have similar or related roles to those of the corresponding A.thaliana member (Lijavetzky et al. 2003).

Genome comparisons between animals provide important insights into the structure and evolution of genes (Yandell et al. 2006). Transferring genetic information from well-researched species to less-studied ones is much easier with the use of this comparative approach (Hewitt 2004). Gene duplication is largely a result of evolutionary modifications. Genes on different chromosomes reflect a segmented duplication process, whereas duplicates on the same chromosome suggest tandem duplication (Panchy, Lehti-Shiu et al. 2016). Our study identified several pairs of DcPBS3 genes that, based on their placement on different chromosomes, most likely resulted from segmental duplication. The predicted timing of divergence or duplication in the homologs was revealed by estimating the Ka/Ks ratio for the predicted duplicated genes in Daucus carota using the λ value ($\lambda = 6.5 \times 10{-}9) \times 10{-}6$ (Baloglu et al. 2014). MicroRNAs (miRNAs) serve as key regulatory elements in plants, impacting different biological processes including plant growth, development, and responses to both biotic and abiotic challenges (Lima et al. 2012). Highly conserved and specialized in function, miRNAs play a vital part in the complicated regulatory networks of plants (Samad et al. 2017).

Conclusion

In conclusion, this work investigates the Daucus carota TcPBS3 genes. To comprehend their evolution, function, and possible regulatory mechanisms, it employs a multi-pronged approach that includes phylogenetic analysis, gene structure evaluation, cis-element identification, and miRNA prediction. The results show structural complexity, light-driven expression, extensive gene duplication, evolutionary variety, and precisely controlled regulation by miRNAs (Alberts 2017). Within the DcPBS3 family, the study identified many clades, indicating functional diversity and possible functional specialization. Light-responsive cis-elements provide support for possible photosynthetic activities (Asayama 2006). The study also discovered that several miRNA families target the DcPBS3 genes, indicating their possible importance in stress responses, cell division, and plant development and that numerous paralogous genes imply previous duplication occurrences.

Future Aspects

Improved carrot production, stress tolerance, and nutritional content may result from research on the DcPBS3 gene. Through the application of CRISPR-Cas9 technology, researchers may manipulate certain genes to gain insight into their regulatory networks and potential for adaptation (Razzaq et al. 2021). By using this in precision breeding, cocoa cultivars with improved attributes can be created (Wickramasuriya and Dunwell 2018). To lessen dependency on chemical inputs, the project also intends to generate carrot plants that can withstand stress (Shukla et al. 2019). By comprehending the interactions between DcPBS3 genes and advantageous soil bacteria, microbiome management techniques that improve soil fertility and carrot plant health may be developed. Additionally, this research can help develop sustainable farming methods that maximize carrot yield while reducing their negative effects on the environment (Thorup-Kristensen et al. 2012).

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Declaration

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Authors' Contribution

AR: Originated the study idea, proposed the method for the research, and oversaw the entire research endeavor. **MA**: She analyzed the data, made interpretation of the results and was involved in the preparation of the manuscript. **AA**: Participated in the literature search, data gathering and review and writing of the manuscript. **AB, AR**: With regard to the above analysis, she played the major role in data collection, contributed to data analysis and interpretation, and finalized the manuscript for overall ideas and contents. **AR**: Assisted in the conception, data analysis and drafting of the manuscript so as to maintain cogency within the study.

Conflict of Interest

The authors state that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial or personal manner concerning the development of this project, the gathering of data, the interpretation of the data, or the writing and publishing of this paper.

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All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable