Analysis of the transcriptome of the needles and bark of *Pinus radiata* induced by bark stripping and methyl jasmonate

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**Abstract**

**Background:** Plants are attacked by diverse insect and mammalian herbivores and respond with different physical and chemical defences. Transcriptional changes underlie these phenotypic changes. Simulated herbivory has been used to study the transcriptional and other early regulation events of these plant responses. In this study, constitutive and induced transcriptional responses to artificial bark stripping are compared in the needles and the bark of *Pinus radiata* to the responses from application of the plant stressor, methyl jasmonate. The time progression of the responses was assessed over a 4-week period.

**Results:** Of the 6312 unique transcripts studied, 86.6% were differentially expressed between the needles and the bark prior to treatment. The most abundant constitutive transcripts were related to defence and photosynthesis and their expression did not differ between the needles and the bark. While no differential expression of transcripts were detected in the needles following bark stripping, in the bark this treatment caused an up-regulation and down-regulation of genes associated with primary and secondary metabolism. Methyl jasmonate treatment caused differential expression of transcripts in both the bark and the needles, with individual genes related to primary metabolism more responsive than those associated with secondary metabolism. The up-regulation of genes related to sugar break-down and the repression of genes related with photosynthesis, following both treatments was consistent with the strong down-regulation of sugars that has been observed in the same population. Relative to the control, the treatments caused a differential expression of genes involved in signalling, photosynthesis, carbohydrate and lipid metabolism as well as defence and water stress. However, non-overlapping transcripts were detected between the needles and the bark, between treatments and at different times of assessment. Methyl jasmonate induced more transcriptional responses in the bark than bark stripping, although the peak of expression following both treatments was detected 7 days post treatment application. The effects of bark stripping were localised, and no systemic changes were detected in the needles.

**Conclusion:** There are constitutive and induced differences in the needle and bark transcriptome of *Pinus radiata*. Some expression responses to bark stripping may differ from other biotic and abiotic stresses, which contributes to the understanding of plant molecular responses to diverse stresses. Whether the gene expression changes are heritable and how they differ between resistant and susceptible families identified in earlier studies needs further investigation.

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Introduction

Plants have evolved a variety of constitutive and inducible defences to resist and tolerate herbivory. An assessment of the genetic mechanisms that influence these defences will enhance our understanding of their evolution [1]. Although structural changes in DNA are the major source of genetic variation [2, 3], the phenotypic outcomes of several traits can be linked to gene expression [4–8]. However, the genes and genetic pathways that underlie most phenotypes are still unknown [2]. To date, most gene expression studies have focussed on identifying transcripts (different RNA products a single gene) or genes showing differential expression, or pathways associated with a phenotype (case/control) or condition (treated/untreated). In conifers, for example, transcript abundance has been examined with respect to biotic and abiotic environmental factors such as herbivory [9–11], pathogens [12], artificial wounding [13], drought [14], light intensity [15], seasonal changes [16], chemical stressors like methyl jasmonate [17], as well as associated phenotypic traits such as resistance and chemical composition [9, 10]. Studies in conifer and non-conifer species that have simultaneously compared the expression from different stressors, such as mechanical wounding and methyl jasmonate, indicate both overlapping and non-overlapping gene expression and suggest that molecular mechanisms associated with varying stressors may differ [18–20].

In conifer-herbivory studies, most gene expression studies have focused on understanding induced defence responses, with a premise that these may be more important than constitutive defences as they are metabolically cost effective and expressed only when required [21, 22]. Global transcriptome responses have been studied in both needles and bark, monitoring the expression of a wide range of genes related to the biosynthesis of primary and secondary compounds, and structural components [13, 23–28]. Most of these genes are expressed at basal levels in plants but some are only expressed in the presence of an appropriate stimulus. Some of the genes significantly respond to herbivory cues, by increasing or reducing their expression either locally at the site of the perceived effect or systemically throughout the plant [23, 29, 30]. Studies also show a high overlap in the genes that are differentially expressed when plants are subjected to different biotic and abiotic stresses [31, 32]. However, the genes that show differential expression differ within and between target plant species [10, 26], between plant tissues [23, 33], as well as between biotic agents [34] and applied treatments [35]. Intra-specific differences in the timing of transcript expression have also been observed, where plants may respond to injury within hours or days, with short, or long, lasting effects [17, 23, 25, 33]. Plant responses to different classes of herbivores may differ due to differences in herbivore oral secretions or mode of feeding and the amount of plant tissue damage [34, 36, 37]. While available conifer studies have documented changes in gene expression in response to insect herbivory [13, 32], there are no studies from the perspective of mammalian herbivory, and none that link changes in gene expression to changing chemistry. Mammalian bark herbivory is fundamentally different from insect herbivory in the mode of feeding [22] and possibly the oral secretions. This particularly applies to mammalian bark stripping, which is of increasing concern to managers of conifer forests world-wide, including Pinus radiata plantations in Australia [38–40].

Pinus radiata is native to California [41], but is now a major plantation species in Australia (ABARES 2018) where it is subject to bark stripping, mainly by native marsupials (wallabies and kangaroos) [42]. The bark is stripped from the base of the trees during the early stages of growth [43–45], reducing tree growth rate, distorting stems and, in extreme cases, causing death [38, 42]. The levels of bark stripping within plantations may be highly variable and progeny trials have shown a genetic, physical and chemical basis to this variation [42, 46, 47]. Further, chemical profiling in P. radiata shows that needles and bark respond differently to bark stripping and other forms of real and simulated herbivory, mostly by increasing levels of secondary compounds, especially terpenes and phenolics [48, 49], and reducing levels of sugars and fatty acids [46, 50]. This suggests changes in the expression of underlying genes that subsequently transforms the chemical phenotype. Indeed, the differences in timing of the induced changes in terpenes, phenolics and sugars [50–52] suggest corresponding differences in the expression of the underlying genes. However, while transcriptomic changes have been studied in P. radiata associated with ontogeny, wood formation [53–55] and fungal infections [56], those underlying the induced chemical changes to bark stripping have not been characterised.

The present study aims to quantify and compare the transcriptome changes that occur in response to artificial bark stripping of P. radiata and whole plant stress induced by application of the chemical stressor, methyl jasmonate. The longer-term goal is to identify genes that specifically mediate the previously shown induced
chemical responses to bark stripping in _P. radiata_, which may help develop strategies to reduce bark stripping. The specific aims of the study are to: 1) characterise and compare the constitutive transcriptome of _P. radiata_ needles and bark; 2) identify genes which are differentially expressed following artificial bark stripping (aimed at mimicking mammalian bark stripping); and 3) identify genes which are differentially expressed following whole plant application of methyl jasmonate and compare these induced responses with those of bark stripping. The results are discussed in view of the holistic chemistry that has been characterised on the same individuals with the same treatments [50].

**Materials and methods**

**Experimental design**

In 2015, 6-month-old seedlings from 18 full-sib families (each with 4 seedlings; total number of seedlings = 72) of _P. radiata_ (D. Don) originating from the Radiata Pine Breeding Company deployment population, were obtained from a commercial nursery. Seedlings were transferred into 145 mm × 220 mm pots containing 4L of basic potting mix (composted pine bark 80% by volume, coarse sand 20%, lime 3 kg/m³ and dolomite 3 kg/m³) and raised outdoors in a common fenced area (to protect against animal damage) at the University of Tasmania, Hobart. At 2 years of age, plants were moved to a shade house and an experimental design established by randomly allocating the 18 families to three treatment groups (methyl jasmonate [MJ], artificial bark stripping [strip] and control), each with 6 families. The three treatment groups were arranged in a randomized block design of 3 blocks, each block comprised a treatment plot of two families, with the treatment plots separated within each block to minimise any interference among treatments. Each family was represented by four plants arranged linearly, and randomly allocated to four sampling times (T0-T21). T0 represents the time immediately before treatment applications. T7, T14 and T21 represent respective sampling times at 7, 14 and 21 days after treatment (MJ and strip) application. All T0 seedlings (n = 18), irrespective of group allocation, were not treated and were used to compare the constitutive transcriptome of the needles and bark (i.e. plant parts). Additionally, all seedlings allocated to the control were not treated throughout the experimental period. One seedling from each family in the control and treated groups was destructively sampled at each sampling time to estimate differential expression (n = 18; Table 1). For each plant part, comparisons were made between the control (n = 6) and methyl jasmonate (MJ, n = 6) and between the control (n = 6) and bark stripping (strip, n = 6) treatments at each sampling time (T7, T14, T21) (Table 1). Methyl jasmonate (MJ) was applied in a 25 mM solution by spraying the whole plant with a fine mist from a hand sprayer until ‘just before run-off.’ The treated seedlings were sprayed in a well-ventilated area away from untreated seedlings to avoid cross contamination [57]. For bark stripping (strip), 18 plants were artificially stripped by removing a 30 cm vertical strip of bark, beginning 2 cm from the ground and covering 50% of the stem circumference, which is the average upper threshold of browsing observed in natural field conditions.

Up to 20 young needles were randomly collected per seedling from different parts of the crown. The bark was sampled from different points of the stem, above and besides the area where the bark stripping treatment was applied, carefully avoiding the wood, following Nantongo et al. [50]. Individual samples were kept separate providing 144 samples for sequencing (2 plant

| Control # seedlings | MJ # seedlings | Strip # seedlings | Total # seedlings sampled at each time |
|---------------------|---------------|-------------------|---------------------------------------|
| T0                  | 6             | 6                 | 18                                    |
| T7                  | 6             | 6                 | 18                                    |
| T14                 | 6             | 6                 | 18                                    |
| T21                 | 6             | 6                 | 18                                    |
| Total # seedlings for each treatment | 24 | 24 | 24 | 72 |

Table 1: The treatments, sample size and pairwise comparisons that were made for each time and for the two treatments - bark stripping (strip) and methyl jasmonate (MJ). The seedlings of each family were grown in a line-plot and one was chosen at random for destructive harvesting at each time (T7 to T21). At T0, the sampled seedlings were destructively harvested just before treatment applications. At 7 (T7), 14 (T14) and 21 (T21) days after treatment, one seedling from each family (total number of seedlings per sampling time = 18, equivalent to the number of families and n = 6 are seedlings selected from each treatment) was destructively harvested.
parts × 72 seedlings). The needles and bark samples were snap frozen in liquid nitrogen and were stored at −80°C until RNA extraction. The 6 families sampled from each treatment at each time point were treated as biological replicates. No technical replicates were included. This sampling occurred at the same time when the tissue for the chemistry assays reported in Nantongo et al. [50] was sampled.

RNA extraction and sequencing
RNA from all the 144 bark and needle samples was extracted using the Spectrum™ Plant Total RNA kit (Sigma Aldrich, St. Louis, Missouri, USA, lot # SLBW2113). The RNA extraction was random with respect to part, sampling time, treatment, family and shade house replicate. The quality and quantity of the RNA extracts were assessed with an Agilent 5200 Fragment Analyzer (Palo Alto, California, USA). One sample had poor quality RNA and was excluded from further processing. Using the high-quality RNA samples, 143 separate libraries were prepared with a 6-bp nucleotide bar-coding tag for each library. To construct the library, approximately 1 μg of total RNA was used following the MGIEasy RNA Directional Library Prep Kit (MGI, China). Paired-end sequencing was performed using the Beijing Genomics Institute, (BGI, China) MGISEQ-2000 sequencer according to the manufacturer’s instructions, yielding 100-bp paired-end reads and a total of 20 m reads per sample. Tagged cDNA libraries were sequenced in separate lanes. The library for each lane was selected at random. The quality of RNAseq sequences was assessed using FastQC version 0.11.8 [58]. Quality trimming and filtering of data was performed using Trimmomatic v 0.39 [59]. On average, 99.9% of the sequences were retained at phred33 [60].

A de novo assembly of the pooled transcriptome was attempted using TRINITY v2.9.0 using default parameters [61], however due to the excessive computation requirements, it could not be completed with the available resources in the required timeframe. Accordingly, the filtered reads were aligned to the *P. radiata* reference transcriptome that is harboured at Scion (the New Zealand Forest Research Institute trading as Scion, Rotorua New Zealand) [54] with SALMON v0.14.1 using default parameters [62]. This reference transcriptome (www.ncbi.nlm.nih.gov/bioproject/482145) was assembled from a range of *P. radiata* genotypes and tissue types that were collected at different developmental and temporal stages. Most of the samples were from healthy seedlings under normal growth conditions but also included some pathogen infected seedlings [54]. The reference transcriptome has a total of 279,510 unique transcripts.

Differential transcripts expression analysis
Statistical analysis of differential expression was performed using the edgeR v3.24.3 package in R (v3.6.0) [63] using default parameters [64], except for the cut-off of false discovery rate (FDR) in treated samples that was modified as described below. EdgeR uses the Poisson distribution model to examine differential expression of replicated count data, which makes it simpler than methods that use other statistical distributions [65]. Transcripts were first filtered retaining only those with a minimum expression change of 2 fold and with a minimum of 100 counts per million of a single transcript in at least two part x treatment x time groups. To adjust for library sizes and skewed expression of transcripts, the estimated abundance values were normalized using the trimmed mean of M-values normalization method included in edgeR. To detect differential transcript expression between the needles and the bark, the samples taken at T0 were used as these comprised a single plant from each of the 18 families (as treatments were not applied at this stage) and an FDR value of 0.05 was used. However, to establish transcript expression after treatment, instead of using an FDR of 0.05, a more conservative sample-specific approach was used [66], where transcript expression was initially compared between the samples collected from the control plants (*n* = 6), MJ-allocated (*n* = 6) or strip-allocated (*n* = 6) groups at T0 (before treatment) to check the inherent (potentially random) differences between sample groups. The *p*-values at which no differential expression was detected between these groups was set as the FDR for downstream pairwise comparisons. Accordingly, the *p*-value for detecting differentially expressed transcripts (DET) in the treated needles following both MJ and bark stripping was set at 1.0 × 10⁻¹¹. A *p*-value of 1.0 × 10⁻¹⁸ was set to detect DET in MJ treated bark and 1.0 × 10⁻¹⁰ to detect DET in the bark stripped samples. Twelve pairwise comparisons were performed. An upset diagram was generated using the UpSetR function in R to summarise the transcripts that were identified as significantly differentially expressed across different comparisons.

Principal component and unsupervised cluster analyses were performed to detect the dominant, relative expression patterns across the needles, bark and treatments. Following Ralph et al. [13], a subset of 500 transcripts with the highest variability and highest expression across the 143 libraries were selected in edgeR for this analysis. Principal components analysis (PCA), using FactoMinerR version 1.41 [67] was based on the correlation matrix among all identified transcripts. Clustering and heat maps were generated using the heatmap.2 function from the gplots package in R, with a matrix of Euclidean distances from the log2 counts of normalised transcripts.
Sequence similarity search

For sequence similarity search and functional analysis of differentially expressed transcripts (DETs) the transcripts were blasted against the nucleotide BLAST database using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST analysis revealed that *P. radiata* transcripts were most similar to those predicted from genome sequences of *P. taeda* (BLASTn with e-value < 0.0001). Other species, mostly *P. sylvestris, P. monticola, Picea stichensis and Pseudotsuga menziesii,* showed high similarity with the *P. radiata* transcripts. Annotations of selected transcripts were done by comparing *P. radiata* transcripts to the sequences in the SwissProt database of annotated genes [68] using cut-off values ≤ 1. To gain clear patterns of the responses, only transcripts associated with genes of known function were included. However, there were many uncharacterised transcripts and proteins of unknown functions.

GO classification

Gene ontology (GO) classification was undertaken to understand the biological process, cellular component and molecular function categories represented in the genes exhibiting differential expression. These assignments were done for selected transcripts identified above using protein analysis through evolutionary relationships (PANTHER) version 14.1 [69]. This was first undertaken using transcripts that were differentially up-regulated in the needles over the bark and vice versa, with the aim of understanding the constitutive differences of the GO processes between the transcriptome of the needles and the bark. Secondly, the GO classification was performed on selected T1 transcripts to understand the differences in the up-regulated and down-regulated transcripts after treatment, as well as differences in the induced transcriptome of the strip and MJ treated samples. Due to the limited annotation resources available for conifers, gene family annotations were obtained using genomes of 10 species: *Arabidopsis thaliana, Citrus sinensis, Cucumis sativus, Oryza sativa, Populus trichocarpa, Prunus persica, Saccharomyces cerevisiae, Theobroma cacao, Vitis vinifera* and *Zea mays.* GO term classification was done for the top differentially expressed transcripts in the different conditions (time × treatment × part).

Results

The *Pinus radiata* reference transcriptome and read mapping

RNA-seq of *P. radiata* generated a total of 2860 million 100-bp PE reads with a minimum of 20 million reads from each of the 143 samples. 87.6% of the reference transcriptome was represented among the study transcripts. However, after the filtration criteria described above, only 6312 unique transcripts (2.6% of the reference transcriptome) were retained as the expression of the other transcripts was too low. The analysis was constrained to individual transcripts, which may not be unigenes.

Differential expression of the transcriptome

The overall relationships between the transcriptome from the different samples were visualised using a principal component analysis (PCA) plot (Fig. 1) and the unsupervised hierarchical clustering (Fig. 2) of the top 500 variable transcripts in the transcriptome. Both figures show that the major differences in expression were due to plant parts (differences along the x-axis of Fig. 1 and the top x-axis of Fig. 2). Within plant parts, we noted genes that were:

(i) up-regulated in the needles relative to the bark and generally non-responsive to treatment;
(ii) up-regulated in the bark relative to the needles and generally non-responsive to treatment;
(iii) up-regulated in either the needles or the bark and responsive to treatment; and
(iv) not differentially expressed between the needles and the bark but responded to treatment by up- or down-regulation.

Differences in the constitutive needle and bark transcriptome

Of all 6312 transcripts considered for analysis, 5 transcripts were detected only in the needles and 13 transcripts were detected only in the bark. Most of these part-specific transcripts were uncharacterised (Table 2). Gene level annotation of the top 10 transcripts expressed in each plant part are listed in Table 3 (superscript refers to ID number in Table 3). The type 2 light-harvesting chlorophyll a/b-binding polypeptide[1] that is possibly involved in photosynthesis, was the most expressed gene in both the needles and the bark and was represented by different copies of transcripts (isoforms). The needles had other photosynthesis-related genes expressed such as ribulose bisphosphate carboxylase/oxygenase (RuBisCO)[12] and PSI-D1 precursor[17] possibly due to its major role in photosynthesis. Genes related to secondary metabolism were also detected among these top 10 genes, suggesting that constitutive defence is important in *P. radiata.* These included dehydrin[2], metallothionein[3], chalcone synthase[4], defensin[5] and pathogenesis-related proteins[8] and were represented by more transcripts in the bark than in the needles but their relative expression was not statistically significantly different between the needles and the bark.
At T0, 5469 out of the 6312 transcripts (86.6%) were differentially expressed between the needles and the bark. Of these, 3123 were up-regulated in the bark compared to the needles, while 2346 transcripts were up-regulated in the needles. The top 10 most strongly up-regulated transcripts in each of the bark and needles are shown in Table 4 (superscripts are identifiers to help locate the needle (N) or bark (B) transcripts in the ID column of the table). Besides the general function genes and those related with photosynthesis, there was an up-regulation of genes related to terpene [B9] and lipids biosynthesis [B7] in the bark and those related to sugars [N4] and phenolics biosynthesis [N1] in the needles. Of note is the up-regulation of genes involved in sugar transport in both the needles [N3] and the bark [B2], but these are different genes.

To assess the overall constitutive functional differences in transcripts differentially upregulated in the needles and the bark, the GO annotation of the top 100 differentially upregulated genes in both plant parts was obtained. There were quantitative differences for all the molecular but not biological or cellular GO categories. In the molecular GO category, a greater proportion of the top upregulated genes in the needles were ascribed to catalytic activity in the needles than in the bark (Fig. 3).

**Overall transcript expression in the needles and the bark after treatment**

After treatment, considering all time points, a total of 1479 (23.4%) transcripts were differentially expressed at one time or another. More transcripts responded to treatment in the needles than in the bark and more transcripts were up-regulated than down-regulated (Fig. 4). For both treatments, most differential expression was detected 7 days (T7) after treatment and declined thereafter, although differential expressed transcripts were still evident in both treatments 21 days later (Fig. 4). MJ was applied to both bark and needles and caused more transcript expression than bark stripping in both the needles and the bark (Fig. 4). Indeed, no differential expression of transcripts was detected in the needles following bark stripping. Of the transcripts that were differentially expressed between the bark and needles at T0, only 20% and 1% of those respectively responded following either of the treatments in the bark and needles suggesting that the transcripts that did not differ constitutively (i.e. at T0) between the needles and the bark were more responsive to treatment. One uncharacterised transcript (NZPradTrx091980_C05) that was not present in the transcriptome of untreated samples was present after treatment. One isoform of ribulose bisphosphate...
carboxylase preprotein (NZPradTrx098233_C06) that is involved in photosynthesis was present before treatment but was missing in all the samples in the bark and the needles after treatment, including the untreated control samples.

Annotations of the top ten genes that were up-regulated or down-regulated for each condition (time × treatment × part) are presented in Table 5. Based on these genes, various functions were detected, indicating that multiple genes are involved in coordinating plant responses to stress. Most of the genes were up-regulated, for example genes associated with primary metabolism, secondary metabolism, digestive inhibitors, pathogenesis-related (PR) protein families, genes involved with physical strengthening of the cell-wall, transcription factors, phytohormones and signalling molecules as well as molecules involved in broad biotic and abiotic stress responses and broad function genes. In contrast, the general catalysts as well as molecules involved in transcription were down-regulated. A subset (968 out of 1479 = 64.7%) of the differentially expressed transcriptome studied was differentially expressed in only one treatment (strip or MJ) (Fig. 5, Table 5). Similarly, non-overlapping differentially expressed transcripts, occurring in only one condition, were detected at different times in the needles and bark (Fig. 5, Table 5).

**Gene expression after MJ treatment**

A stronger response to the MJ treatment was detected in the needles than the bark, where 2206 versus 683 out of 6312 transcripts studied were differentially expressed, respectively (Fig. 4). Annotations of the non-overlapping, differentially expressed transcripts showed that MJ caused the unique differential expression of more genes that are directly involved in the metabolism of sugars,
fatty acids and amino acids in both the bark and the needles compared with the bark stripping (Table 6).

Six transcripts were consistently differentially expressed from T7 – T21 (Fig. 5) in the methyl jasmonate-induced transcriptome of the bark (B-MJ) and these were mostly up-regulated. Annotations of these transcripts showed that the genes were mostly involved in generating energy from various substrates, particularly glucose and fatty acids. In the needles treated with methyl jasmonate (N-MJ), 114 transcripts were consistently differentially expressed from T7 - T21 (Fig. 5). These genes were mostly directly associated with defence as well as chemical and physical structures, for example those involved in phenolic biosynthesis and structural components of the cell wall (Table 5).

**Gene expression after bark stripping**

Bark stripping did not cause any systemic response in the needles at any time point (Fig. 4). The strip induced bark transcriptome had, among the top genes, those involved in defence against pathogens, such as chitinases\(^{17}\), PR10\(^{39}\) and defensins\(^{18}\). Bark stripping also caused differential expression of water-stress responsive genes\(^{12,39}\) as well as genes related to replacement of tissues\(^{34}\) (Table 6). The difference in the representation of genes is likely related to the kind of damage incurred by the two stressors.

Both stressors caused differential expression of genes related to secondary metabolism (Table 5), including metabolism of monoterpenes (e.g. geranyl diphosphate synthase), phenolics (e.g. laccases) and alkaloids (e.g. phenylalanine ammonia-lyase). The differential expression of genes associated with lignification of cell walls were also identified for both treatments in the needles and the bark, emphasising the role of cell wall physical properties in stress responses. For some genes, the same gene was represented by different isomorphs in the different conditions such as geranyl diphosphate synthase in B-strip and N-MJ treatment/part combinations shown in Table 5. Only 6 differentially expressed genes were consistently differentially expressed following both treatments across all times and plant parts, except that no differential expression occurred in the needles following the strip treatment. Annotations of these transcripts mostly showed genes related to amino acid synthesis.

### Table 2

| Scion transcript code     | Gene name                  | Gene function                                                                 |
|---------------------------|----------------------------|-------------------------------------------------------------------------------|
| **Transcripts expressed in the needles but not in the bark at T0** |
| NZPradTrx008090_C01       | Unknown                    |                                                                               |
| NZPradTrx102814_C01       | Hypothetical protein 0_2136_01 |                                                                               |
| NZPradTrx14705_C04        | PREDICTED: uncharacterized LOC101213828 | Key determinant of many cell wall proteins [https://www.uniprot.org/uniprot/Q40375](https://www.uniprot.org/uniprot/Q40375) |
| NZPradTrx19356_C01        | Repetitive proline-rich cell wall protein 2 precursor, putative |                                                                               |
| NZPradTrx38443_C01        | Unknown                    |                                                                               |
| **Transcripts expressed in the bark but not in the needles at T0** |
| NZPradTrx105287_C05       | Chloroplast ELIP early light-induced protein | Prevents photooxidative stress (Hutin et al. 2003) |
| NZPradTrx068786_C02       | Unknown                    |                                                                               |
| NZPradTrx110900_C02       | Unknown                    |                                                                               |
| NZPradTrx158724_C01       | Unknown                    |                                                                               |
| NZPradTrx11161_C02        | Embryo-abundant protein    | May act as a cytoplasm protectant during desiccation. [https://www.uniprot.org/uniprot/P46520](https://www.uniprot.org/uniprot/P46520) |
| NZPradTrx032755_C01       | Unknown                    |                                                                               |
| NZPradTrx054373_C01       | Unknown                    |                                                                               |
| NZPradTrx151188_C01       | Unknown                    |                                                                               |
| NZPradTrx007008_C01       | Unknown                    |                                                                               |
| NZPradTrx069030_C01       | Unknown                    |                                                                               |
| NZPradTrx081218_C01       | Unknown                    |                                                                               |
| NZPradTrx154223_C01       | PREDICTED: tetrahydrocannabinolic acid synthase-like | Catalyzes the oxidative cyclization of the monoterpen moiety in cannabigerolic acid [https://www.uniprot.org/uniprot/Q8GTB6](https://www.uniprot.org/uniprot/Q8GTB6) |
| NZPradTrx189870_C01       | Uninformative              |                                                                               |
Table 3  Top most expressed transcripts (identified by the percentage number of transcripts represented) in the constitutive transcriptome of the bark and the needles as assessed at T0 (sampled before treatment), indicating their identification number, Scion transcript code, gene name and predicted function. Some transcripts were represented by different copies of the transcripts (isoforms—represented by different transcript codes in each row) and the percentages of transcripts represented by each isoform are indicated. Each isoform has a superscript linking it to its corresponding percentage number of transcripts identified. Ba = first isoform identified in the bark for the gene, Na = first isoform one identified in the needles etc. The transcripts were not significantly differentially expressed between the bark and the needles. Some transcripts were selected in both plant parts.

| ID number | Scion transcript code (or isoforms) | Gene name | Predicted gene function | Percentages of transcripts (out of 6312) |
|-----------|-------------------------------------|-----------|-------------------------|---------------------------------------|
|           |                                     |           |                         | Bark                                  | Needles                                |
| 1         | NZPradTrx107583_C02^{Ba, Na}         | Light-harvesting chlorophyll a/b-binding polypeptide (Lhcb2) mRNA | Absorb sunlight and transfer the excitation energy to the core complexes of PSII in order to drive photosynthetic electron transport (Liu et al. 2013) [70, 71] | 1.46^{Ba}, 0.28^{Bb}, 0.25^{Bc}, 1.99^{Na}, 0.95^{Nb}, 1.07^{Nc}, 0.51^{Nd}, 0.51^{Ne}, 0.33^{Ne} | |
| 2         | NZPradTrx100458_C02^{Ba}             | Dehydrin 7 | Involved in dehydration stress (Stival Sena et al. 2018) [72] | 1.38^{Ba}, 0.60^{Bb} | |
| 3         | NZPradTrx112612_C02^{Ba, Na}         | Metallothionein 3 | Play important roles in metal homeostasis and protection against heavy metal toxicity (Nevrtalova et al. 2014) [73] | 0.82^{Ba}, 0.29^{Bb}, 0.58^{Nc}, 1.75^{Na}, 0.66^{Nb} | |
| 4         | NZPradTrx052720_C01^{Ba}             | Chalcone synthase | Plays crucial roles in phenolic biosynthesis (Dixon and Paiva 1995) [74] | 0.70^{Ba}, 0.37^{Bb}, 0.35^{Bc}, 0.27^{Bd}, 0.26^{Be}, 0.30^{Be} | |
| 5         | NZPradTrx050994_C02^{Ba}             | Defensin | Inhibit the growth of a broad range of pathogens, including bacteria, fungi and viruses (Ernkalova et al. 2016; Picart et al. 2013) [75, 76]. | 0.61^{Ba}, 0.53^{Bb} | |
| 6         | NZPradTrx076819_C01                  | TCTP-like protein | Implicated in important cellular processes, such as cell growth, cell cycle progression, malignant transformation and in the protection of cells against various stress conditions and apoptosis (Bommer and Theile 2004) | 0.42 | |
| ID number | Scion transcript code (or isoforms) | Gene name | Predicted gene function | Percentages of transcripts (out of 6312) |
|-----------|------------------------------------|-----------|-------------------------|----------------------------------------|
| 7         | NZPradTrx062252_C01 B^a NZPradTrx107621_C01 B^b | Nonspecific lipid transfer protein | Play important roles in resistance to biotic and abiotic stress. Have the ability to bind or transfer various types of hydrophobic molecules in vitro, such as fatty acids, fatty acyl-CoA, phospholipids, glycolipids and cutin monomers (Liu et al. 2015a) | 0.27 B^a, 0.26 B^b |
| 8         | NZPradTrx116410_C12                  | Pathogenesis-related protein 10 | Show biological activities related to disease resistance (Liu and Ekramod-doullah 2006) | 0.26 |
| 9         | NZPradTrx077717_C01                  | LP3-1 | Implicated in water-stress | 0.24 |
| 10        | NZPradTrx100333_C02                  | ASR protein | Involved in sugar and abscisic acid signalling (Çakir et al. 2003) | 0.25, 0.24 |
| 11        | NZPradTrx098632_C01                  | Translation elongation factor-1 alpha | Catalyses the transfer of aminoacylated-tRNAs (Sasikumar et al. 2012) | 1.57 Na, 0.59 Nb, 0.53 Nc, 0.36 Nd, 0.30 Ne, 0.22 Nf |
| 12        | NZPradTrx098233_C03 Na NZPradTrx064995_C01 Nb NZPradTrx064875_C01 Nc NZPradTrx098233_C01 Nd NZPradTrx098233_C05 Ne NZPradTrx064875_C02 Nf | Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) | Catalyses carboxylation of RuBP in the first step of the Calvin cycle of photosynthesis (Tabita 1999) | 0.37 |
| 13        | NZPradTrx098207_C02 Na NZPradTrx098207_C01 Nb | Cysteine proteinase inhibitor CPI-3 | Involved in plant development and defence, especially in the regulation of stress responses (Li et al. 2015) | 0.77 Na, 0.27 Nb |
| 14        | NZPradTrx105813_C01                  | PREDICTED: probable fructose-bisphosphate aldolase 2, chloroplastic-like | Plays a key role in glycolysis and gluconeogenesis | 0.37 |
| 15        | NZPradTrx111299_C01 Na NZPradTrx100425_C01 Nb | PREDICTED: oxygen-evolving enhancer protein 1, chloroplastic-like isoform 2 | Stabilizes the manganese cluster which is the primary site of water splitting | 0.35 Na, 0.32 Nb |
| 16        | NZPradTrx065162_C02                  | Thiazole biosynthetic enzyme | Thiamine synthesis and DNA damage tolerance (Liu et al. 2015b) | 0.34 |
| ID number | Scion transcript code (or isoforms)    | Gene name          | Predicted gene function                                                                 | Percentages of transcripts (out of 6312) |
|-----------|--------------------------------------|--------------------|-----------------------------------------------------------------------------------------|------------------------------------------|
| 17        | NZPradTrx184720_C01                  | PSI-D1 precursor   | PsaD can form complexes with ferredoxin and ferredoxin-oxidoreductase in photosystem I (PS I) reaction centre. [https://www.uniprot.org/uniprot/Q9S7H1](https://www.uniprot.org/uniprot/Q9S7H1) | 0.22                                     |
### Table 4
Top 10 up-regulated genes differentially expressed between the bark and needles at T0 (before treatment) for each plant part. The table also shows the ID of the genes assigned in this study for ease of identification in the tables, Scion transcripts code, predicted gene name and function.

| Part     | ID           | Scion transcript code       | Predicted gene name                        | Predicted gene function                                                                 |
|----------|--------------|-----------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------|
| Bark     | B1           | NZPradTrx054097_C01         | Homeobox transcription factor KN3           | Central regulators of meristem cell identity (Guillet-Claude et al. 2004)                |
|          | B2           | NZPradTrx073079_C03         | Transporter, putative                      | Sugar transport (Weig et al. 1994)                                                     |
|          | B3           | NZPradTrx087709_C01         | Homeobox transcription factor KN1           | Central regulators of meristem cell identity (Namroud et al. 2010)                      |
|          | B4           | NZPradTrx055579_C01         | Mini zinc finger 1                          | Regulates several development aspects, including photomorphogenesis, apical dominance, longevity, flower morphology and fertility, as well as root and stem elongation (https://www.uniprot.org/uniprot/Q9CAS1) |
|          | B5           | NZPradTrx048496_C01         | Plastid phosphate translocator             | Involved in the exchange of metabolites and inorganic phosphate between stroma and cytosol (Bockwoldt et al. 2019) |
|          | B6           | NZPradTrx101882_C02         | Auxin-induced protein SNG4, putative        | Transmembrane transporter activity especially during root formation (Busov et al. 2004)   |
|          | B7           | NZPradTrx103825_C01         | PREDICTED: GDSL esterase/lipase At5g03610-like | Lipid catabolic process (https://www.uniprot.org/uniprot/Q9LZ57)                     |
|          | B8           | NZPradTrx184572_C01         | G1-like protein                            | Polymerizes the backbones of non-cellulosic polysaccharides (hemicelluloses) of plant cell wall  |
|          | B9           | NZPradTrx055645_C01         | PREDICTED: squalene monooxygenase-like      | Converts squalene into oxidosqualene, the precursor of all known angiosperm cyclic triterpenoids (Rasbery et al. 2007) |
|          | B10          | NZPradTrx093053_C01         | Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit | Catalyses carboxylation of RuBP in the first step of the Calvin cycle of photosynthesis (Tabita 1999) |
| Needles  | N1           | NZPradTrx115678_C04         | Anthocyanidin reductase                     | Involved in the biosynthesis of proanthocyanidins (Zhu et al. 2015)                     |
|          | N2           | NZPradTrx115678_C05         | Cytochrome P450 CYP42A                      | Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (https://www.uniprot.org/uniprot/A9F9S4) |
|          | N3           | NZPradTrx114954_C01         | Glucosyltransferase                        | Transfer of glucose (Chen et al. 2016)                                                  |
|          | N4           | NZPradTrx086877_C02         | Glucose-1-phosphate adenylytransferase, putative | Involved in the pathway starch biosynthesis (https://www.uniprot.org/uniprot/Q688T8)  |
|          | N5           | NZPradTrx086324_C01         | PREDICTED: LOB domain-containing protein 1-like | Involved in the repression of the homeobox gene BP (https://www.uniprot.org/uniprot/Q9FKZ3-1) |
|          | N6           | NZPradTrx065580_C01         | Catalase                                   | Crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen (Nandi et al. 2019) |
|          | N7           | NZPradTrx049683_C01         | Photosystem II core complex proteins psbY2C chloroplast precursor | Multi-component pigment-protein complex responsible for water splitting, oxygen evolution, and plastoquinone reduction (Lu 2016) |
|          | N8           | NZPradTrx097448_C02         | ribonucleoprotein, chloroplast, putative   | Involved in chloroplast RNA processing (Tillich et al. 2009)                            |
|          | N9           | NZPradTrx119685_C01         | SOUL heme-binding protein                  | Plays an active role in primary plant metabolic pathways as well as in stress signalling (Shanmugabalaji et al. 2020) |
|          | N10          | NZPradTrx184701_C01         | chloroplast ribosomal protein S1           | Involved in translation initiation via positioning of initiation mRNA–protein complexes (mRNPs), and the potential involvement of these unique domains in the processivity of chloroplast translation (Manuell et al. 2007) |
Time progression of genes

Not only did the treatments differ in the magnitude of their general response through time (Figs. 1, 4 and 5), but the pattern of response of individual genes differed between treatments. For the top ten expressed transcripts in the constitutive transcriptome (assessed at T0) of the bark and the needles (ID numbers 1 to 10 in Table 3), Fig. 6 shows the time progression of differential expression following stripping and methyl jasmonate application.

There was a tendency for genes to be up-regulated or down-regulated following both treatments. Of the three genes (dehydrin, light-harvesting chlorophyll a/b-binding polypeptide and metallothionein) that showed marked down-regulation, only dehydrin showed
Table 5  Top 10 genes differentially expressed in each of the time periods from T7 to T21 in the bark (B) and needles (N) following bark stripping (S) or methyl jasmonate (MJ) treatment of two-year old *Pinus radiata* plants. The Scion transcript code, predicted gene name and predicted functions of the known genes are indicated. Some genes were represented by more than one transcript (isoforms—different Scion *P. radiata* transcript codes that represent one gene in column 1) and multiple copies of an isoform as indicated by the numbers in the parentheses, for example + (2) = two copies of an isoform relating to the gene were identified, where + = up-regulation, – = down-regulation. The superscript following numbers in the parentheses following the gene names represent the core function of the gene among the 11 broad categories listed in the table footnote. For example for the Peptide transporter PTR3-A-like a the superscript a denotes that this gene was associated with primary metabolism (see footnote). However, it is recognised that some genes may fall in more than one category. Gene functions are mostly from Uniprot [77].

| Scion transcript code | Gene name | Function | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|-----------------------|-----------|----------|---------|--------|---------|----------|--------|---------|----------|--------|---------|
| NZPradTrx081530_C01   | Peptide transporter PTR3-A-like a | Facilitates amino acid induction (Barnes et al. 1998) | +       |        |         |         |        |         |         |        |         |
| NZPradTrx115883_C01   | Granule-bound starch synthase, partial b | Responsible for amylose synthesis (Miao et al. 2014) | –       |        |         |         |        |         |         |        |         |
| NZPradTrx13785_C01    | GDP-D-mannose-3',5'-epimerase a | Central enzyme of the major ascorbate biosynthesis pathway in higher plants that converts GDP-D-mannose to GDP-L-galactose (Gilbert et al. 2009) | +       |        |         |         |        |         |         |        |         |
| NZPradTrx065162_C02   | Thiazole biosynthetic enzyme b | Thiamine synthesis and DNA damage tolerance (Liu et al. 2015b) | –       |        |         |         |        |         |         |        |         |
| NZPradTrx083866_C01   | 1-aminocyclopropane-1-carboxylate oxidase 3 g | Production of ethylene, that functions as a mediator of responses to external stimuli, such as wounding (Houben and Ván de Poel 2019) | +       | +      | +       | +       |        |         |         |        |         |
| NZPradTrx17447_C01    | PREDICTED: transcription factor bHLH126-like a | Transcription factors play a central role in a number of biological processes, producing, for example, the induction of specific genes in response to particular stimuli as well as controlling the cell type specific or developmentally regulated expression of other genes (Latchman 2008) | + (2)   | + (2)  |         |         |        |         |         |        |         |
| NZPradTrx091619_C02   | Oleoyl-acyl carrier protein thioesterase, partial b | Plays an essential role in chain termination during de novo fatty acid synthesis [https://www.uniprot.org/uniprot/Q42561](https://www.uniprot.org/uniprot/Q42561) | – (2)   | –      |         |         |        |         |         |        |         |
| Scion transcript code | Gene name | Function | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|-----------------------|-----------|----------|---------|--------|---------|----------|---------|---------|----------|---------|---------|
| NZPradTrx111880_C01   | Cell wall invertase<sup>a</sup> | Mediates reduced export of sucrose or enhanced import of hexoses at the site of infection (Proels and Hückelhoven 2014) [78] | + | + | + | (2) |
| NZPradTrx132560_C01   | DNA binding protein, putative<sup>i</sup> | DNA binding proteins serve two principal functions: to organize and compact the chromosomal DNA and to regulate and effect the processes of transcription, DNA replication, and DNA recombination (Travers 2001). | + | – | – | – |
| NZPradTrx186688_C01   | DNA binding protein, putative<sup>i</sup> | DNA binding proteins serve two principal functions: to organize and compact the chromosomal DNA and to regulate and effect the processes of transcription, DNA replication, and DNA recombination (Travers 2001). | + | – | – | – |
| NZPradTrx065807_C02   | PREDICTED: cleavage and polyadenylation specificity factor subunit 5-like<sup>i</sup> | Component of the cleavage factor Im (CFIm) complex that functions as an activator of the pre-mRNA 3'-end cleavage and polyadenylation processing required for the maturation of pre-mRNA into functional mRNAs [https://www.uniprot.org/uniprot/Q16630] | – |
| NZPradTrx095732_C01   | Thaumatin-like protein<sup>d</sup> | Involved in local responses of roots to colonization by non-pathogenic plant growth-promoting rhizobacteria (PGPR) fluorescent Pseudomonas spp. (Léon-Kloosterziel et al. 2005) | –;+ (2) | + | + | + | + |
| NZPradTrx064724_C01   | Chloroplast threonine deaminase 1 precursor<sup>c</sup> | Useful in isoleucine (Ile) biosynthesis and impairing digestive processes in the insect gut (Chenet et al. 2007) | + | + | + | + | + | + | + |
| NZPradTrx111230_C01   | Triacylglycerol lipase, putative<sup>a</sup> | Releases fatty acids from a number of different substrates (Padham et al. 2007) | – |
| Scion transcript code     | Gene name                                    | Function                                                                                                                                                                                                                                                                                                                                 | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|---------------------------|----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------|---------|---------|---------|---------|---------|---------|---------|
| NZPradTrx084103_C02       | PREDICTED: glutamate-cysteine ligase, chloroplastic-like<sup>9</sup> | Seems to play an important role in controlling the expression of resistance responses like the regulation of salicylic acid (SA) and phytoalexin (camalexin) production. Involved in resistance to fungal and bacterial pathogens. [https://www.uniprot.org/uniprot/P46309](https://www.uniprot.org/uniprot/P46309) | +       |        |         |         |         |         |         |         |
| NZPradTrx074370_C02, NZPradTrx132647_C01 | PREDICTED: lysine histidine transporter 2-like<sup>8</sup> | Amino acid-proton symporter. Transporter with a broad specificity for neutral and acidic amino acids [https://www.uniprot.org/uniprot/Q9LRB5](https://www.uniprot.org/uniprot/Q9LRB5) | +       | +      | +       | +       | +       | +       | (2)     |         |
| NZPradTrx098051_C01       | PREDICTED: endo-1,3,1,4-beta-D-glucanase-like<sup>1</sup> | Implicated in responses to stress, wounding, and pathogen infection (Rezzonico et al. 1998)                                                                                                                                       | +       |        |         |         |         |         |         |         |
| NZPradTrx053937_C01       | 2-methyl-6-phytylbenzoquinone methyltransferase<sup>1</sup> | One of the regulators of the composition of tocopherols-class of compounds that function as lipid soluble antioxidants that are extremely potent quenchers of singlet oxygen and free radical species (Shintani et al. 2002) | –       |        |         |         |         |         |         |         |
| NZPradTrx119228_C01       | 4-hydroxyphenyl-pyruvate dioxygenase<sup>a</sup> | Plays an important role in degrading aromatic amino acids (Fritze et al. 2004)                                                                                                                                                                                                 | +       |        |         |         |         |         |         |         |
| NZPradTrx184501_C01       | PREDICTED: S05 ribosomal protein L6, chloroplastic-like<sup>1</sup> | Binds directly to 23S ribosomal RNA and is located at the aminoacyl-tRNA binding site of the peptidyltransferase centre. [https://www.uniprot.org/uniprot/O23049](https://www.uniprot.org/uniprot/O23049) | –       |        |         |         |         |         |         |         |
| NZPradTrx186075_C01       | PREDICTED: hexokinase-1-like<sup>a</sup> | Fructose and glucose phosphorylating enzyme [https://www.uniprot.org/uniprot/Q42525](https://www.uniprot.org/uniprot/Q42525)                                                                                                                                                                        | –       |        |         |         |         |         |         |         |
| NZPradTrx105399_C03       | PREDICTED: leucine-rich repeat-containing protein 40-like<sup>a</sup> | Plays crucial roles in development and stress responses (Liu et al. 2017) [17, 79]                                                                                                                                                                                            | –       |        |         |         |         |         |         |         |
| Scion transcript code | Gene name | Function | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|-----------------------|-----------|----------|---------|--------|---------|----------|---------|---------|----------|---------|---------|
| NZPradTrx051602_C02   | Sodium-bile acid cotransporter, putative<sup>a</sup> | Is involved in photorespiratory metabolism (South et al. 2017) | – |
| NZPradTrx082621_C01   | Mitogen activated protein kinase 6<sup>a</sup> | Involved in oxidative stress-mediated signalling cascade (such as ozone) | + |
| NZPradTrx093779_C01   | PREDICTED: pentatricopeptide repeat-containing protein At1g62670, mitochondrial-like<sup>1</sup> | Binds one or several organelar transcripts, and influences their expression by altering RNA sequence, turnover, processing, or translation (Barkan and Small 2014) | – |
| NZPradTrx184660_C01   | PREDICTED: PGR5-like protein 1A, chloroplastic-like<sup>a</sup> | Ferredoxin-plastoquinone reductase involved in cyclic electron flow (CEF) around photosystem | – |
| NZPradTrx097586_C01   | Type III chlorophyll a/b-binding protein<sup>a</sup> | Functions as a light receptor, capturing and delivering excitation energy to photosystems with which it is closely associated | – |
| NZPradTrx101698_C02   | PrMC3<sup>b</sup> | Predicted to encode a chalcone-synthase-like protein (Walden et al. 1999) | – | – |
| NZPradTrx117804_C07   | PREDICTED: probable carboxylesterase 2<sup>a</sup> | Carboxylesterases hydrolyse esters of short-chain fatty acids (Marshall et al. 2003) | – |
| NZPradTrx100227_C01   | PREDICTED: medium-chain-fatty-acid–CoA ligase<sup>a</sup> | Catalyses the esterification, concomitant with transport, of exogenous fatty acids into metabolically active CoA thioesters for subsequent degradation or incorporation into phospholipids | + |
| NZPradTrx081530_C01   | PREDICTED: peptide transporter PTR3-A-like<sup>a</sup> | Facilitates amino acid induction (Barnes et al. 1998) | + |
| Scion transcript code | Gene name | Function | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|-----------------------|-----------|----------|---------|--------|---------|----------|---------|---------|----------|---------|---------|
| NZPradTrx192941_C01   | Beta-amylase | Involved in starch breakdown in plants (Kaplan and Guy 2004) | +       |        |         |          |         |         |         |         |         |         |
| NZPradTrx052040_C01   | PREDICTED: oleosin 16kDa-like | May have a structural role to stabilize the lipid body during desiccation of the seed by preventing coalescence of the oil. [https://www.uniprot.org/uniprot/Q42980](https://www.uniprot.org/uniprot/Q42980) | -       |        |         |          |         |         |         |         |         |         |
| NZPradTrx108711_C04   | PREDICTED: putative UDP-rhamnose:rhamnosyltransferase 1-like | Involved in fatty acid metabolism (van der Sluis and Erasmus 2016) | +       |        |         |          |         |         |         |         |         |         |
| NZPradTrx112833_C07   | Tify domain containing protein | Found in a variety of plant transcription factors [https://pfam.xfam.org/family/PF06300](https://pfam.xfam.org/family/PF06300) | +       | +      | +       |          |         |         |         |         |         |
| NZPradTrx071306_C02   | PREDICTED: transmembrane ascorbate ferrireductase 1-like | Catalyses ascorbate-dependent trans-membrane ferric-chelate reduction [https://www.uniprot.org/uniprot/Q8L856](https://www.uniprot.org/uniprot/Q8L856) | +       |        |         |          |         |         |         |         |         |         |
| NZPradTrx12833_C08    | Tify domain containing protein | Found in a variety of plant transcription factors [https://pfam.xfam.org/family/PF06300](https://pfam.xfam.org/family/PF06300) | +       | +      | +       |          |         |         |         |         |         |         |
| NZPradTrx119456_C01   | PR10-1.13 | Involved in defence against pathogen infection and other environmental stresses (Liu et al. 2005) | +       |        |         |          |         |         |         |         |         |         |
| NZPradTrx051982_C01   | PREDICTED: histone H2B 2-like isoform 2 | Play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability [https://www.uniprot.org/uniprot/Q5QNW6](https://www.uniprot.org/uniprot/Q5QNW6) | -       |        |         |          |         |         |         |         |         |         |
| NZPradTrx119456_C01   | PR10-1.13 | Involved in defence against pathogen infection and other environmental stresses (Liu et al. 2005) | +       |        |         |          |         |         |         |         |         |         |
| NZPradTrx053878_C02   | Aldehyde dehydrogenase | Involved in plant metabolism and contributes to aldehyde homeostasis to eliminate toxic aldehydes (Zhao et al. 2017) | +(3)    | +      | +       |          |         |         |         |         |         |         |
| NZPradTrx053878_C03   | PREDICTED: lanC-like protein 2-like | May play a role in abscisic acid (ABA) signalling [https://www.uniprot.org/uniprot/F4IEM5](https://www.uniprot.org/uniprot/F4IEM5) | +       |        |         |          |         |         |         |         |         |         |
| Scion transcript code     | Gene name                                      | Function                                                                 | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|---------------------------|------------------------------------------------|--------------------------------------------------------------------------|---------|--------|---------|----------|---------|---------|----------|---------|---------|
| NZPradTrx115807_C06       | Hydrolase, putative                        | Enzyme which catalyses hydrolysis reaction, i.e. the addition of the hydrogen and hydroxyl ions of water to a molecule with its consequent splitting into two or more simpler molecules. [https://www.uniprot.org/keywords/KW-0378](https://www.uniprot.org/keywords/KW-0378) | +       |        |         |          |         |         |          |         |         |
| NZPradTrx112951_C03       | Embryo-abundant protein                    | May act as a cytoplasm protectant during desiccation. [https://www.uniprot.org/uniprot/P46520](https://www.uniprot.org/uniprot/P46520) |         | +      |         |          |         |         |          |         |         |
| NZPradTrx097637_C01       | PREDICTED: leucoanthocyanin dioxygenase-like | Involved in anthocyanin and protoanthocyanidin biosynthesis by catalysing the oxidation of leucoanthocyanidins into anthocyanidins. [https://www.uniprot.org/uniprot/Q96323](https://www.uniprot.org/uniprot/Q96323) | +       |        |         |          |         |         |          |         |         |
| NZPradTrx112166_C01       | Peroxidase-like protein, partial            | Response to oxidative stress. [https://www.uniprot.org/uniprot/Q24925](https://www.uniprot.org/uniprot/Q24925) | +       | +      |         |          |         |         |          |         |         |
| NZPradTrx082621_C01       | Mitogen activated protein kinase 6          | Plays a key role in the transduction of environmental and developmental signals through phosphorylation of downstream signalling targets. (Jagodzik et al. 2018) |         |        | +       |          |         |         |          |         |         |
| NZPradTrx110107_C07       | PREDICTED: transcription factor aborted microspores-like | Required for male fertility and pollen differentiation, especially during the post-meiotic transcriptional regulation of microspore development within the developing anther. [https://www.uniprot.org/uniprot/Q9ZVX2](https://www.uniprot.org/uniprot/Q9ZVX2) | +       |        |         |          |         |         |          |         |         |
| NZPradTrx112236_C02       | Laccase                                    | Involved in phenolic metabolism and functioning of cell wall (Ranocha et al. 2002) | +       |        |         |          |         |         |          |         |         |
| NZPradTrx089433_C01       | Lipoxygenase 2                             | Essential for formation of green leaf volatiles and five-carbon volatiles (Mochizuki et al. 2016) | +       |        |         |          |         |         |          |         |         |
| Scion transcript code | Gene name | Function | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|----------------------|-----------|----------|---------|--------|---------|---------|--------|---------|---------|--------|---------|
| NZPradTrx109272_C04  | Malic enzyme, putative\(^a\) | Catalyses the oxidative decarboxylation of malate to form pyruvate, a reaction important in a number of metabolic pathways (Zhang et al. 2016) | – | – |     |
| NZPradTrx107808_C01  | Putative flavoprotein-containing polyamine oxidase, partial\(^b\) | Involved in drought stress response and flavonoid biosynthesis (Kamada-Nobusada et al. 2008) | + |     |
| NZPradTrx049513_C01  | Putative proline-rich arabinogalactan protein 4\(^c\) | Contributes to the strengthening of cell walls of quickly growing organs (Hijazi et al. 2014) | + |     |
| NZPradTrx049513_C02  |          |          |         |        |         |         |        |         |         |        |         |
| NZPradTrx079868_C01  | PREDICTED: (RS)-norcoclaurine 6-O-methyltransferase-like\(^b\) | Involved in the biosynthesis of (S)-coclaurine, the common precursor of all benzylisoquinoline alkaloids. [https://www.uniprot.org/uniprot/Q6WUC1](https://www.uniprot.org/uniprot/Q6WUC1) | – |     |
| NZPradTrx054832_C01  | Aquaporin-like protein\(^b\) | Involved in transport of water and other small neutral molecules across cellular biological membranes (Kapilan et al. 2018) | + |     |
| NZPradTrx069597_C01  | Acetyl-CoA carboxylase BCCP subunit\(^a\) | Catalyses the first committed step of fatty acid synthesis, the carboxylation of acetyl-CoA to malonyl-CoA (Sasaki and Nagano 2004) | – |     |
| NZPradTrx117954_C05  | E-alpha-bisabolene synthase\(^b\) | Involved in defensive oleoresin formation in conifers in response to insect attack or other injury. Involved in sesquiterpene (C15) olefins biosynthesis [https://www.uniprot.org/uniprot/O81086](https://www.uniprot.org/uniprot/O81086) | + |     |
| NZPradTrx087252_C01  | TPA: putative GID1-like gibberellin receptor\(^a\) | Involved in gibberellin signaling (Sun 2011) | + |     |
| NZPradTrx074370_C01  | Putative proline transporter\(^b\) | Mediates the amino acid proline and glycine betaine transport [https://www.uniprot.org/uniprot/P92961](https://www.uniprot.org/uniprot/P92961) | +\(^2\) |     |
| Scion transcript code | Gene name                                                                 | Function                                                                 | T7-B-MJ | T7-B-S | T7-N-MJ | T14-B-MJ | T14-B-S | T14-N-MJ | T21-B-MJ | T21-B-S | T21-N-MJ |
|-----------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|---------|--------|---------|----------|---------|---------|----------|---------|---------|
| NZPradTrx113904_C06/  | PREDICTED: clavaminic synthase-like protein At3g21360-like  
                      | NZPradTrx101343_C01                                                      | Associated with metal ion binding and oxido-reductase activity  
                                                                 | https://www.uniprot.org/uniprot/Q9ULG0 | +       |         |          |         |         |         |         |

* primary metabolism  
* secondary metabolism  
* digestive inhibitors  
* pathogenesis-related (PR) protein families  
* genes involved with physical strengthening of the cell-wall  
* transcription factors  
* phytohormones and signalling molecules  
* general catalysts  
* molecules involved in transcription  
* molecules involved in broad biotic and abiotic stress responses  
* broad function genes
significant down-regulation at T7 in both strip and MJ treated samples.

**Functional classification of differentially expressed transcripts**

To assess the overall effect of the treatments across different gene families and molecular processes, the GO terms were determined for the up-regulated and down-regulated transcripts for each condition (time × treatment × plant part). There was an overall similarity in the GO terms for genes that were up- and down-regulated in the strip and methyl jasmonate treatments. For example, in the GO-molecular processes, differentially expressed genes were associated with catalytic activity both in the needles and the bark (Fig. 7, Supplementary Fig. 1). However, the proportion of the top 100 differentially expressed genes in the catalytic activity category varied markedly. For example in the bark, a great percentage of top down-regulated genes following bark stripping were in the catalytic activity category (72%) compared with the up-regulated genes (28%).

Comparing GO terms for the top differentially expressed genes in the constitutive (needles versus bark) and induced transcriptome, indicated that some gene functions that were not strongly expressed in the constitutive state (T0) were notably up-regulated or down-regulated after treatment, and this differential expression appears to be treatment specific (Fig. 7). For example, genes related to response to stimulus (GO:0050896), plasmodesma (GO:0009506) and cell junction (GO:0030054) were strongly up-regulated at T7 in the transcriptome of the bark stripped samples but not the methyl jasmonate samples. Accordingly, transcripts of many of the other GO categories were under expressed in the transcriptome of the bark stripped samples.

**Discussion**

We aimed to understand the differences in the constitutive needle and bark transcriptomes, the changes that occur following bark stripping and how they compare with those of methyl jasmonate that have been most commonly reported for conifer species [17, 24, 35, 80]. While the results are based on a partial transcriptome, comparing the needle and bark transcriptome as assessed prior to treatment (T0) showed that there were minimal qualitative differences in terms of the transcripts found
**Table 6** Number of differentially expressed (DETs) transcripts (up to a maximum of top10) that were unique (non-overlapping) for each condition (time × treatment × plant part) category. The table also shows the ID of the genes assigned in this study for ease of identification in the tables, Scion transcripts code, predicted gene name and function. These transcripts were not expressed at any other time or treatment. T7, T14 and T21 represents respectively 7, 14 and 21 days after application of methyl jasmonate (MJ) and bark strip (strip) treatments in the bark (B) or needles (N). (+) = up-regulated and (−) = down-regulated. Only transcripts with predicted gene functions are included. The predicted gene functions are mostly from UniProt [77].

| Condition | No. unique DETs | ID | P. radiata code | Gene name | Predicted gene function | Direction |
|-----------|----------------|----|-----------------|-----------|-------------------------|-----------|
| T7-B-MJ   | 96             | U1 | NZPradTrx115883_C02 | granule bound starch synthase 1a precursor | Involved in the pathway starch biosynthesis [https://www.uniprot.org/uniprot/P0C585](https://www.uniprot.org/uniprot/P0C585) | −         |
|           |                | U2 | NZPradTrx184661_C01 | PREDICTED: putative caffeoyl-CoA O-methyltransferase At1g67980-like | Involved in the reinforcement of the plant cell wall. Also involved in the responding to wounding or pathogen challenge by the increased formation of cell wall-bound ferulic acid polymers [https://www.uniprot.org/uniprot/Q9CW83](https://www.uniprot.org/uniprot/Q9CW83) | −         |
|           |                | U3 | NZPradTrx108036_C04 | Cytochrome b reductase | Required for the NADH-dependent electron transfer involved in the desaturation and hydroxylation of fatty acids and in the desaturation of sterol precursors [https://www.uniprot.org/uniprot/Q92N1](https://www.uniprot.org/uniprot/Q92N1) | −         |
|           |                | U4 | NZPradTrx19186_C01 | DEAD-box RNA helicase | Ubiquitous in RNA-mediated processes and function by coupling cycles of ATP binding and hydrolysis to changes in affinity for single-stranded RNA [https://ncbi.nlm.nih.gov/pmc/articles/PMC3032546/](https://ncbi.nlm.nih.gov/pmc/articles/PMC3032546/) | −         |
|           |                | U6 | NZPradTrx060156_C02 | PREDICTED: probable rhamnose biosynthetic enzyme 1 | Involved with nucleotide-sugar metabolic process [https://www.uniprot.org/uniprot/A0A1IU7W6H4](https://www.uniprot.org/uniprot/A0A1IU7W6H4) | +         |
|           |                | U7 | NZPradTrx119948_C01 | PREDICTED: protein HOTHEAD-like | Required to limit cellular interactions between contacting epidermal cells during floral development [Krolkowski et al. 2003](Krolkowski et al. 2003) | +         |
|           |                | U8 | NZPradTrx119070_C01 | PREDICTED: mitochondrial-processing peptidase subunit alpha-like | Substrate recognition and binding subunit of the essential mitochondrial processing protease (MPP), which cleaves the mitochondrial sequence off newly imported precursors proteins [https://www.uniprot.org/uniprot/P29677](https://www.uniprot.org/uniprot/P29677) | +         |
|           |                | U9 | NZPradTrx10606_C03 | snakin | Active against fungal and bacterial plant pathogens [Berrocal-Lobo et al. 2002](Berrocal-Lobo et al. 2002) | −         |
|           |                | U9 | NZPradTrx10606_C04 | snakin | Active against fungal and bacterial plant pathogens [Berrocal-Lobo et al. 2002](Berrocal-Lobo et al. 2002) | −         |
|           |                | U10| NZPradTrx094750_C01 | PREDICTED: zinc finger CCCH domain-containing protein 20-like | Known to play important roles in RNA processing as RNA-binding proteins in animals [Wang et al. 2008](Wang et al. 2008) | −         |
| Condition   | No. unique DETs | ID          | P. radiata code     | Gene name                                                                 | Predicted gene function                                                                 | Direction |
|-------------|----------------|-------------|---------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|-----------|
| T7-B-strip  | 39             | U11         | NZPradTrx111276_C02 | low molecular weight heat-shock protein                                  | Expressed in plants experiencing high-temperature stress (Hernandez and Vierling 1993)    | –         |
|             |                | U12         | NZPradTrx09179_C02  | LP3-1                                                                      | Shown to be up-regulated in response to water deficit stress and to also act as transcription factors for genes likely involved in hexose transport (Lecoy and García-Gil 2020) | –         |
|             |                | U13         | NZPradTrx12152_C04  | PREDICTED: L-type lectin-domain containing receptor kinase IV.1-like     | Involved in resistance response to the pathogenic oomycetes, promotes hydrogen peroxide production and cell death [https://www.uniprot.org/uniprot/Q9LXAS](https://www.uniprot.org/uniprot/Q9LXAS) | +         |
|             |                | U14         | NZPradTrx082734_C01 | Casparian strip domain-like gene                                          | Recruit the lignin polymerisation machinery necessary for the deposition of Casparian strips in the endodermis [https://www.ebi.ac.uk/interpro/entry/InterPro/IPR006459/](https://www.ebi.ac.uk/interpro/entry/InterPro/IPR006459/) | –         |
|             |                | U15         | NZPradTrx05759_C05  | Methyl esterase 13                                                        | Involved in jasmonic and salicylic acid metabolic process [https://www.uniprot.org/uniprot/F4IE65](https://www.uniprot.org/uniprot/F4IE65) | +         |
|             |                | U16         | NZPradTrx042090_C01 | Geranyl diphosphate synthase                                              | Catalyses the condensation of dimethylallyl diphosphate and isopentenyl diphosphate to geranyl diphosphate, the key precursor of monoterpene biosynthesis (Burke et al. 1999) | +         |
|             |                | U17         | NZPradTrx064702_C01 | Class II chitinase                                                        | Involved in the defence response against pathogen and fungal infection (de A. Gerhardt et al. 1997) | –         |
|             |                | U18         | NZPradTrx05720_C01  | Defensin                                                                   | Inhibits the growth of a broad range of pathogens, including bacteria, fungi and viruses (Ernokova et al. 2016; Picart et al. 2012) [75, 76]. | –         |
|             |                | U19         | NZPradTrx119059_C01 | Annexin p33                                                                | Central regulator or effector of plant growth and stress signalling (Mortimer et al. 2008) | –         |
|             |                | U20         | NZPradTrx118949_C01 | Peroxiredoxin                                                             | Guardian against oxidative stress and modulator of peroxide signalling (Perkins et al. 2015) | –         |
Table 6 (continued)

| Condition | No. unique DETs | ID          | P. radiata code | Gene name                                      | Predicted gene function                                                                 | Direction |
|-----------|----------------|-------------|-----------------|------------------------------------------------|----------------------------------------------------------------------------------------|-----------|
| T7-N-MJ   | 751            | U21         | NZPradTrx10565_C01 | UDP-sulfoquinovose synthase                     | Involved in the biosynthesis of sulfolipids found in thylakoid membranes. Converts UDP-glucose and sulfite to the sulfolipid head group precursor UDP-sulfoquinovose [https://www.uniprot.org/uniprot/O48917](https://www.uniprot.org/uniprot/O48917) | -         |
| U22       |                | NZPradTrx064995_C02 | Chloroplast ribulose bisphosphate carboxylase/oxygenase activase alpha1, partial | Catalyses carboxylation of RuBP in the first step of the Calvin cycle of photosynthesis [Tabita 1999](https://www.uniprot.org/uniprot/P00579) | -         |
| U23       |                | NZPradTrx088104_C02 | RNA polymerase sigma factor rpoD, putative | Initiation factor that promotes the attachment of RNA polymerase to specific initiation sites [https://www.uniprot.org/uniprot/P00579](https://www.uniprot.org/uniprot/P00579) | -         |
| U24       |                | NZPradTrx081803_C01 | PREDICTED: mitochondrial carnitine/acylcarnitine carrier-like protein-like | Mediates the transport of acylcarnitines of different length across the mitochondrial inner membrane from the cytosol to the mitochondrial matrix for their oxidation by the mitochondrial fatty acid-oxidation pathway [https://www.uniprot.org/uniprot/O43772](https://www.uniprot.org/uniprot/O43772) | -         |
| U25       |                | NZPradTrx086144_C02 | Chloroplast omega-6 fatty acid desaturase | Introduces the second double bond in the biosynthesis of 16:3 and 18:3 fatty acids, important constituents of plant membranes. It is thought to use ferredoxin as an electron donor and to act on fatty acids esterified to galactolipids, sulfolipids and phosphatidylglycerol [https://www.uniprot.org/uniprot/P46312](https://www.uniprot.org/uniprot/P46312) | -         |
| U26       |                | NZPradTrx065194_C01 | Glutamate–ammonia ligase | Key enzyme of ammonium assimilation and recycling in plants where it catalyses the synthesis of glutamine from ammonium and glutamate [Lothier et al. 2011](https://www.uniprot.org/uniprot/P46312) | -         |
| U27       |                | NZPradTrx077590_C01 | PREDICTED: ATP synthase gamma chain, chloroplastic-like | Produces ATP from ADP in the presence of a proton gradient across the membrane. The gamma chain is believed to be important in regulating ATPase activity and the flow of protons through the CF<sub>0</sub> complex [https://www.uniprot.org/uniprot/Q01908](https://www.uniprot.org/uniprot/Q01908) | -         |
| U28       |                | NZPradTrx064646_C01 | PREDICTED: photosystem I reaction center subunit XI, chloroplastic-like | Involved in photosynthesis [https://www.uniprot.org/uniprot/Q41385](https://www.uniprot.org/uniprot/Q41385) | -         |
| U29       |                | NZPradTrx115121_C05 | glutathione peroxidase-like protein, partial | Protects cells from phospholipid hydroperoxides and nonphospholipid peroxides during oxidative stress [https://www.uniprot.org/uniprot/P36014](https://www.uniprot.org/uniprot/P36014) | +         |
| U30       |                | NZPradTrx186664_C01 | F353614_1 senescence-associated protein SPA15 | May be involved in the regulation of leaf senescence [https://www.uniprot.org/uniprot/Q65X5F](https://www.uniprot.org/uniprot/Q65X5F) | -         |
| Condition | No. unique DETs | ID                  | P. radiata code | Gene name                             | Predicted gene function                                                                                     | Direction |
|-----------|----------------|---------------------|----------------|---------------------------------------|-------------------------------------------------------------------------------------------------------------|-----------|
| T14-B-MJ  | 18             | U31 NZPradTrx1 92941_C01 | Beta-amylase    | Involved in starch breakdown in plants (Kaplan and Guy 2004) |
|           |                | U32 NZPradTrx076831_C01 | UV-B receptor 1 | Involved in response to UV-B (Loyola et al. 2016) | +                                                                                                             |
|           |                | U33 NZPradTrx044917_C01 | Putative cyclophilin | Involved in various physiological processes including transcriptional regulation, organogenesis, photosynthetic and hormone signalling pathways, stress adaptation and defence responses (Barbosa dos Santos and Park 2019) | -                                                                                                             |
|           |                | U34 NZPradTrx1 19079_C01 | Xyloglucan endotransglucosylase/hydrolase 13 | Cleaves xyloglucan polymers, an essential constituent of the primary cell wall, and thereby participates in cell wall construction of growing tissues. [https://www.uniprot.org/uniprot/Q9FKL8](https://www.uniprot.org/uniprot/Q9FKL8) | -                                                                                                             |
|           |                | U35 NZPradTrx037564_C01 | PREDICTED: bidirectional sugar transporter SWEET3-like | Mediates both low-affinity uptake and efflux of sugar across the plasma membrane [https://www.uniprot.org/uniprot/Q6NQN5](https://www.uniprot.org/uniprot/Q6NQN5) | -                                                                                                             |
|           |                | U36 NZPradTrx1 18938_C01 | Glycine-rich RNA-binding protein | Plays a role in RNA transcription or processing during stress. Binds RNAs and DNAs sequence with a preference to single-stranded nucleic acids. [https://www.uniprot.org/uniprot/Q03250](https://www.uniprot.org/uniprot/Q03250) | -                                                                                                             |
|           |                | U37 NZPradTrx09658_C01 | Probable aquaporin | Involved in transport of water and other small neutral molecules across cellular biological membranes (Kapilan et al. 2018) | -                                                                                                             |
|           |                | U38 NZPradTrx094541_C02 | PREDICTED: methionine gamma-lyase-like | Involved in amino acid catabolism (Ravanel et al. 1998) | +                                                                                                             |
| Condition     | No. unique DETs | ID          | P. radiata code | Gene name                     | Predicted gene function                                                                 | Direction |
|--------------|----------------|-------------|----------------|-------------------------------|----------------------------------------------------------------------------------------|-----------|
| T14-B-strip  | 12             | U39         | NZPradTrx1 19456_C01 | PR10-1.13 | Involved in defence against pathogen infection and other environmental stresses (Liu et al. 2005) | +         |
|              |                | U40         | NZPradTrx098320_C05 | PREDICTED: LOB domain-containing protein 1-like | Controls the proximal-distal patterning in petals and the adaxial-abaxial determination of leaves. Involved in the repression of the homeobox gene BP. [https://www.uniprot.org/uniprot/Q9FKZ3-1](https://www.uniprot.org/uniprot/Q9FKZ3-1) | +         |
|              |                | U41         | NZPradTrx073494_C01 | TPA: putative ARF GTPase-activating domain family protein | Have potential roles in cell migration, central to normal physiology in embryogenesis, the inflammatory response and wound healing (Campa and Randazzo 2008) | –         |
|              |                | U42         | NZPradTrx103835_C01 | 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase | Involved in the biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), two major building blocks of terpenoid compounds. [https://www.uniprot.org/uniprot/P62617](https://www.uniprot.org/uniprot/P62617) | +         |
|              |                | U43         | NZPradTrx102746_C02 | S6 ribosomal protein | Key downstream effector of the target of rapamycin (TOR) kinase pathway that regulates various biological processes, including translation, synthesis of ribosomal proteins, and transcription of rRNA (Kim et al. 2014) | +         |
|              |                | U44         | NZPradTrx094959_C01 | Pathogenesis-related protein 10 | Involved in a cell wall rigidification to signal transduction and antimicrobial activity (Liu and Ekramoddoolah 2006) | +         |
|              |                | U45         | NZPradTrx096309_C03 | Dirigent-like protein | Predominant roles in defence responses, secondary metabolism, and fiber biosynthesis (Li et al. 2017) | +         |
|              |                | U46         | NZPradTrx10315_C01 | PREDICTED: uncharacterized LOC101219508 | Catalyses a wide variety of redox reactions with many different substrates (Sellés Vidal et al. 2018) | –         |
|              |                | U47         | NZPradTrx077043_C01 | FAD/NAD(P)-binding oxidoreductase domain-containing protein | Catalyses a wide variety of redox reactions with many different substrates (Sellés Vidal et al. 2018) | –         |
|              |                | U48         | NZPradTrx110593_C01 | PREDICTED: chaperonin CPN60-2, mitochondrial-like | Implicated in mitochondrial protein import and macromolecular assembly. May facilitate the correct folding of imported proteins. May also prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions in the mitochondrial matrix. [https://www.uniprot.org/uniprot/O05046](https://www.uniprot.org/uniprot/O05046) | +         |
| Condition | ID          | P. radiata code | Gene name                                      | Predicted gene function                                                                 | Direction |
|-----------|-------------|-----------------|------------------------------------------------|----------------------------------------------------------------------------------------|-----------|
| T14-N-MJ  | U49 NZPradTrx1 18421_C03 | Caffeic acid O-methyltransferase | Catalyses the conversion of caffeic acid to ferulic acid and of 5-hydroxyferulic acid to sinapic acid. The resulting products may subsequently be converted to the corresponding alcohols that are incorporated into lignins. [https://www.uniprot.org/uniprot/Q06509](https://www.uniprot.org/uniprot/Q06509) | +         |
|           | U50 NZPradTrx079649_C05 | Geranyl diphosphate synthase | Catalyses the condensation of dimethylallyl diphosphate and isopentenyl diphosphate to geranyl diphosphate, the key precursor of monoterpenoid biosynthesis (Burke et al. 1999) | +         |
|           | NZPradTrx079649_C03 | NZPradTrx079649_C02 |                                           |                                                                                        |           |
| U51 NZPradTrx1 22822_C01 | PREDICTED: F-box protein GID2-like |                                           | Essential component of the SCF-type E3 ligase complex, SCF(GID2), a complex that positively regulates the gibberellin signaling pathway [https://www.uniprot.org/uniprot/Q9STX3](https://www.uniprot.org/uniprot/Q9STX3) | +         |
| U52 NZPradTrx083848_C01 | Chlorophyllase |                                           | The first enzyme involved in chlorophyll (Chl) degradation and catalyzes the hydrolysis of ester bond to yield chlorophyllide and phytol (Tsuchiya et al. 1999) | +         |
| U53 NZPradTrx1 03321_C01 | Phenylalanine ammonia-lyase |                                           | Phenylalanine aminomutase that catalyzes the rearrangement of L-phenylalanine to R-beta-phenylalanine. Catalyzes the first committed step in the biosynthesis of the side chain of the alkaloid taxol (paclitaxel) [https://www.uniprot.org/uniprot/Q68G84](https://www.uniprot.org/uniprot/Q68G84) | +         |
| U54 NZPradTrx071573_C01 | Starch synthase isoform II |                                           | Contributes to the extension of glucan chains in the synthesis of starch (Edwards et al. 1999) | +         |
| U55 NZPradTrx1 05898_C01 | Glutamate-1-semialdehyde 2,1-aminomutase |                                           | Essential enzyme in the pathway that leads to the synthesis of chlorophyll and other tetrapyrroles in plants and some bacteria (Tyacke et al. 1995) | -         |
| U56 NZPradTrx1 82827_C01 | PREDICTED: LRR receptor-like serine/threonine-protein kinase FLS2-like |                                           | Constitutes the pattern-recognition receptor (PPR) that determines the specific perception of flagellin (flg22), a potent elicitor of the defence response to pathogen-associated molecular patterns (PAMPs) [https://www.uniprot.org/uniprot/Q9FL28](https://www.uniprot.org/uniprot/Q9FL28) | +         |
| U57 NZPradTrx1 84681_C01 | FK506 binding-like protein |                                           | Involves in diverse cellular functions including protein folding, cellular signalling, apoptosis and transcription (Tong and Jiang 2016) | +         |
| U58 NZPradTrx094486_C01 | Putative UDP-glucose-flavonoid glucosyltransferase |                                           | Enhances the solubility of flavonoids (Chen et al. 2011) | +         |
| Condition | No. unique DETs | ID          | P. radiata code | Gene name                                      | Predicted gene function                                                                 | Direction |
|-----------|----------------|-------------|-----------------|------------------------------------------------|------------------------------------------------------------------------------------------|-----------|
| T21-B-MJ  | 4              | U59         | NZPradTrx083714_C01 | PREDICTED: protein GLUTAMINE DUMPER 1-like   | Involved in the regulation of amino acid metabolism, in the salicylic acid (SA) pathway and in the geminivirus-host interaction | +         |
|           |                | U60         | NZPradTrx053990_C01 | PREDICTED: cytochrome P450 71A1-like          | Involved in the metabolism of compounds associated with the development of flavour in the ripening fruit process, possibly by acting as trans-cinnamic acid 4-hydrolase | +         |
|           |                | U61         | NZPradTrx105443_C01 | GMP synthase [glutamine-hydrolyzing] subunit A, putative | Involved in de novo biosynthesis of guanosine nucleotides                                   | +         |
|           |                | U62         | NZPradTrx112336_C01 | Laccase                                       | Involved in phenolic metabolism and functioning of cell wall (Ranocha et al. 2002)         | +         |
| T21-B-S   | 13             | U63         | NZPradTrx087634_C02 | Properoxidase                                 | Involved in lignification, cell elongation, stress defence and seed germination (Shigeto and Tsutsumi 2016) | +         |
|           |                | U64         | NZPradTrx103699_C01 | Oxidoreductase, 2OG-Fe(II) oxygenase family protein | Involved in defence against pathogens (Van Damme et al. 2008)                              | +         |
However, after treatment there was strong transcriptional response of the basal transcripts in both the needles and the bark, with the expression being different and with sometimes non-overlapping transcripts between plant parts, treatments and at each sampling timepoint. While the effects of methyl jasmonate have been previously reported in various pine species [17, 24], this is the first study to illustrate transcriptional responses to bark stripping. The response to bark stripping was less than that to methyl jasmonate and was localised, as no systemic response extending to the needles was detected at any time point. Differences in responsiveness to both treatments were also detected between the classes of genes, where genes related to primary metabolism responded to treatments with a greater magnitude of up-regulation or down-regulation compared to genes associated with secondary metabolism.

Among the genes that were homogeneously expressed between the bark and the needles were those related to basic life functions especially those related to primary and secondary metabolism. For example, ribulose bisphosphate carboxylase/oxygenase (RuBiSCo) and a chlorophyll a/b binding protein were dominant both in the transcriptome of the needles and the bark. Similar observations were made in the needles of other P. radiata populations [81] and Pinus monticola [70], although these studies did not analyse how the transcriptomes change with treatment and the observations were limited to one plant part. Genes directly related to secondary metabolism, for example chalcone synthases, dehydrins and defensins, were among the basal genes, highlighting the importance of constitutive defences in P. radiata. Chalcone synthase has been identified in other conifers [82, 83] and plays crucial role in phenolic biosynthesis [74]. Defensins have also been detected in various conifers where they inhibit the growth of a broad range of pathogens, including bacteria, fungi and viruses [75, 76]. Dehydrins that represent a family of genes for drought tolerance have been detected in spruces and in other Pinaceae [72]. Metallothioneins that were strongly expressed both in the bark and the needles are important in protection against heavy metal toxicity [73] and have been documented mainly in Pseudotsuga menziesii [84, 85]. They could reflect an adaptation to leached, heavy metal enriched soils in the coastal sites of California where P. radiata originates [86]. However, while the above genes are expressed at high amounts equally in the bark and needles, some transcripts were up-regulated in the needles or the bark. More up-regulation was detected in the bark, which contrasted with the higher expression

![Fig. 6 Time progression in the differential expression (control versus treatment) of the top 10 most expressed genes in the constitutive transcriptome of Pinus radiata. The genes are detailed in Table 3 and their differential expression in bark is shown following a bark strip and b methyl jasmonate treatments. The average change in expression was estimated at each time point by comparing the raw counts for the bark strip or methyl jasmonate induced transcripts and the transcripts from control treatments (mean of treatment – mean of control) for a specific time and were adjusted according to the differences in basal expression of the treatment groups at T0. T0 is before treatment applications and T7, T14 and T21 correspond to 7, 14 and 21 days after treatment application, respectively.](image-url)
of transcripts in the needles than the bark reported in other *P. radiata* populations [81]. In both plant parts up-regulated genes were predominantly related to the synthesis and transfer of macro- and micro-molecules, as well as transcription factors which are the key molecular switches orchestrating the regulation of plant responses to a variety of stresses.

After treatment with methyl jasmonate and bark stripping, there was an up-regulation and down-regulation of several genes involved in both primary and secondary metabolism both in the bark and needles, consistent with other studies that have characterised responses to other stressors in conifers [24, 79]. The top genes that were up- or down-regulated in the present study overlap with those observed in similar studies with contrasting sources of stress in conifers [13, 70, 79, 80, 87], suggesting that changes in gene expression following stress are relatively conserved. Among the top expressed genes, results showed a down-regulation of hexokinases, granule-bound starch synthase and sodium-bile acid cotransporter as well as genes related with photosynthesis, suggesting reduction in sugar metabolism in the treated plants. However, cell wall invertase that mediates export of sucrose or enhanced import of hexoses at the site of damage was up-regulated in both methyl jasmonate and strip treated plants. Cell wall invertase (CWI) is an enzyme that cleaves sucrose, the major transport sugar in plants, irreversibly yielding glucose and fructose, which can be taken up by plant cells [78, 88]. An increase in CWI should ideally lead to a reduction in sucrose, which is consistent with the drastic reduction in the amounts of sucrose that has been observed following methyl jasmonate and strip treatments in *P. radiata*. The up-regulation of CWI would also suggest an increase of glucose and fructose, but this was not the case as a strong reduction in the amounts of glucose and fructose was observed in treated samples [50]. This suggests that although fructose and glucose may be potentially enhanced by an increased break down of sucrose, their utilisation for energy and carbon skeletons for other organic compounds or for tissue recovery exceeds their production, supporting the concept that defence is costly in terms of energy [89]. Gould, Reglinski [90] detected a repression of photosynthesis in *P. radiata* as a response to stress that
could lead to a reduction of sugars. Sugars have also been shown to function as signalling molecules, in a manner similar to hormones [88, 91], but their down-regulation contrasts to the up-regulation of other signalling molecules. However, according to Eveland and Jackson [92] sugar signals are generated either by relative ratios to other metabolites, such as C:N, not necessarily carbohydrate concentration.

In addition to the sugar-related genes, the other primary metabolism genes that were responsive to the treatment included those genes related to fatty acid metabolism such as the medium-chain-fatty-acid-CoA ligase and UDP-rhamnose:rhamnosyltransferase that were up-regulated and those related to fatty acid synthesis, such as carboxylesterase, that were down-regulated. Observations on the same population showed a reduction in fatty acids following treatment, consistent with their potential use as precursors to the formation of secondary compounds [93]. Accumulating evidence has suggested lipids and lipid metabolites as important regulators of plant defence [94]. Genes related to amino acid synthesis were also among the top expressed genes.

Increase in amino acid levels have been detected in plants under stress and is hypothesized to protect plant cells against dehydration [95, 96]. Amino acid accumulation has been observed to be strongly related to abscisic acid signalling [95]. Molecules related to abscisic acid signalling were also strongly up-regulated similar with pathogenicity response in the Pinus pinaster - Fusarium circinatum pathosystem [97]. This study contributes to the body of literature demonstrating the crucial role of phytohormones in host defense response [98].

Genes related directly to secondary metabolism were not detected among the top differentially expressed genes following treatment although they are abundant in the constitutive transcriptome of both the needles and the bark, consistent with the observations in spruce [10]. However, the relatively weak transcriptional response to treatment of individual genes related to secondary metabolism in this study contrasts with other studies [13, 17] and could possibly be due to the timing of the sampling, which was done 7 days after treatment application. In various studies, maximum expression of genes is shown to be attained within 5 days after treatment application [13, 17]. On the same population, a weak response of terpenes and phenolics was observed following similar treatments [50], which probably suggests an inherently weak response of secondary compounds and associated genes to stress in P. radiata. Defence genes being strongly expressed in the constitutive but not in the induced transcriptome may suggest existence of trade-offs in induced gene expression [99], analogous to the trade-offs in constitutive versus induced chemical responses that have been detected in P. radiata [21]. Although alkaloids have not been well researched as important defence compounds in conifers, genes related to alkaloid biosynthesis such as RS-norcoclaurine 6-O-methyltransferase were among the top expressed genes but were down-regulated after treatment. There were also many proteins of unknown functions that were up-regulated or down-regulated at various time points, which potentially explains the many unknown chemical compounds that were quantified on the same plants.

Considerable overlap was observed between the methyl jasmonate and the strip induced transcriptome. However, results also indicate that bark stripping can induce transcripts that are not induced with methyl jasmonate and vice versa. Defence responses for bark stripping may differ from methyl jasmonate since bark stripping causes tissue and water loss at the injured sites, and damaged plants are also easily infected by pathogens through these wounds. In this case both defence and repair responses are required. Hence the dominant genes in the strip-induced transcriptome involved pathogenesis-related (PR) genes and those related to fibre synthesis. The expression of PR genes could also be related to the historical relationship between P. radiata and various pathogens [100]. No systemic transcript responses were observed in the needles to bark stripping. Coupled with the chemical changes that were observed in the needles following bark stripping on the same population, for example the reduction of glucose and fructose at T7 and T14 [50], this observation suggests that some chemical stress responses, possibly those involving sugars, may not involve on-site gene expression changes and may result from passive reallocation of chemistry within the plant. For other compounds like terpenes, it has been indicated that passive changes normally occur only in the constitutive environment and that stress-induced changes in terpenes are entirely of a de novo nature [101].

A key finding from this study is that the main transcriptome change associated with either treatment was clearly earlier than the main chemical changes observed on the same population [50]. The maximum differential expression of the transcripts was observed 7 days after treatment whereas most chemical change were detected 14 and 21 days after treatment, consistent with a time-lag between gene and phenotypic expression. This discrepancy may be associated with trade-offs between gene expression and other cellular resources, including the nutritional quality of the plant [99]. One GO-term that was significantly enriched after treatment was response to stimuli and, consistently, genes related to signalling were among the top expressed genes. For example, 1-aminocyclopropane-1-carboxylate oxidase, which is related to production of
ethylenes; lanC-like protein 2-like for abscissic acid and Tify domain containing protein for jasmonates were strongly responsive. Ethylene is one of the major signaling molecules in plant defences in addition to others, such as jasmonic acid, salicylic acid and abscisic acid [102]. Ethylene can act synergistically or antagonistically with jasmonic acid in the regulation of both stress and developmental responses. The connection between these two signalling pathways has been demonstrated genetically to be the transcription factor for the ethylene response [103], that was also strongly expressed. This suggests that jasmonates, abscisic acid and ethylene are involved in induced responses of *P. radiata* under different stresses. The involvement of jasmonates and ethylene in induced defence responses has been shown in other pine species [20]. In other species, abscisic acid has been shown to be involved in defence responses and has been reported to play a negative role in the regulation of the major photosynthesis gene — type 2 light-harvesting chlorophyll a/b-binding polypeptide [71] — which was reduced after treatment in this current study.

**Conclusion**

There are marked quantitative differences in the needle and bark transcriptome of *Pinus radiata* both in the constitutive and induced states. The transcriptome triggered by bark stripping substantially differed from methyl jasmonate triggered responses suggesting that some molecular aspects of bark stripping may differ from other biotic and abiotic responses. Gene annotation revealed that some of the differentially expressed transcripts have putative functions in plant defence signalling, transcription regulation, biosyntheses of primary and secondary metabolites and other biological processes. The diversity of these genes reflects the complexity of stress responses. The expressed genes provide a basis for further identification of candidate genes that affect bark stripping through variation in their expression levels while the uncharacterized genes that responded to simulated herbivory and methyl jasmonate provide a rich resource for future studies. Gene expression can be used by breeders to exploit phenotype variability among individuals within or between populations. It also remains to be tested whether variations in the transcript levels, particularly the differentially expressed components in response to the artificial stress treatments can be linked to the susceptibility classes identified in the field [46] and whether they are heritable.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12864-021-08231-8.

**Additional file 1: Supplementary Figure 1.** Number of transcripts in each cellular, biological and cellular categorization of up-regulated and down-regulated genes in *Pinus radiata* needles (N) at T0 and after treatment with methyl jasmonate (MJ) or bark stripping (strip) at T7. The categorization is based on gene ontology (GO) annotations of the top 100 differentially expressed transcripts in each category. Go terms with < 2% gene enrichment were excluded. (−) = down-regulated, (+) = up-regulated transcripts.

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**Authors’ contributions**

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**Availability of data and materials**

The datasets supporting the results of this article are available on reasonable request from Assoc. Prof Julianne O’Reilly-Wapstra, School of Natural Sciences, University of Tasmania, Australia. The expressed transcripts can be accessed on the ncbi website (Sequence Read Archive (SRA) submission: SUB10571957).

**Declarations**

**Ethics approval and consent to participate**

The experimental research on *P. radiata* including the collection of plant material was compliant with the ethical guidelines and legislation of the University of Tasmania. The pine seedlings used in the study were provided by industrial partners; Timberlands Pacific Pty Ltd. and the Radiata Pine Breeding Company.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest.

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