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Identification of Wound Responsive Genes in *Brassica juncea* by Transcriptome Analysis

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ABSTRACT: *Brassica juncea* (L.) is economically important oilseed crop, next to groundnut, due to its high oil content and superior oil quality. Wounding caused due to agricultural practices and insect attack is the most common stress, resulting in extensive loss of vegetative tissues. To understand the mechanism of wound response in *B. juncea* wound treatment was given and samples were collected at three different time point intervals (control and wound samples). RNAs of all three-time points interval was pooled together and RNA sequencing was done. RNA sequencing gave transcriptome data, in *B juncea* 1503 genes were upregulated and 1769 genes were downregulated genes (DEGs). In this study transcriptome analysis of wounded *B. juncea* deciphered the upregulation of stress proteins including vegetative cell wall protein gp1-like, protein MLP1-like isoform X1, F-box/LRR-repeat protein 7, protein TIFY 10B, myb-like protein Xin wounded *B. juncea* compared to control *B. juncea*. Transcription factor bHLH92, bHLH149, bHLH74, WRKY transcription factor 6-like, WRKY transcription factor 18-like, 75, transcription factor MYB6-like and transcription factor PosF21, signalling kinases like mitogen-activated protein kinase ANP1-like, serine/threonine-protein kinase NAK, receptor like protein kinase S.2-like, glycerol kinase-like, serine/threonine-protein kinase Nek6, metabolite synthesizing enzymes cellulose synthase, galactinol-sucrose galactosyltransferase 2, callose synthase 5, flavonol synthase/flavanone 3-hydroxylase were upregulated in wounded *B. juncea* compared to control *B. juncea*. ROS scavenging enzymes, methionine sulfoxide reductase A4, glutathione S-transferase U24, peroxidase 71, L-ascorbate peroxidase 1, catalase were upregulated while superoxide dismutase catalase-2 and catalase-3 were down regulated in wounded *B. juncea* compared to control leaves. Upregulation of these significant genes suggests that they have potential role in wound stress recovery and these genes can be employed for transgenic crop development.

Keywords: *B. juncea*, wound stress, Transcription factors, MAP kinases, ROS.

INTRODUCTION

B. juncea (L.) is economically important oilseed crop, next to groundnut, due to its high oil content and superior oil quality and is grown in about 6.09-million-hectare area with average productivity of 12000 (kg/ha) (DAC, 2017). The average yield per hectare of the crop is very low when compared to the world average production of 1300 kg/ha. Wound stress is one of the most common stresses to which plants are constantly exposed. Plant tissue is damaged or lost when it is crushed by treading, grazing, insect feeding, or cutting. The majority of these wound stresses result in extensive loss of vegetative tissues and rapid regrowth. The molecular characterization of the wound stress response has been studied in dicotyledonous plant systems (Cheong *et al.,* 2002), but it has not been studied as thoroughly in monocots and oilseed crops. Plants respond to wounding by generating a diverse array of signals, which in turn activate complex, integrated signalling networks (Maffei *et al*., 2007).

Wound-generated signals are systemically transmitted to distal portions of the plant via hydraulic, electrical, and chemical signals. In addition to proteins kinases, receptors, calmodulin, and calcium-binding proteins, mall molecules such as reactive oxygen species (ROS), small calcium, ethylene, salicylic acid (SA), and jasmonic acid (JA) derivatives are also important in wound response (Wang *et al.,* 2016). JA and its bioactive derivatives are critical in wound signalling (Wasternack and Feussner 2018). JA has vital role in many aspects of plant growth, development, and environmental responses (Cheong and Do Choi 2003). JA-independent signalling pathways modulates the expression of JA-responsive genes and regulate the expression of distinct sets of wound-related genes (Lebrasseur *et al*., 2002). After wounding, signalling elements and various components of oxylipin biosynthesis were induced in maize and rice

(Szczegielniak *et al.*, 2012), electrical and hydraulic signals were identified in barley (Felle and Zimmermann 2007), mitogen-activated protein kinases (MAPKs) in rice (Sinha *et al.*, 2011); wound-inducible genes and proteins in maize and rice (Van Loon *at al.*, 2006) and defense-related proteins. In plants wound stress response and disease resistance response shares common genes and pathway. To identify wound specific genes in *B. juncea* transcriptome analysis was done. In present study the differentially induced genes in *B. juncea* after severe wounding were investigated. The wound-induced transcriptome of *B. juncea* revealed a diverse set of proteins involved in signalling, growth and stress-related component.

MATERIALS AND METHODS

A. Sample collection and RNA Extraction

B. juncea plants were grown in net house under controlled environment of 16hr/8hr light and dark cycle at temperature 24°C/ 20°C for day /night and relative humidity of 78 %. Forty-five days old plants were selected and small injury was made with the help of a glass cover slip on third and fourth leaves at bottom of *B. juncea* plants. Control plants were maintained separately. Control and wounded leaves of *B. juncea* were collected at three time point intervals i.e. 24 hpi, 48 hpi and 96 hpi. Using NucleoSpin plant RNA kit RNA was extracted from 100 mg of frozen leaves of wound and control leaf samples. Extracted RNAs of stage 24 hpi, 48 hpi and 96 hpi were pooled together and send to Clever gene Banglore for RNA sequencing. RNA sequencing gave transcriptome data.

B. De novo assembly and data analysis

De novo transcriptome assembly was done using Trinity v2.11.0. with default parameters. The assembled transcripts were used to predict protein coding sequences using Trans Decoder. The protein sequences were annotated against NCBI nr, using BLASTp module of diamond. The QC passed reads were mapped to the assembled transcriptome using Bowtie2 and read counts per gene were extracted using feature Counts. The differential expression analysis was performed using DESeq2. The genes with adj pvalue<=0.05 and log2 fold change >=2 and <<=2 were considered significant for comparison. Blast2GO was used to identify GO category differences between the upregulated and down regulated DEGs.

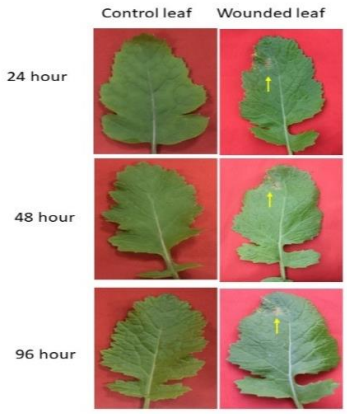

Fig. 1. Control and wounded leaf of *B. juncea.*

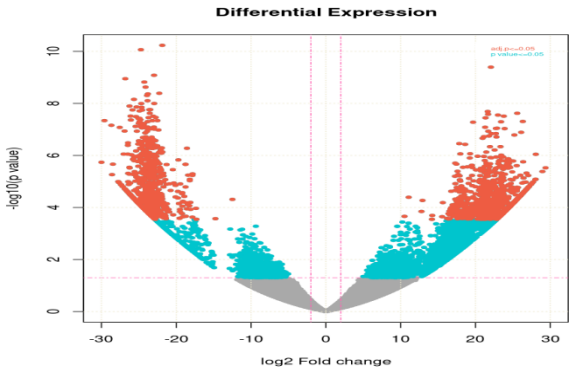

Fig. 2. Differentially induced genes in wounded *B. juncea* leaf compared to *B. juncea* control leaf.

Table 1: Details of raw sequence data of control and wounded *B. juncea* sample.

Sample Name	No. of Reads	Data In GB	GC%	Read length	Q20	Q30
B. juncea control	44109120	6.618	51	150	99.96	97.87
B. juncea control	39231120	5.865	49	150	99.97	97.98
B. juncea wounded	36999952	5.86	51	150	99.955	97.865
B. juncea wounded	34461634	5.227	50	150	99.965	97.94

Table 2: Differentially induced significant genes in wounded *B. juncea* compared to control *B. juncea.*

Comparison: *B. juncea* control Vs *B. juncea* wounded sample			
Sr. No.	**Types of Gene**	**Up regulated genes**	**Down regulated genes**
1.	Oxidative stress enzymes	11	28
2.	Stress related proteins	45	55
3.	Transcription factors	18	37
4.	Signalling and Metabolite synthesizing enzymes	12	34
	Total	**86**	**154**

RESULTS AND DISCUSSION

RNA sequencing data given 5 to 6 million reads in each sample. In wounded *B juncea*1503 genes were upregulated and 1769 genes were downregulated (DEGs) compared to control samples. In wounded *B. juncea*, stress proteins including vegetative cell wall protein gp1-like, protein MLP1-like isoform X1, F-box/LRR-repeat protein 7, 1F-box/LRR-repeat protein 2-like , protein TIFY 9-like isoform X1, 6B, 10B, stress-associated endoplasmic reticulum protein 2 isoform X1, MYB-like protein X, protein 78-like isoform X1, UPF0664 stress-induced protein C29B12.11c-like, 18 isoform X1, plant intracellular Ras-group-related LRR protein 8, rhodanese-like domain-containing protein 15, chloroplastic isoform, SelR-like protein, 1BTB/POZ and TAZ domain-containing protein 5, pentatricopeptide repeat-containing protein At1g06580, 1F-box/LRR-repeat protein 2-like, 1leucine-rich repeat protein 1 isoform X2, RING-H2 finger protein ATL17, protein EXORDIUM-like 4, protein NUCLEAR FUSION DEFECTIVE 4-like, lectin-like protein At3g16530, were upregulated. Additionally, disease resistance protein TAO1-like, DUF4228 domain protein, universal stress protein PHOS32-like, disease resistance protein RML1A-like, universal stress protein PHOS34, universal stress protein A-like protein isoform X1, pathogenesis-related protein 1A-like, ABC transporter G family member 40, RING-H2 finger protein ATL17, leucine-rich repeat extensin-like protein 3, pfkB-like carbohydrate kinase family protein were upregulated as compared to control leaf sample. Whlie disease resistance protein RPS4-like, Disease resistance protein (TIR-NBS-LRR class) family, stress response protein nst1, RRS1-like, protein At5g47250, protein downy mildew resistance 6, stress enhanced protein 2, protein At1g12290, RPS5-like, protein At1g12280, protein-like HSPRO2, disease resistance protein RPS4, protein At5g45510 isoform X1, RPP8-like protein, endoglucanase IV precursor, protein enhanced disease resistance 2-like, hypersensitive-induced response protein 4, endochitinase At2g43610 were down regulated in wounded *B. juncea* compared to control leaves. In *Aquilaria sinensis* after mechanical injury JAZ proteins like TIFY7, TIFY9, TIFY10A, and TIFY11B were upregulated (Ruyue Du *et al.,* 2022). Expression of various genes, plant growth, development, and plant responses to environmental changes all are regulated by molecular switches that are activated by transcription factors (TFs). Transcription factor bHLH92, bHLH149, bHLH74, bHLH92-like isoform X2, JUNGBRUNNEN 1, KUA1, ethylene-responsive transcription factor ERF113-like, ERF113-like, transcription factor DIVARICATA-like, MADS-box transcription factor 23, GATA transcription factor 17, transcription factor LUX-like, WRKY transcription factor 15, 6-like, 18-like, 75, transcription factor MYB6-like and transcription factor PosF21 were upregulated in wounded *B. juncea* but NAC transcription factor 47, NAC transcription factor 29, trihelix transcription factor ASIL1, bzip transcription factor protein, GATA transcription factor 1, MADS-box transcription factor 23, transcription factor bHLH149, transcription factor bHLH101, transcription factor bHLH3-like, transcription factor bHLH92, transcription factor bHLH47-like isoform X4, transcription factor TCP8, transcription factor TGA1 isoform X1, transcription factor MYB1R1-like, transcription factor MYC3, WRKY transcription factor 18, 15, 63, 46, 54, 62 were down regulated compared to control leaf. Transcription factors AP2, WRKY, MYB, and MYC significantly contribute to the defense against wounds.

Wound stress in *A. sinensis* elevated the expression of WRKY, AP2, NAM, MYB, HLH, bZIP1 transcription factors (Ruyue Du *et al.,* 2022). Genes involved in stress response and plant defence, were differentially elevated in *A. sinensis* (Xu *et al.,* 2013). Signal transduction caused by wound in plants is mediated by mitogen-activated protein kinases (MAPKs). Signalling kinases like mitogen-activated protein kinase ANP1-like, serine/threonine-protein kinase NAK, receptor like protein kinase S.2-like, glycerol kinase-like, serine/threonine-protein kinase Nek6, LRR receptor-like serine/threonine-protein kinase At4g20940 were upregulated in wounded *B. juncea* While arginine kinase, casein kinase 1-like protein 8 in isoform X1, serine/threonine-protein kinase CTR1-like, serine/threonine-protein kinase SRK2A-like, serine/threonine-protein kinase At1g01540 isoform X2, CDPK-related kinase 6-like, serine/threonine-protein kinase WNK4, receptor like protein kinase S.2-like, serine/threonine-protein kinase SRK2I, CBL-interacting protein kinase, mitogen-activated protein kinase kinase 9, mitogen-activated protein kinase 6 were down regulated in wounded *B. juncea* as compared to control leaf. In tobacco plants, wounding activated two distinct MAPKs, namely WIPK (wound-induced protein kinase) and SIPK (salicylic acid-induced protein kinase) (Seo *et al*., 1999). In wounded B. juncea, cellulose synthase, galactinol--sucrose galactosyltransferase 2, callose synthase 5, flavonol synthase/flavanone 3-hydroxylase these metabolite synthesizing enzymes were upregulated but monooxygenase 2, chalcone--flavonone isomerase, methylesterase 4-like were down regulated in wounded *B. Juncea* compared to control leaves. In *Dalbergia odorifera* and *Dracaena cochinchinensis* wounding stress up-regulated CHS (chalcone synthase and other gene families related to the phenylpropanoid and the flavonoid pathways ultimately promoted flavonoid biosynthesis (Sun *et al.,* 2021).

To maintain the redox homeostasispeptide methionine sulfoxide reductase A4, glutathione S-transferase U24, peroxidase 71, L-ascorbate peroxidase 1, Catalase were upregulated while superoxide dismutase catalase-2 and catalase-3 were down regulated in wounded *B. juncea* compared to control leaves. After six hours in wounded leaves of rice, superoxide dismutase activity was increased. Wound stress altered the activity of copper/zinc superoxide dismutase and manganese superoxide dismutase (Chandru *et al.,* 2003). Extracellular peroxidase (ECPOX) activity was

increased and soluble peroxidases with molecular weights of 37 KD, 40 KD, and 136 KD were upregulated during wound stress in wheat (Minibayeva *et al.*, 2009).

CONCLUSIONS

The transcriptome analysis of the wounded *B. juncea* revealed the upregulation of genes encoding a wide array of proteins involved in signalling, defense, and metabolic processes. In response to wounding plant produces signals that rapidly activate MAPK. These MAPK signalling proteins could be activated by reactive oxygen species. MAPK and receptor kinases were found to be upregulated in the wound transcriptome. Wounding in plant results in the activation and transcription of genes coding for proteins involved in numerous cellular and molecular functions. Signalling kinases, stress-related proteins, transcription factors, metabolic enzymes and ROS scavenging enzymes together interplay vital role to recover damage caused by wounding.

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Conflict of Interest. None.

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