EVALUATION OF DIFFERNTIALLY EXPRESSED TRANSCRIPTS OF CANOLA (BRASSICA NAPUS L.) GROWN UNDER SALT STRESS

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ABSTRACT

Objective

For improved understanding of stress mechanism and machinery, analysis of regulatory pathways of multiple transcripts (i.e., non-coding RNAs) and salt stress-responsive genes is a vital need.

Methodology

In the current study, seeds of two cultivars of *Brassica napus* Dunkled and Cycloned were used in completely randomized design with three replicates. Plants were categorized into two S1 and So treatments of NaCl. Growth parameters of plants were measured, and effects of salinity were observed. Transcriptome analysis was carried out after isolation of total RNA by analyzing protein coding 10 upregulated and 10 downregulated genes on NCBI, KEGG, UniProt, Interpro. Phylogenetic analysis of genes was done by using different analytical software i.e., NCBI BLAST, MUSCLE for alignment, BioEdit analytical tool for editing the sequences and MEGA7 was used for phylogenetic tree to describe the ancestral relationship of plant genes with other identified genes.

Results

Results showed that the CRM-domain protein was involved in the mitochondrial splicing introns, which was ultimately very crucial for growth and stress responses of plants; and, for all stress reducing mitochondrial functions.

Conclusion

According to the results, functions of selected upregulated and downregulated genes of *Brassica napus* were predicted.

Keywords: Canola, non-coding RNAs, Brassica napus, salt stress.

INTRODUCTION

Area-wise, Pakistan is the 8th country that is affected with salinity (Khan *et al*., 2017). Every year, it has been estimated that 10 million hectares of agricultural land throughout the globe are destroyed by salinity (Machado and Serralheiro, 2017). Salinity is also dominantly disturbing the ecological environment causing degradation to the fertile soil specifically to highly irrigated lands globally (Sohaib AU et al., 2023) (Akram *et al*., 2017: Kumar *et al*., 2017).

Studies have revealed how plants sense and respond to abiotic stresses through the various physiological and metabolic processes (. This study helps to understand the coping mechanisms of plants against stresses and to plan strategies to develop salt-tolerant plants (Munns *et al*., 2016). When plants are found-out to salt stress their epidermal cells and membrane proteins at root hairs sense the extent of salt stress and send signals to plant parts and the salt stress-responsive genes express themselves and instantly triggers the related biochemical pathways, ultimately balancing the changes in CO_2 assimilation, growth regulatory hormones ion homeostasis and detoxification of RO Species, (Hasegawa *et al*., 2002; Munns and Tester, 2008). Saltoverly sensitive (SOS) pathway genes and their transporters have been identified, which are found to be associated with calcium-binding proteins and control ionic homeostasis under salt stress (Ishitani *et al*., 2000, Ismail *et al*., 2017). Over the last few decades, proteomic profiling and transcriptomic mapping in plants have attained huge attention for the identification of the physiological processes and salt tolerance mechanisms in plants (Guo *et al*., 2015, Iqbal *et al*., 2018). For the identification of transcriptome variations in plants and for the evaluation of molecular profiles for food and oiled crops, a technique known as next-generation sequencing (NGS) has been introduced.

Various approaches are being and have been introduced to facilitate production of crops in saline soils; improvement of crops through breeding is possibly among the best approaches to achieve this goal (Wani *et al*., 2016). Genetic engineering approaches offer a viable alternative to standard plant breeding for some crops and now a days extensively utilized throughout the globe to harvest salt stress tolerant crops. To produce stress tolerant plants different identified approaches like co-expression of various genes, pre- and post-transcriptional modifications (small/microRNAs) and epigenetic control of gene expression have been introduced in the world of genetic engineering (Kumar *et al*., 2018; Shriram *et al*., 2016).

Kyoto Encyclopedia of Genes and Genomes i.e., KEGG is a resource that hugely covers 15 main databases, or it is a bioinformatics analyses tool which in cooperates all the genomic (Orthology, Genome and Genes) information, chemical (compounds, Reactions, Enzymes, Glycan, Rpair and Rclass) and systemic functional (DISEASE, BRITE, MODULE, PATHWAY, DRUG and ENVIRON) information regarding genes (kanehisa *et al*., 2012). Its main objective is to collect the set of information of genes at genomic level for their high-level structure sequences from KEGG Genes and functioning from KEGG Pathway (kanehisa

et al., 2016). European Life Science Infrastructure ELIXIR introduced an analytical tool known as g: profiler who is commonly used for enriched biological entries like proteins and genes for their computational analysis. It provides highly qualified data to measure the functional terms for the given gene list, source types, organisms, and identifier spaces (Raudvere *et al*., 2019).

A branching diagram that demonstrates the evolution and relationship among biological species; biological tree is based on the physical or molecular characteristics of species keeping in view the similarities and differences (Felsenstein, 2004). It tells the common ancestor and relation between species and taxa by using Fasta sequences of DNA and Protein (Hall and Barlow, 2006). MUSCLE is an alignment software which can align the multiple sequences of Protein or DNA in a single go (Edgar, 2004). BioEdit analytical tool is used to trim or edit the aligned sequences to run it on MEGA for phylogenetic analysis. MEGA7 is the analytical tool that undergoes the process of phylogenetic analysis following another analytical tool (Tamura *et al.*, 2011).

MATERIAL AND METHODOLOGY

2.1 Seeds collection and Experimental Design

Seeds of salt tolerant cultivars (Dunkled) and salt sensitive cultivars (Cyclone) were procured from oil seed section of Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Experiments were carried out according to the completely randomized design (CRD) with three replicates.

2.1.1 Sowing

Seeds were sown in sand culture (25 seeds per plant) supplied with Hoagland nutrient solution.

2.1.2 Thinning

After five days of germination, thinning was done leaving 5 plants per pot.

2.1.3 Application of salt stress

Plants were divided into two treatments:

- S_o or Control supplied with nutrient solution containing 0 mmol NaCl
- S_1 is supplied with a nutrient solution containing 200 mmol NaCl.

2.2 Data Collection and Growth analysis

Plants responses towards salinity were observed thoroughly and recorded accordingly for growth parameters.

2.2.1 Plant fresh weight

From each replicate, fresh weights using electric balance and mean values of both types of plants were calculated.

2.2.2 Plant dry weight

Harvested plants were air-dried for 2 weeks. Their weights were calculated to determine the mean.

2.2.3 Plant height

The height of both plants was measured using a meter rod from base of the plant to the tip, furthermore, mean was determined.

2.3 Transcriptome analysis

Transcriptome data was obtained from a previous study (Ulfat *et al*., 2020). Respective transcriptome analysis was

carried out after isolation of total RNA. Leave samples were harvested right after 24 hours of salinity and afterward leaves were stored in RNA-Later solution. NGS sequencing was carried out at Macrogen (Korea). Raw data was analyzed for differential gene expression as non-characterized differentially expressed upregulated and downregulated genes were analyzed using appropriate tools of bioinformatics.

Gene Description from NCBI

Gene description, symbol, gene location, gene type, length and exon count of uncharacterized upregulated differentially expressed genes were collected online from NCBI (https://www.ncbi.nlm.nih.gov/).

2.3.1 Data retrieval of protein coding genes

The selected upregulated and downregulated genes of *Brassica napus L*. were subjected to KEGG genomics database tool (https://www.kegg..jp/) and orthology (https://www.kegg.jp/ssdb-bin/ssdb_best?org_gene) was individually explored online. Furthermore, details of their protein families were collected from **Pfam** search tool (https://www.genome.jp/dbget-bin/www_bget?pfam) given on the same KEGG site. Protein domains and details of their sub-domains were studied from **Interpro analytical tool** (https://www.genome.jp/dbget-bin/get_linkdb?-t+interpro). Accession number, entry, sequences, and consensus of these proteins families were taken from **NCBI-CDD search tool** (https://www.genome.jp/dbget-bin/get_linkdb?-t+ncbi-cdd). 3D structures, structural functions and Gene ontology of varying hits from dependent databases were overviewed and their of each upregulated gene were also predicted using Uniprot analytical tool (https://www.genome.jp/dbget-bin/get_linkdb?). For computational analysis, an online analytical tool g: profiler (https://biit.cs.ut.ee/gprofiler/gost) was used to measure the molecular, cellular, and biological highly up-to-date functions of all the given sources like genes and proteins of organisms.

2.4 Statistical Analysis

Growth data were subjected to statistical analysis using CoStat statistical software.

2.5 Phylogenetic Analysis

For the phylogenetic analysis of selected upregulated and downregulated genes of *Brassica napus*, four steps were carried out. BLAST was carried out for the selected gene individually on NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The most probable identical sequences were selected and their complete Fasta sequences were aligned to get clustal free sequences on MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). The aligned sequences were further edited and cropped on BioEditor analytical tool (https://bioedit.software.informer.com/7.2/) for phylogenetic analysis. Lastly, the aligned and cropped sequences were run on MEGA7 analytical tool (https://www.megasoftware.net/) to analyze the ancestor and evolutionary history of selected genes, out of them genes with strong bootstrap were identified and compared to predict their physical and molecular

functions of selected up and downregulated genes.

RESULTS

3.1 Growth analysis

3.1.1 Fresh weight, Dry weight, and Height of canola plant

The results of fresh weight, dry weight and height of plants are presented in table 3.1. The data regarding interactive effects indicated the co-relation between salinity and cultivars that seemed to be significant or non-significant sources of variation (figure 3.1; 3.2). In the saline cultivars the height of plant is seemed to retard and in control cultivars height increased accordingly. The meaning, by comparing both cyclone and dunkled cultivars of *Brassica napus* L. were determined and shown in table 3.2.

Table 3.1: Analysis of variance (ANOVA) for the data of fresh weight (g), dry weight (g)and height of two cultivars of canola (Brassica napus L.) plants grown under control (0 mMNaCl) and saline (200 mMNaCl) condition.

SOV	DF			MS			F		
	Fresh weight	Dry weight	height	Fresh weight	Dry weight	Height	Fresh weight	Dry weight	Height
Main Effects									
Cultivars	1	1	2	25	3.96	0.27	20^{**}	0.0002^{***}	0.937^{ns}
Salinity	1	1	1	180	5.46	169	145^{***}	0.0001^{***}	0.0007^{***}
Interactive Effects									
Cultivar x Salinity	1	1	2	4	0.0075	0.77	3^{ns}	0.79^{ns}	0.835^{ns}
Error	8	8	6	1	0.095	4.20			
Total	11	11	11	220	10.3	196			

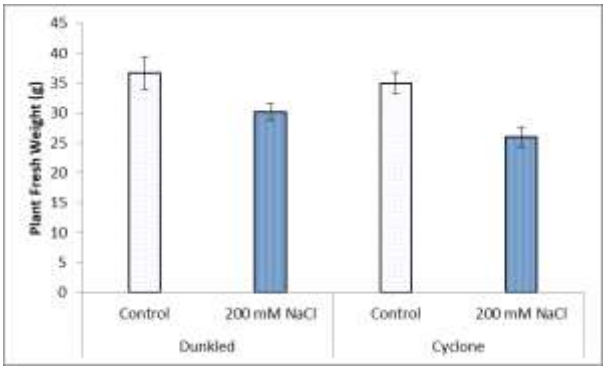
Key:
ns = non-significant (p>0.05)
* = significant (p≤0.05)

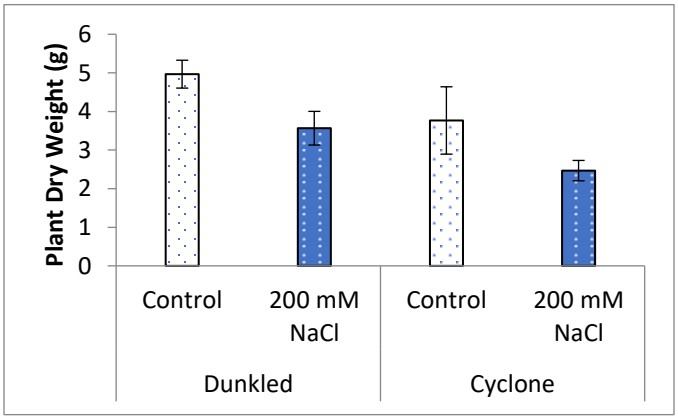
*** = Significant (p≤0.001)

Table 3.2: Fresh weight (g/plant), Dry weight and height for two cultivars of canola (Brassica napus L.) plants grown under control (0 mMNaCl) and saline (200 mMNaCl) conditions (Mean ± SD; n=3)

	Control (0 mMNaCl)			**Saline (200 mMNaCl)**		
	Mean of Fresh weight	**Mean of dry weight**	**Mean of height**	**Mean of Fresh weight**	**Mean of dry weight**	**Mean of height**
Dunkled	ax 36.66 ± **1.52**	ax 4.9 ± 0.20	ax 37.04± 1.00	bx 30.13 **± 0.80**	bx 3.5 ± 0.25	bx 30.1 ± 1.00
Cyclone	ax35.00 **± 1.00**	ax3.7 ±0.50	ax34.9 ±1.00	by 26.00 ± **1.00**	by 2.4 ±0.15	by 27.45 ±0.84
LSD $_{(0.05)}$(fresh weight) = 1.48 LSD $_{(0.05)}$(Dry weight)= 0.412 LSD $_{(0.05)}$(Height)= 3.54						

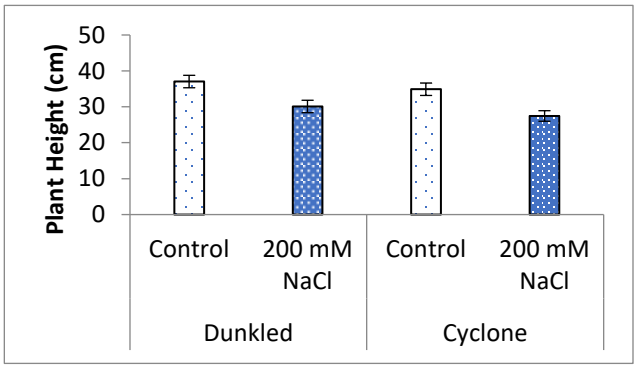
Key:
a,b= letters used to distinguish means in comparison of cultivars
x,y= letters used to distinguish means in comparison of control vs saline





Graph 3.1: Plant Fresh weight (g) for two cultivars (85 days old) of canola grown under 0 and 200 mM NaCl stress

Graph 3.2: Plant dry weight (g) for two cultivars (85 days old) of canola grown under 0 and 200 mMNaCl stress.



Graph 3.3: Plant height for two cultivars (85 days old) of canola grown under 0 and 200 mM NaCl stress.

4.2 Selected Upregulated and Downregulated genes of *Brassica napus* L.

Our study comprised of 20 genes and their varying expressions from RNA-seq data from the previous study on canola (*Brassica napus* L.) by Ulfat (2020) submitted to NCBI among them; 10 were upregulated and 10 were downregulated under NaCl stress. Selection was done among uncharacterized genes. Following are the genes that were subjected to the present study:

The upregulated gene **106401437** was worked out to be uncharacterized aarF domain-containing protein kinase, chloroplastic-like (At1g71810). It was located on chromosome C6 of *Brassica napus* L. It was 7,713 nt long a protein coding gene with 20 exons. This gene had 3 families: ABC1, WaaY and APH with 2, 1 and 2 protein domains respectively.

For the phylogenetic analysis initially, BLAST was carried out and relevant 19 sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.3). It formed a sister clade with gene XM 013841954 with strong bootstrap of *Brassica napus* having aarF domain-containing chloroplast protein kinases. This gene consists of several protein families acting to be chaperonins, resisting antibiotics, phosphorylates Hep II in plants and helps to suppress the effect of accumulated Reactive Oxygen Species. This upregulated gene is predicted to be involved in protein phosphorylation, inducing biosynthetic pathways and suppression of oxidative species during salinity.

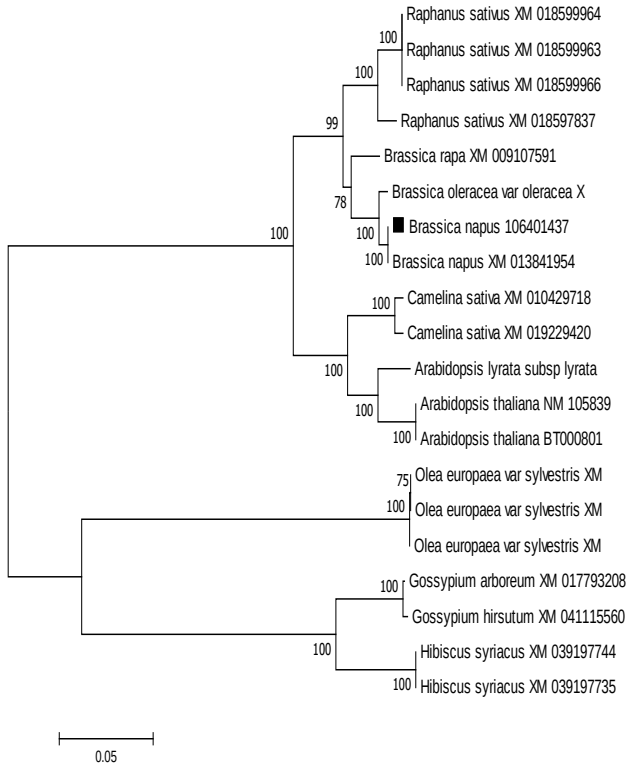


Figure 3.1: Phylogenetic analysis of Brassica napus gene 106401437 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated gene **106353398** was worked out to beprotein Activity of BC1 Complex Kinase 3, chloroplastic-like. It was located on chromosome A7 of *Brassica napus* L. It was 3,435nt long, a protein coding gene with 4 exons. This gene had 5 families: ABC1, APH, PKianse, HEAT_2 and WaaY with 2, 2, 1, 0 and 1 protein domains respectively.

For the phylogenetic analysis of upregulated gene 106353398 initially BLAST was carried out and 23 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.4). It formed a sister clade with two genes: XM 013793154 and XM 009106267 with strong bootstrap of *Brassica napus*. Both these sister genes have aarF protein domains which are chloroplast type in nature. It is predicted that this gene is involved in the regulation of phosphorylation of proteins, helps in ionic and metallic binding and cell division during retarded growth phases of plants life cycle.

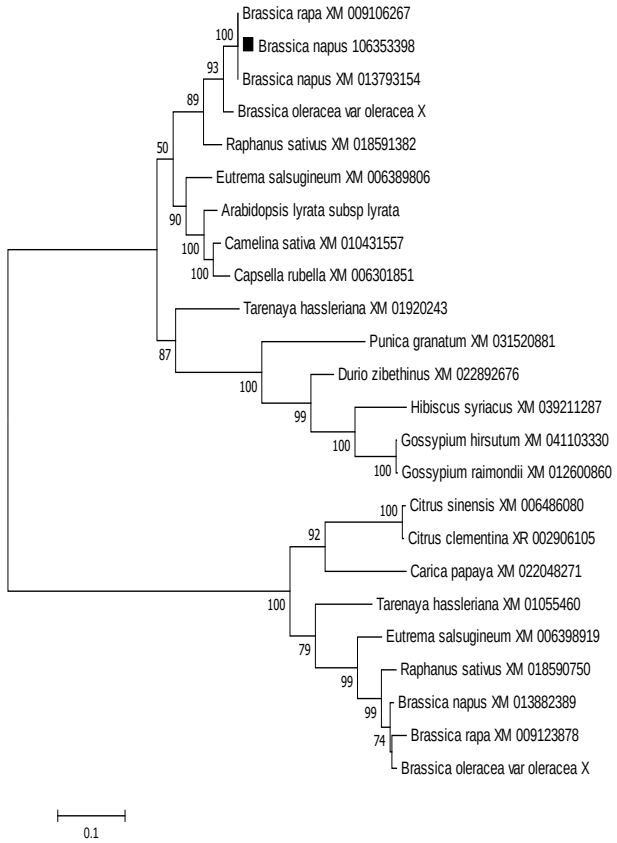


Figure 3.4: Phylogenetic analysis of Brassica napus gene 106353398 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated gene **106440663** was worked out to beprotein Uncharacterized aarF domain-containing protein kinase At5g05200, chloroplastic-like. It was located on chromosome Un of *Brassica napus* L. It was 3,586 nt long a protein coding gene with 11 exons. This gene had 2 families: ABC1 and APH with 1 protein domain each.

For the phylogenetic analysis of upregulated gene 106440663 initially BLAST was carried out and 22 different predicted plant sequences were selected. Fasta sequences were aligned and edited on analytical tools

and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.5). It formed a sister clade withgene XM 013882389 with strong bootstrap of *Brassica napus.* It consists of aarF protein domain which includes mitochondrial and chaperonin proteins. It is predicted that genes are involved in activation of photosynthesis and enhance the resistance activity of plants against microorganisms.

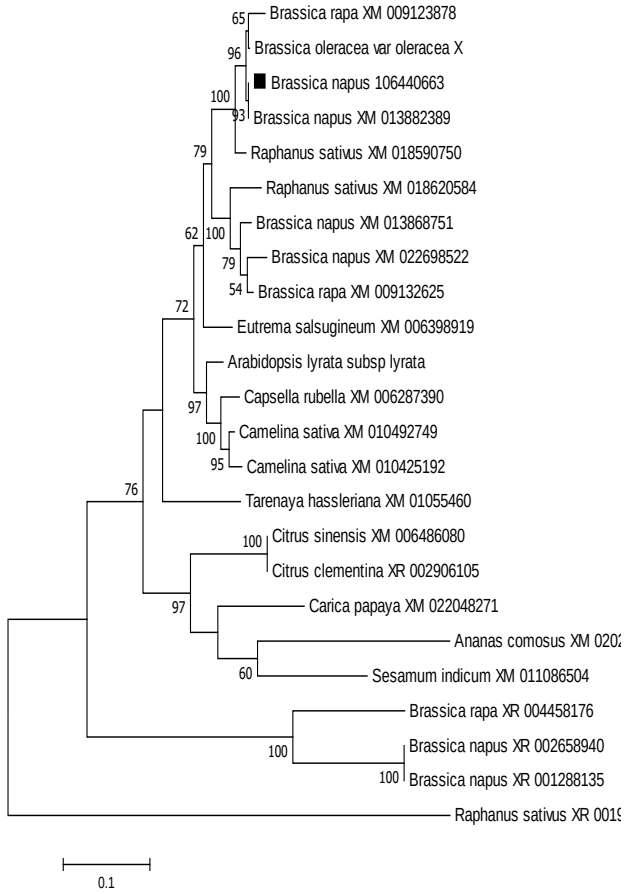


Figure 3.5: Phylogenetic analysis of Brassica napus gene 106440663 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated **106353810** gene was worked out to be protein ENHANCED PSEUDOMONAS SUSCEPTIBILTY 1-like. It was located on chromosome A7 of *Brassica napus* L. It was 2,179 nt long a protein coding gene with 2 exons. This gene had 1 family: Transferase with 1 protein domain.

For the phylogenetic analysis of upregulated gene 106353810 BLAST was initially carried out and 22 different predicted plant sequences were selected. Fasta sequences were aligned and edited on analytical tool and was run on MEGA 7 and it has fallen in the sub-clade 1 out of 3 (figure 3.6). It formed a sister clade with gene XM 013793612 with strong bootstrap of *Brassica napus.* Its enzymatic proteins help to catalyze the biosynthetic chemical reactions in plants as well as in fungal isolates. It reduces the effect of mycological infections and accumulation of salicylic acid on the membranes during salinity. It is predicted that this analyzed upregulated gene is involved in biosynthetic pathways, helping to reduce the accumulation of toxic metals and salicylic acid during salt stress.

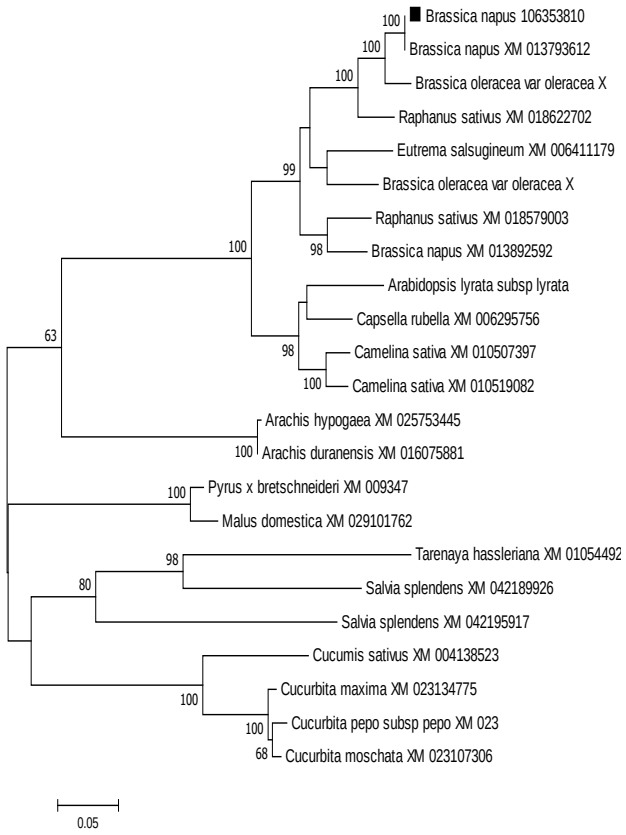


Figure 3.6: Phylogenetic analysis of Brassica napus gene 106353810 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

Another upregulated gene **106384994** was worked out to be protein uncharacterized acetyltransferase At3g50280-like. It was located on chromosome A3 of *Brassica napus* L. It was 2,065 nt long, a protein coding gene with 1 exon. This gene had 1 family: Transferase with 1 protein domain.

For the phylogenetic analysis, initially BLAST was carried out and 21 different predicted plant sequences were selected. Fasta sequences were aligned and edited on analytical tool and was run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.7). It formed a sister clade with gene XM 013824948 with strong bootstrap of *Brassica napus*. This gene worked out to be protein Enhanced Pseudomonas Susceptibility 1, having catalyzing enzymes performing same functions as mentioned in above gene 106353810.

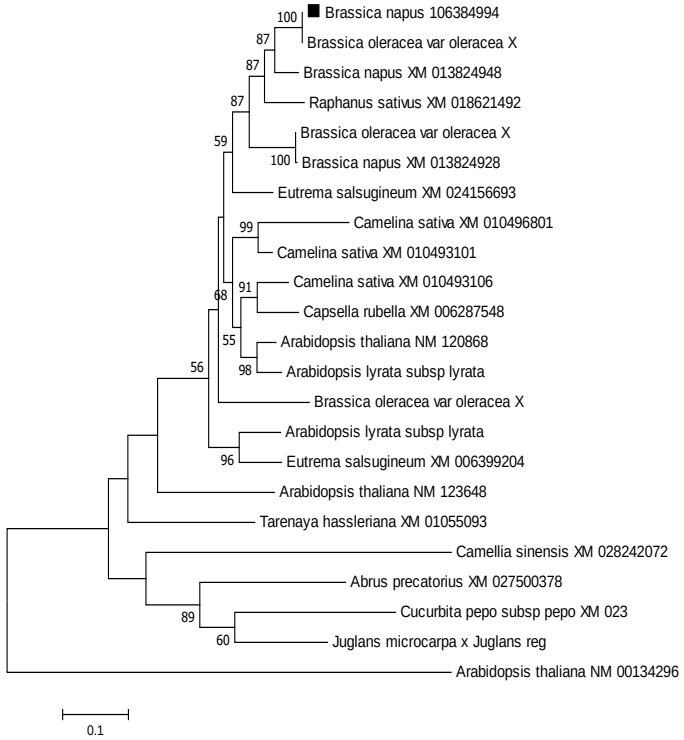


Figure 3.7: Phylogenetic analysis of Brassica napus gene 106384994 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

Similarly, upregulated gene **106422599** was worked out to be protein uncharacterized acetyltransferase At3g50280-like. It was located on chromosome A2 of *Brassica napus*. It was 2,217 nt long, a protein coding gene with 2 exons. This gene had 1 family: Transferase with 1 protein domain.

For the phylogenetic analysis initially, BLAST was carried out and 19 different predicted plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.8). It formed a sister clade with gene XM 009127455 with strong bootstrap of *Brassica napus.* This gene worked out to be protein Enhanced Pseudomonas Susceptibility 1, having catalyzing enzymes performing same functions as mentioned in above gene 106353810.

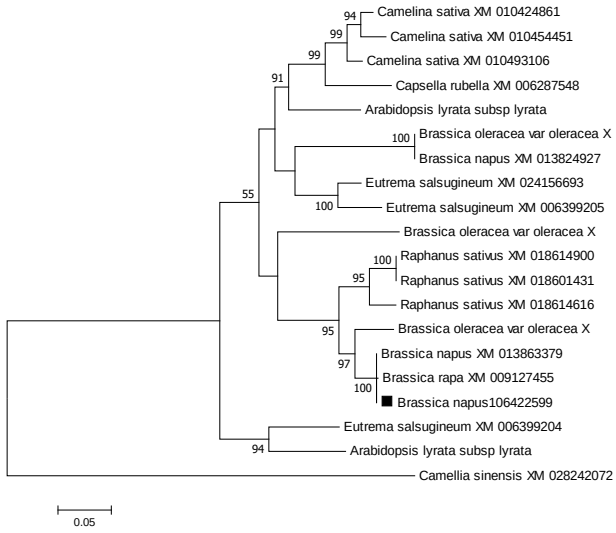


Figure 3.8: Phylogenetic analysis of Brassica napus gene 106422599 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

Lastly, the upregulated gene **106447447** was worked out to be protein Enhanced Pseudomonas Susceptibility 1-like. It was located on chromosome A4 of *Brassica napus* L. It was 2,191 nt long, a protein coding gene with 2 exons. This gene had 1 family: Transferase with 1 protein domain.

For the phylogenetic analysis, BLAST was carried out and 21 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in clade 1 out of 3 (figure 3.9). It formed a sister clade with gene XM 013793612 with strong bootstrap of *Brassica napus.* This gene worked out to be protein Enhanced Pseudomonas Susceptibility 1, having catalyzing enzymes performing same functions as mentioned in above gene 106353810.

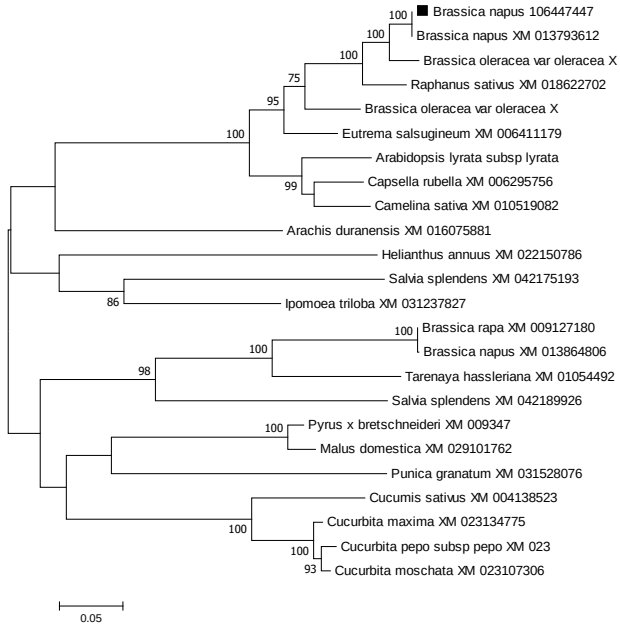


Figure 3.9: Phylogenetic analysis of Brassica napus gene 106447447 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated gene **106349247**was worked out to be protein uncharacterized acetyltransferase At3g50280-like. It was located on chromosome A6 of *Brassica napus* L. It was 3,629 nt long a protein coding

gene with 4 exons. This gene had a CRS1_YhbY family with 1 domain.

For the phylogenetic analysis, BLAST was carried out and 23 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 4 (figure 3.10). It formed a sister clade with gene XM 013848100 with strong bootstrap of *Brassica napus*. It consists of CRM proteins which reside in the cell organelles i.e., mitochondria, chloroplast, nucleus, and cytoplasm of plant cells. It is involved in RNA binding, ribosomal assembly and intron splicing of RNA in chloroplast. It is predicted here that this gene is involved in assembly of small and large ribosomal subunits and in the synthesis of proteins.

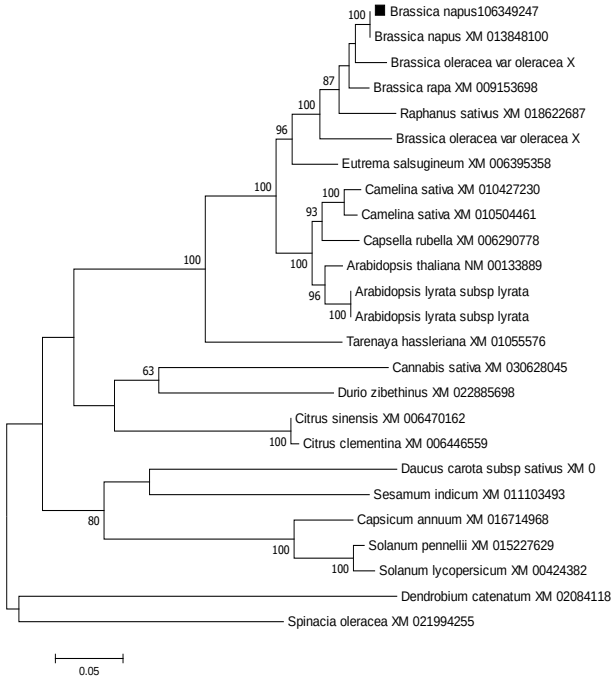


Figure 3.10: Phylogenetic analysis of *Brassica napus* gene 106349247 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated gene **106401786** was worked out to be protein uncharacterized CRM domain-containing protein At3g25440, chloroplastic-like. It was located on chromosome A2 of *Brassica napus* L. It was 3,859 nt long a protein coding gene with 4 exons. This gene had a CRS1_YhbY family with 1 domain.

For the phylogenetic analysis, BLAST was carried out and 21related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 3 (figure 3.11). It formed a sister clade with gene XM 013842370 with strong bootstrap of *Brassica napus*, having CRM protein domain residing in the cell organelles i.e., mitochondria, chloroplast, nucleus, and cytoplasm of plant cells. It is involved in RNA binding, ribosomal assembly and intron splicing of RNA in chloroplast. It is predicted here that this gene is involved in assembly of small and large ribosomal subunits and in the synthesis of proteins.

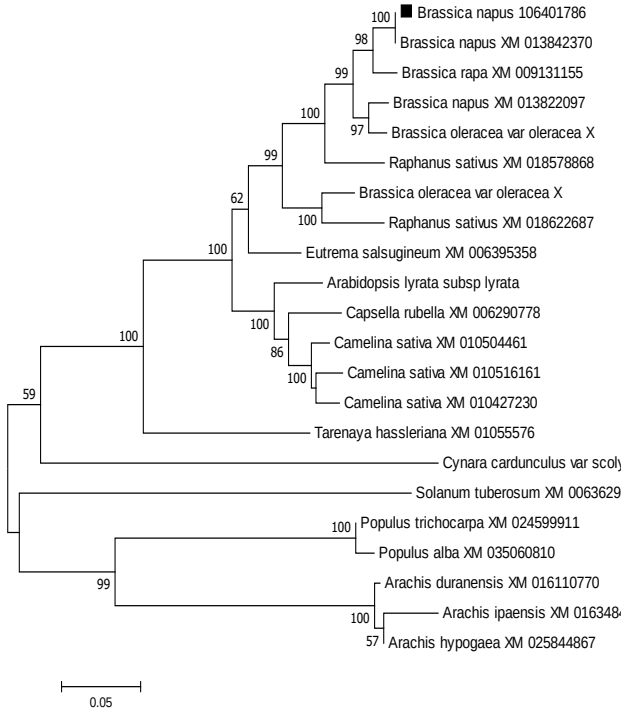


Figure 3.11: Phylogenetic analysis of *Brassica napus* gene 106401786 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The upregulated gene **106366679** was worked out to be protein uncharacterized CRM domain-containing protein At3g25440, chloroplastic-like. It is located on chromosome A7 of *Brassica napus* L. This gene had 3 families: CRS1_YhbY, NikR_C and DASH_Spc19 consisting of 1 protein domain each.

For the phylogenetic analysis, BLAST was carried out and 22 different predicted plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and was run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.12). It formed a sister clade withgene XM 009104164 with strong bootstrap of *Brassica napus.* It consists of several protein families which are involved in ribosomal assembly, helps in the regulation and uptake of heavy metals during salt stress. It is specifically involved in cell division and chromosomal segregation. Accordingly, its sister clad is expected to be involved in metallic binding during salinity, cell division, chromosomal segregation, and protein binding.

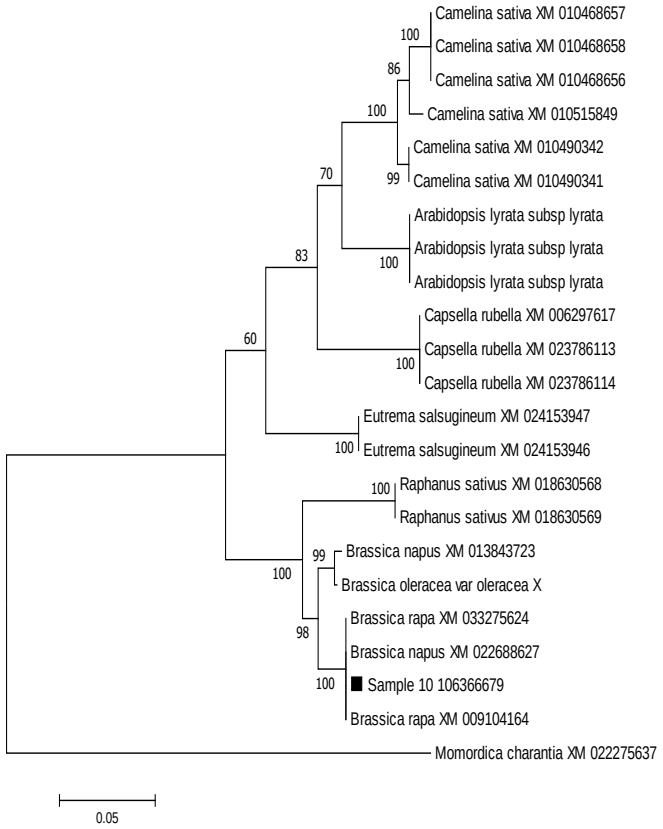


Figure 3.12: Phylogenetic analysis of Brassica napus gene 106366679 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The downregulated gene **106420779**was worked out to be Nematode resistance protein-like HSPRO2. It was located on chromosome C3 of *Brassica napus* L. It was

1,896 nt long a protein coding gene with 1 exon. This gene had 2 families: Hs1pro-1_C and Hs1pro-1_N with 1 protein domain each. For the phylogenetic analysis, BLAST was carried out and 19 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and was run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.13). It formed a sister clade with gene XM 013879102 with strong bootstrap of *Brassica napus*. It consists of nematode resistance proteins which provide resistance against nematodes and also confer the biotic and abiotic stresses. It is predicted here this downregulated gene is involved in resistance mechanism against biotic and abiotic stress and microbial attack on plants.

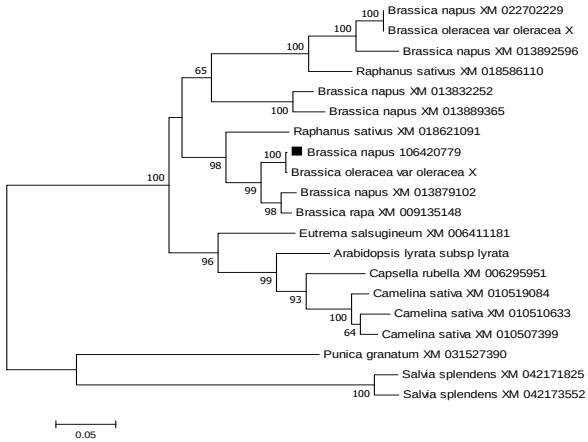


Figure 3.13: Phylogenetic analysis of Brassica napus gene 106420779 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The downregulated gene **106357317** was worked out to be Ethylene-responsive transcription factor ERF018. It was located on chromosome A7 of *Brassica napus* L. It was 1,196 nt long a protein coding gene with 1 exon. This gene had 1 family: AP2 with 1 protein domain.

For the phylogenetic analysis, BLAST was carried out and 21 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.14). It formed a sister clade with gene XM 013796997 with strong bootstrap of *Brassica napus*. It consists of an Ethylene-responsive transcription factor that helps to induce the defense mechanism of ethylene. It regulates the expression of genes under salinity and against pathogenic attack. It is predicted that this downregulated gene is involved in inducing the pathogenesis-related genes to combat the pathogenic attack and to activate the expression of genes by enhancing the ethylene response in plants during stress.

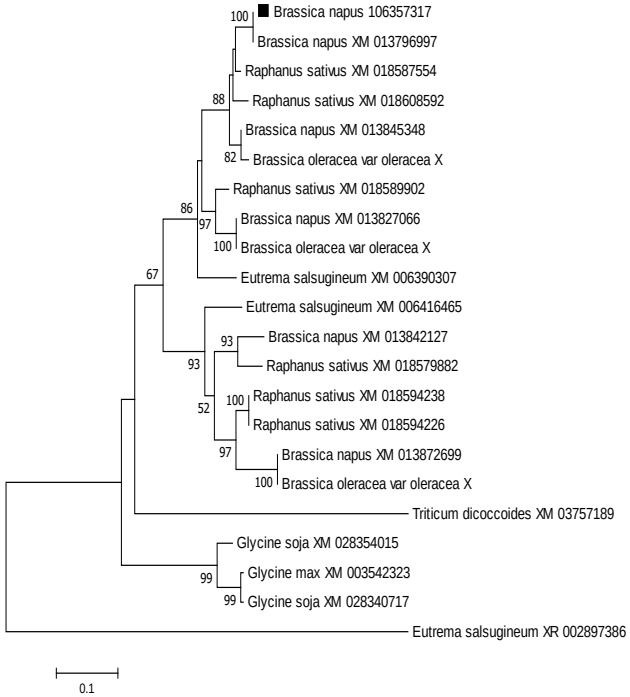


Figure 3.14: Phylogenetic analysis of Brassica napus gene 106357317 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank

accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

Another downregulated gene **106397199** was worked out to be Ethylene-responsive transcription factor ERF104-like. It was located on chromosome C7 of *Brassica napus* L. It was 1,439 nt long a protein coding gene with 1 exon. This gene had 1 family: AP2with 1 protein domain.

For the phylogenetic analysis, BLAST was carried out and 23 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 2 (figure 3.15). It formed a sister clade with gene XM 013837776 with strong bootstrap of *Brassica napus.* It consists of Ethylene-responsive transcription factor helps to induce the defense mechanism of ethylene. It regulates the expression of genes under salinity and against pathogenic attack by. It is predicted that this downregulated gene is involved in activation of the expression of genes by enhancing the ethylene response in plants during stress.

Figure 3.15: Phylogenetic analysis of *Brassica napus* gene 106397119 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

The downregulated gene **106379918** was worked out to be Dehydration-responsive element-binding protein 1F. It was located on chromosome A8 of *Brassica napus* L. It was 1,179 nt long a protein coding gene with 1 exon. This gene had 1 family: AP2with 1 protein domain.

For the phylogenetic analysis, BLAST was carried out and 22 related plant sequences were selected. Fasta sequences were aligned and edited on analytical tools and were run on MEGA 7 and it has fallen in the clade 1 out of 3(figure 3.16). It formed a sister clade with gene XM 013819765 with strong bootstrap of *Brassica napus.* It consists of Dehydration-responsive element-binding proteins which are said to be involved in regulating the development of seed coat, specifying floral and vegetative organs of plants and separating the identified structures of meristems. It is predicted that this downregulated gene is involved in the differentiation of parts of plant body and enhances the development of plant parts.

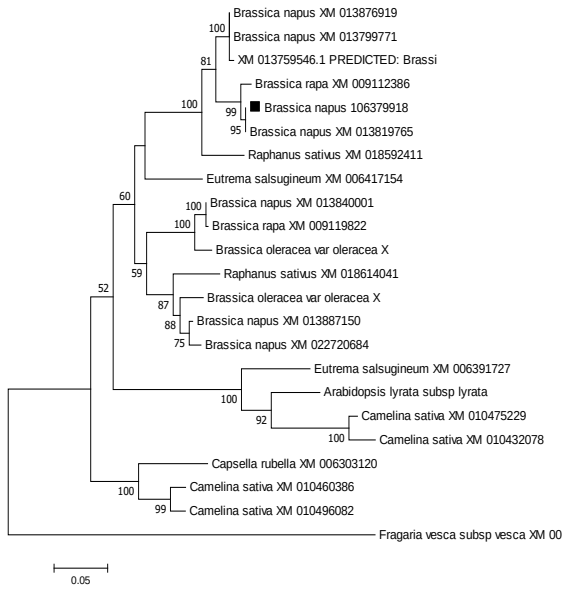


Figure 3.16: Phylogenetic analysis of *Brassica napus* gene 106397198 with respect to other genes. Tree inferred maximum likelihood. The numbers against the branches indicate the percentage (>50%) at which the branch supported in 1000 bootstrap replications. Genebank accession numbers are given at the end of species name. Highlighted taxa indicated the species to be reported.

DISCUSSION

The growing stage of plants under salinity stress affected seed at germination stage, developing stage of seedlings, vegetative and floral developmental stage and even fruiting stage of plants. This abiotic salinity stress ultimately declined crop production. Canola was the most well-known salt tolerant crop among others (Wang *et al*., 2009).

In this current review, treated canola plants were subjected to salinity resistant parameters and selected upregulated and downregulated genes were studied molecularly, functionally, metabolically, and physiologically. These genes and their protein families were predicted which possibly be engaged in regulating plant adaptation mechanisms and tolerance to salinity stress. In the same way Gupta and Huang, 2014 studied food crops like wheat, barley, rice, maize etc. under salinity stress. They reviewed ion uptake and transport, harmonic modulation, activation or deactivation of enzymes and synthesis of amines during stress to tolerate salt.

In the selected upregulated gene, gene ABC1 was a residue in the form of novel chaperonins and functions diplomatically in mitochondria, it also suppressed a cytochrome b complex during mRNA translation and reviewed previously (Poon *et*

al., 2000; Macinga *et al*., 1998; Chehade *et al*., 2013) and according to the phylogenetic analysis it is important for e- transport in bc 1 complex as a cofactor as concluded in the review of Bousquet *et al*., 1991. WaaY family was in the waa locus of bacterial strain of *E. coli*, which consisted of bacterial lipopolysaccharide; WaaY also acted as an enzyme which played an important role in phosphorylation of HepII in plants (Yethon *et al*., 1998). Phylogenetically it is predicted that Gene APH and its family consisted of kinases like fructosamine, homoserine and protein kinases having bacterial antibiotic resistance, which specifically acted to suppress the effect of accumulated Reactive Oxygen Species (Trower and Clark, 1998).

Furthermore, Pkinase family was found in Eukaryotes like plants, Pkinase was an enzymatic protein family which shared common catalytic core consisting of serine and tyrosine kinases (Hanks, 2003). NikR_C family contains NikR DNA binding transcription factor that helped to bind and regulate nickel uptake during saline stress (Schreiter *et al*., 2003) and DASH_Spc19 family consisted of DASH complex which contained a component; Spc19 specifically involved in thorough cell division. It also acted as a microtubule-binding subcomplex, which was essential for chromosome segregation, performed specific role in cell division for spindle and kinetochore integrity (Janke *et al*., 2002; Li *et al*., 2002).

In downregulated genes a family P450s ishaem-thiolateproteinic which was involved in the degradation of oxidative toxins and many other environmental mutagens of plants. They enzymatically catalyzed oxidation of non-activated hydrocarbons at specific optimal temperatures. Its domains were involved in biosynthesis of antibiotics and activated proteomic activity also reviewed previously by Munro *et al*., 2007; McLean *et al*., 2005; Nguyen *et al*., 2010.
According to the review of a current study, the CRM-domain protein was involved in the mitochondrial splicing introns, which was ultimately very crucial for growth and stress responses of plants; and, for all stress reducing mitochondrial functions. However, the role of many CRM-domain proteins was still unknown which were said to be doing splicing of introns and rRNA processing in chloroplasts or mitochondria and their role in the growth, development, and stress responses of plants. Lee *et al.*, 2019 reported its role in splicing of RNA transcripts and its promotional role in cis and trans-splicing in cellular poraganlles of plants.

CONCLUSION

Today's world has declared that *Brassica napus* is certainly the most salt tolerant family among the food crops. These results showed the most capable genes are involved in reducing the salt tolerance efficiently and some genes worked out to improve salt stress tolerance capacity of plants by the regulated expressions of genes.

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