Differential gene expression analysis of the resprouting process in Pinus canariensis provides new insights into a rare trait in conifers

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Abstract

Resprouting, one of the main fire-adapted traits, is crucial in population dynamics in response to wildfires or herbivory. This trait, common in angiosperms, is rare in conifers, being *Pinus canariensis* one of the few species able to resprout. We analyzed gene expression during wound-induced resprouting in 5 years-old Canarian pines. RNA was extracted at three dates, including control samples from remote branches, representing immediate response to wounding (R0), resprouting initiation (R1), and elongation (R2), and then hybridized to a microarray designed with 15000 cDNAs from *Pinus canariensis* transcriptome of meristematic activity. We found 1164 Differentially Expressed Genes (DEGs) as response to wounding grouped in 6 clusters across time points. Genes related to defense- and stress-response were mainly found to be overexpressed at R0, including disease resistance response protein 206-like, or pathogenesis-related proteins PR-4b-like, among others. We also found DEGs coding for transcription factors such as GROWTH-REGULATING FACTOR (GRF), FLOWERING-PROMOTING FACTOR (FPF), and the HOMEODOMAIN LEUCINE ZIPPER Class IV (HD-ZIP IV), mainly related to outgrowth processes and lateral organogenesis in plants, showing overexpression at R1 and R2, when new shoots were emerging. This data was compared to differential gene expression during apical growth in *P. canariensis*, suggesting similarities and differences between vegetative apical growth and regulation of lateral shoot development in response to mechanical wounding.

Introduction

Resprouting is a tolerance trait that confers resilience to plant species due to the presence of vegetative latent buds that are able to produce regrowth and *de novo* develop into a new epicormic shoot. This trait is especially important in response to traumatisms and mechanical stress caused by forest fires or herbivory, among other disturbance factors (Clarke et al. 2013). Depending on the position of resprouts, species can be classified as stem, apical, basal or root resprouters (Clarke et al. 2013). For the latter, below-ground tissues are well protected and isolated by soil. However, intense fires such as crown-fires can damage aerial organs in above-ground tree resprouters, causing necrosis and stem hydraulic failures (Fernandes et al. 2008; Midgley et al. 2011). Many of these tree species present self-pruning trunks, preventing canopy fires and therefore avoiding damages to their shoot apical meristems (SAM), located in the top part of the crown. On its side, thick barks confer a better protection for lateral meristematic tissues and latent buds, allowing them to resprout (Schwilk & Ackerly 2001).

Resprouting is not rare in flowering plants, and the term and process were recognized years ago (Noble & Slatyer 1980) and suggested as an important ecological factor for population dynamics (Loehle 2000; Hoffmann et al. 2009), as well as the basis of silvicultural practices such as coppicing and pollarding. However, not much research has been done on this feature. For instance., this ability is common in shrub species from fire-prone chaparral, including the crown-resprouters *Ceanothus roderickii* W.Knight (Boyd 2007), and some subspecies of *Arctostaphylos glandulosa* Eastw. (Keely et al. 2007). Resprouting has been proposed to be the response to a physiological disequilibrium in the plant due to the necessity of capturing nutrients and resources by expanding foliar coverage and recovering canopy (Meier et al. 2012), with plant hormones playing key roles in signaling during the process (Nicolini et al. 2001; Sprugel 2002; Ishii 2011). But also, small injuries not compromising the photosynthetic capacity of the plant can induce local resprouting. In these cases, new shoots may be formed by dedifferentiation of mature cells into stem cells with capacity of reverting into new cell-types (Savidge 2001). Additionally, in several species new shoots develop from buds located in leaf axils. For example, in Wollemi Pine (*Wollemia nobilis* W.G.Jones, K.D.Hill & J.M.Allen) epicormic shoots arise from small meristems located in axils (Burrows et al. 2003).

From a molecular point of view, the outgrowth of axillary buds and the emergence of new branches is a topic of great interest in breeding and crop science, always looking for an enhanced production. For instance, some studies have focused on the plant architecture of *Brassica napus* L., including genome-wide association studies (GWAS) using a single nucleotide polymorphism (SNP) array of candidate genes for the regulation of flower development (Li et al. 2016). Moreover, transcriptomics of the axillary bud development under sugar and hormone signaling in *B. napus* revealed important roles of key regulators and different transcription factor families (Li et al. 2020). Similarly, gene expression of axillary bud outgrowth in response to exogenous application of strigolactone, auxin and auxin transport inhibitor naphtyl phthalamic acid was studied in the ornamental crop *Chrysanthemum morifolium* Ramat. (Dierck et al. 2018).

In gymnosperms, resprouting has been suggested to be scarce by many authors, as reviewed recently by Burrows (2021). Not without reason, resprouting has been considered an ancestral condition that may have played a key role in adaptation to recurrent fires in the Paleozoic Era (He et al. 2016), but probably lost in modern conifers (Bond & Midgley 2003). Canary Island pine (*Pinus canariensis* C.Sm. ex DC) is one of the few conifers that are able to resprout in both juvenile and adult stages. In this species, the resprouting capability has been proposed to be linked to its evolutionary history in a volcanic environment under selective pressures exerted by perturbation regimes and driving the dynamics of its populations (Climent et al. 2004). In this work, we aim at a better understanding of the gene expression dynamics of local resprouting response to mechanical damage in *P. canariensis*. For this purpose, we used a microarray designed using a normalized transcriptome of the meristematic activity (Chano et al. 2017). We studied gene expression profiles after wounding and during the very early stages of the resprouting process and compared these results with those obtained during apical growth, co-analyzing

expression profiles of genes suggested to be involved in one or both processes as a first approximation to identify resprouting specific genes.

Material And Methods

Plant material, wounding, and sample collection

Using a sterile scalpel, we produced mechanical injuries in three 5-year-old Canary Island pines growing in an experimental garden at UPM facilities under environmental conditions and regular watering. At the beginning of the experiment, trees were about 2.5 m high and the diameter at breast height was 5 to 7 cm. Wounding was performed by making two rectangular windows 10 cm high and covering half of the stem circumference in each tree, placed on opposite sides of the main trunk and with a vertical separation of three times the window height between them. We removed bark, phloem, vascular cambium and first layers of xylem (Online Resource Figure S1). Wounds were produced on April 9th, when cambial activity was ongoing, and sampling was carried out at three time points after wounding: i) one week after wounding, a frame of tissue from the margins of both wounds was collected on April 16th (R0; this sample was analogous to H1 sample from Chano et al (2017) ; ii) the newly emerged resprouts close to wound margins, and surrounding tissue (phloem, cambium and first layers of xylem) were harvested on June 3th (R1), when trees were still forming early wood (Chano, López de Heredia, et al. 2017); and iii) other new resprouts that were left from R1 and continued developing were collected on June 25th (R2). Control samples were also harvested from remote branches of the same trees, and all the collected samples were frozen in liquid nitrogen and stored at -80 ºC until further processing.

RNA isolation and microarray analysis

RNA extraction from each individual sample was performed as described in Chang et al. (1993; (Chang et al. 1993)). Total RNA was then purified with the RNeasy Plant Mini Kit (Qiagen, Dussldorf, Germany), and quantified with Nanodrop ND-1000 (Thermo Scientific, MA, USA). RNA from the three biological replicates of each sampling point (R0, R1 and R2) was hybridized with a two-color microarray (Agilent, USA) as described in Chano et al. (2017b (Chano, Collada, et al. 2017)), including background correction and normalization of expression values using LIMMA (Linear Models for Microarray Data; (Smyth & Speed 2003)), and selection of Differentially Expressed Genes (DEGs) by means of “Rank Products” algorithm available for R Bioconductor (Gentleman et al. 2004). Those transcripts showing a FC value (Fold Change, defined as the logarithmic ratio between experimental and control conditions for each biological replicate and sampling time) over 2 (overexpressed) or below − 2 (underexpressed), with a significance level p-value corrected by False Discovery Rate (FDR) below 0.05, were considered as DEGs. Expression values were clustered using the *pheatmap* package in R (Warnes et al. 2015). Microarray data was uploaded to the Gene Expression Omnibus database (GEO; https://www.ncbi.nlm.nih.gov/geo; Accession number GSE205852). By using Omicsbox following the Blast2GO methodology (Conesa & Götz 2008), we also performed an enrichment analysis based on Fisher’s exact test of GO terms associated to these DEGs and using the whole *P. canariensis’* transcriptome as background.

Validation of expression profiles by qPCR

Validation of microarray expression data was performed using quantitative Real Time PCR (qRT-PCR) analysis of 11 DEGs (Table 1) covering the main profiles obtained from the analysis. The gene specific primers were designed with the Primer 3 webtool (Untergasser et al. 2012) with melting temperatures between 60 and 65 ºC, and producing amplicons ranging between 80 and 120 bp. We also included the Ri18S as housekeeping gene for CT normalization between different qRT-PCR runs (Table 1). First strand cDNA synthesis was performed using the SuperScript™ III reverse transcriptase (Invitrogen™) following the protocol from the manufacturer, and the SsoFast™ EVAgreen® Supermix (Biorad, USA) was used as fluorescent DNA stain. The qPCR reactions were performed in a CFX96™ Real-Time PCR Detection System (Biorad, USA), following a thermal profile of 95 ºC for 3 min, 40 cycles of 95 ºC for 10 s, and 60 ºC for 10 s. Expression values were obtained using the Δ-Δ-CT method with PCR efficiency correction (Pfaffl 2001), and the statistical analysis of qPCR data included Shapiro-Wilk test for distribution, and Bartlett test for homoscedasticity. Statistical differences between groups were inferred following ANOVA. As well, correlation between microarray and qPCR data was assessed by means of Pearson’s correlation test.

Table 1
Primers used for qRT-PCR.

Contig name	Oligo name	Description	Fwd/Rev	bp	Tm	GC	Sequence (5′-3′)
Congit00654	Pc_00654_CESA_F1	cellulose synthase a-like protein	Forward	20	63.0	55	GGACCACACTCCTCATTCCT
	Pc_00654_CESA_R1		Reverse	20	63.0	45	ACCCCATGACTGAAATCCAT
Contig12050	Pc_12050_MYB_F1	MYB46-like protein	Forward	20	62.8	45	ATTCCCAACATGGAAGAAGC
	Pc_12050_MYB_R1		Reverse	20	63.7	50	CTGCATCACCATCACACTCA
Contig20304	Pc_20304_ATHB13_F1	ATHB13-like protein	Forward	20	63.2	50	CCCATTCTCATGATGTCTGC
	Pc_20304_ATHB13_R1		Reverse	20	63.1	50	CAGAACTGCCTTCACTTCCA
Contig00787	Pc_00787_NAC_F1	NAC2-like prtoein	Forward	20	62.5	45	CTAAATGGCCCTGGGTAAAA
	Pc_00787_NAC_R1		Reverse	20	62.8	50	CCCCTTCTTCTTACCAACCA
Contig20555	Pc_20555_PAL_F1	phenylalanine ammonia-lyase-like protein	Forward	20	63.1	50	GAATTGACGTCCTGGTTGTG
	Pc_20555_PAL_R1		Reverse	20	62.7	50	CAGCCTGGACTATGGTTTCA
Contig05551	Pc_05551_WRKY_F1	WRKY51-like protein	Forward	20	62.5	45	ACGCAGAGGGGAATAAGAAA
	Pc_05551_WRKY_R1		Reverse	20	63.2	50	CAGAAAACGTTCACCCACAG
Contig06476	Pc_06476_CCoAOMT_F1	CCoAOMT-like protein	Forward	20	64.0	50	GATTGAACAACCGAGGTGCT
	Pc_06476_CCoAOMT_R1		Reverse	20	63.6	45	TGCAACACCTGAATTCCAAC
Contig05410	Pc_05410_PECTINESTERASE_F1	pectinesterase 2-like	Forward	20	63.1	55	GTACTCTCGCACGGTCTTCA
	Pc_05410_PECTINESTERASE_R1		Reverse	20	62.5	45	ATAATAAAGCGTCCCCAACG
Contig09007	Pc_09007_EXO_F1	exordium 2-like protein	Forward	20	62.9	45	TACCCGATCATGCAAGACAT
	Pc_09007_EXO_R1		Reverse	20	62.7	55	GCGCCTAAATCTACCTGCTC
	Pc_12414_EXPANSIN _R1		Reverse	20	62.9	50	AAGGGTTCCATTCTCCACTG
Contig22185	Pc_22185_PR_F1	Major allergen PRU-like protein	Forward	20	65.0	60	GTGGAGGCAAGGAGACTGTG
	Pc_22185_PR_R1		Reverse	19	64.9	63.2	CTGCCTACGCCTCCATCTC
Contig13239	Pc_13239_YABBY_F1	axial regulator yabby 5-like	Forward	20	62.4	45	AGAGGATCAAAGCCCACAAT
	Pc_13239_YABBY_R1		Reverse	20	63.4	45	TGTCCATCATCATCCCAAAG
Housekeeping	Ri18S_FW	18S robosomal	Forward	19	62.4	53	GCGAAAGCATTTGCCAAGG
	Ri18S_RV		Reverse	21	62.4	48	ATTCCTGGTCGGCATCGTTTA

Tm: Melting temperature. GC: guanine-cytosine content. bp: base pair

Results

Differentially Expressed Genes during resprouting in response to wounding

Transcriptomic analysis of resprouting induced by mechanical wounding in *P. canariensis* was performed by means of a two-color cDNA microarray (Agilent, USA). Samples were taken during three stages over the responsive period, namely R0, R1 and R2, and transcripts with a Fold Change over 2 and below − 2 and significance level adjusted by FDR below 0.05, at least at one sampled date over the time series were selected for downstream analysis (Fig. 1). In total, 1164 DEGs were identified, of which 903 were found in R0, 278 in R1, and 261 in R2. Comparative analysis by groups resulted in 63, 67 and 76 DEGs that were exclusively shared between R0 and R1, R1 and R2, and R0 and R2, respectively, and 36 that were common for the three sampled dates (Fig. 2). On its side, the GO enrichment analysis by using Omicsbox did not yield any statistically enriched term.

Clustering of DEGs by Gene Expression Profiles

According to their transcriptional variations during the analyzed period, six co-expressed gene clusters were identified (Fig. 3; clusters 1 to 6). Cluster 1 showed genes that were mainly repressed at R0, and roughly recover basal expression at R1 and R2. Genes included in cluster 5 presented a similar expression profile, although showing slight overexpression at R2. On the other hand, clusters 2 and 3 showed induced genes at R0, declining at R1 and R2. Cluster 4 included genes mainly repressed during the whole response. And finally, genes grouped in cluster 6 showed overexpression at R1 and R2 from a basal level at R0. The complete list of DEGs can be found in Online Resource Table S1, while a selection of 134 DEGs is shown in Table 2, classified by their functional role, and including the top-hit description from BLASTx, the FC values, and significance level after the statistical analysis.

Table 2
Selected Differentially Expressed Genes during resprouting in the Canary Island pine in response to wounding. Significant values are represented in bold.

Cluster	ID	Seq. Description	R1		R2		R3	
			FC	FDR	FC	FDR	FC	FDR
Cell-wall matrix development and/or carohydrates metabolism								
1	Contig00260	cellulose synthase a catalytic subunit 4	**-11.21**	**0.00**	-1.58	0.36	**-3.94**	**0.04**
1	Contig00654	cellulose synthase a catalytic subunit 3	**-10.66**	**0.00**	-1.65	0.21	**-3.16**	**0.05**
1	Contig01025	fasciclin-like arabinogalactan protein 10-like	**-4.14**	**0.03**	-1.62	0.23	**-3.18**	**0.04**
1	Contig01405	protein cobra-like	**-8.51**	**0.00**	-1.53	0.39	**-3.73**	**0.05**
1	Contig02447	caffeoyl- o-methyltransferase-like	**-10.28**	**0.00**	-1.54	0.37	1.68	0.50
1	Contig02909	mannose-1-phosphate guanyltransferase alpha-like	**-7.90**	**0.01**	-1.41	0.60	-3.01	0.07
1	Contig05066	probable pectate lyase 15-like	**-4.35**	**0.03**	-0.39	0.91	0.26	0.95
1	Contig05410	pectinesterase 2-like	-1.71	0.56	**-8.61**	**0.00**	**-8.48**	**0.00**
1	Contig06476	caffeoyl- o-methyltransferase	-1.58	0.55	**-2.42**	**0.03**	**-5.63**	**0.02**
1	Contig09025	xyloglucan endotransglucosylase hydrolase protein 9-like	**-9.47**	**0.00**	**-2.74**	**0.01**	**-3.97**	**0.04**
1	Contig10110	xyloglucan galactosyltransferase katamari1-like	1.10	1.16	**-9.10**	**0.01**	**-5.00**	**0.03**
1	Contig11436	probable polygalacturonase non-catalytic subunit jp650-like	**-14.26**	**0.00**	-0.42	0.96	-1.78	0.49
1	Contig11855	galactan synthase 1	0.39	0.96	**-16.84**	**0.00**	**-9.68**	**0.00**
1	Contig12072	glucomannan 4-beta-mannosyltransferase 9-like	**-4.51**	**0.03**	-1.25	0.84	-2.06	0.32
1	Contig12945	germin-like protein 2-1-like	-0.34	1.20	**-8.71**	**0.01**	**-6.08**	**0.01**
1	Contig13778	probable polygalacturonase at1g80170-like	**-7.29**	**0.01**	-1.25	0.91	-3.12	0.13
1	Contig20721	cellulose synthase a catalytic subunit 3	**-3.65**	**0.05**	-1.26	0.72	-1.01	1.00
1	Contig41981	cellulose synthase a catalytic subunit 3	**-9.20**	**0.00**	**-2.14**	**0.05**	**-4.67**	**0.03**
1	Ppnisotig01389	mannan endo- -beta-mannosidase 7-like	**-7.65**	**0.01**	-1.19	0.87	-1.72	0.41
2	Contig08531	probable pectate lyase 15-like	**39.65**	**0.00**	1.50	0.52	-0.34	0.76
2	Contig17005	probable pectate lyase 12-like	**41.21**	**0.00**	1.55	0.43	0.36	0.94
2	Contig21204	alpha-expansin 8	**21.30**	**0.00**	2.55	0.09	1.18	1.05
2	Ppnisotig00677	expansin-a8-like	**39.38**	**0.00**	1.86	0.26	1.57	0.61
4	Contig12739	expansin-like a1-like	1.48	0.45	**-2.00**	**0.05**	**-4.81**	**0.04**
4	Contig17013	probable xyloglucan endotransglucosylase hydrolase protein 23	-1.22	0.90	**-2.26**	**0.05**	-1.31	1.04
4	Contig17876	expansin-like a1-like	1.53	0.43	-1.98	0.09	**-4.95**	**0.04**
4	Ppnisotig08645	expansin-b3-like	**-4.48**	**0.03**	1.07	1.04	0.33	1.01
5	Contig08356	udp-glycosyltransferase 85a2-like	**-51.71**	**0.00**	-1.29	0.77	**-4.17**	**0.03**
5	Contig12414	expansin-b3-like	-2.73	0.13	1.71	0.30	**3.70**	**0.03**
5	Contig23434	cinnamoyl- reductase 1-like	-2.34	0.19	1.30	0.79	**3.98**	**0.01**
6	Contig20345	wat1-related protein at5g07050-like	4.13	0.10	**3.24**	**0.03**	**18.49**	**0.00**
Defense and stress response genes								

Cluster	ID	Seq. Description	R1		R2		R3	
			FC	FDR	FC	FDR	FC	FDR
1	Contig01914	chitinase-like protein 1	**-12.06**	**0.00**	-1.59	0.26	**-4.08**	**0.04**
2	Contig00126	basic endochitinase a-like	**22.86**	**0.00**	1.97	0.09	-1.52	0.63
2	Contig00602	defensin ec-amp-d2-like	**14.04**	**0.00**	**3.62**	**0.01**	2.14	0.27
2	Contig02383	peroxidase 44-like	**3.13**	**0.05**	**3.30**	**0.00**	2.35	0.18
2	Contig02586	probable glutathione s-transferase	**4.50**	**0.03**	1.21	0.94	1.29	0.97
2	Contig06170	peroxidase 12-like	**10.44**	**0.00**	**4.43**	**0.00**	**0.40**	1.08
2	Contig06950	probable glutathione s-transferase-like	2.28	0.16	**2.39**	**0.03**	2.56	0.11
2	Contig08118	cationic peroxidase 1-like	**64.34**	**0.00**	1.93	0.20	1.55	0.51
2	Contig08224	probable glutathione s-transferase gstu6-like	**5.50**	**0.02**	**-2.67**	**0.02**	-0.35	0.95
2	Contig12556	probable 1-deoxy-d-xylulose-5-phosphate synthase chloroplastic-like	2.64	0.11	-1.07	1.03	**2.78**	**0.04**
2	Contig13391	cationic peroxidase 1-like	**5.19**	**0.02**	-1.27	0.66	-2.21	0.25
2	Contig14047	disease resistance response protein 206-like	**3.89**	**0.04**	-1.11	0.84	1.37	0.80
2	Contig16597	phenylalanine ammonia-lyase	**4.35**	**0.04**	1.90	0.34	-0.38	1.02
2	Contig17069	endochitinase a-like	**4.77**	**0.03**	**5.19**	**0.00**	1.18	0.53
2	Contig17710	basic endochitinase a-like	**4.59**	**0.03**	**2.78**	**0.04**	1.40	0.91
2	Contig20552	peroxidase 12-like	**3.99**	**0.03**	2.23	0.11	1.57	0.75
2	Contig20555	phenylalanine ammonia-lyase-like	**36.91**	**0.00**	**3.64**	**0.04**	-1.42	0.68
2	Contig20817	phenylalanine ammonia-lyase-like	**6.67**	**0.02**	2.06	0.21	1.09	1.10
2	Contig21755	peroxidase 12-like	**24.74**	**0.00**	**4.38**	**0.01**	1.15	1.03
2	Contig23442	chitinase 1-like	**44.00**	**0.00**	**3.48**	**0.04**	1.10	1.01
2	Contig40036	peroxidase 55	**4.91**	**0.02**	1.26	0.89	1.21	1.02
2	Ppnisotig08058	chitinase 6-like	**83.29**	**0.00**	1.44	0.59	1.11	1.05
2	Ppnisotig10090	endochitinase ep3-like	**8.50**	**0.01**	1.35	0.64	1.13	1.01
2	Ppnisotig11516	pathogenesis-related protein pr-4-like	**7.13**	**0.01**	1.52	0.29	1.24	0.96
3	Contig10307	endochitinase a-like	**88.54**	**0.00**	**9.25**	**0.00**	1.84	0.20
3	Contig17617	defensin ec-amp-d2-like	**45.72**	**0.00**	**2.98**	**0.01**	-1.11	1.00
3	Contig18804	disease resistance response protein 206-like	**450.31**	**0.00**	**15.09**	**0.00**	1.46	0.77
3	Contig19053	pathogenesis-related protein pr-4b-like	**161.48**	**0.00**	**10.17**	**0.00**	**6.14**	**0.01**
3	Contig21216	endochitinase a-like	**163.54**	**0.00**	**6.78**	**0.00**	-1.15	1.02
3	Contig22185	major allergen pru ar 1-like	**212.01**	**0.00**	**10.10**	**0.00**	**3.14**	**0.03**
3	Contig22375	pathogenesis-related protein pr-4-like	**475.82**	**0.00**	**19.71**	**0.00**	**3.95**	**0.04**
3	Ppnisotig00751	endochitinase a-like	**156.62**	**0.00**	**8.46**	**0.00**	2.03	0.20
3	Ppnisotig01747	peroxidase 12-like	**161.51**	**0.00**	**5.71**	**0.00**	1.10	1.09
3	Ppnisotig02901	glutathione s-transferase f9-like	**110.72**	**0.00**	**6.95**	**0.00**	**2.91**	**0.03**
3	Ppnisotig06171	glutathione s-transferase f9-like	**91.32**	**0.00**	**7.76**	**0.00**	**2.64**	**0.05**
3	Ppnisotig12265	antimicrobial peptide 1-like	**158.54**	**0.00**	**84.25**	**0.00**	**16.86**	**0.00**

Cluster	ID	Seq. Description	R1		R2		R3	
			FC	FDR	FC	FDR	FC	FDR
3	Ppnisotig13133	pathogenesis-related protein pr-4-like	**277.44**	**0.00**	**13.79**	**0.00**	**3.87**	**0.05**
3	Ppnisotig13431	disease resistance response protein 206-like	**143.94**	**0.00**	**11.34**	**0.00**	1.54	0.77
6	Ppnisotig00872	endoglucanase 17-like	-1.92	0.36	**4.37**	**0.00**	**26.49**	**0.00**
Transcriptional regulation of meristem activity								
1	Contig01739	homeobox-leucine zipper protein athb-14-like	**-5.43**	**0.02**	-1.18	0.97	-2.06	0.27
1	Contig02588	transcription repressor myb5-like	**-4.46**	**0.03**	-1.62	0.21	-1.41	0.72
1	Contig12050	transcription factor myb46-like	**-10.79**	**0.00**	-1.27	0.71	-2.86	0.13
1	Contig12416	transcription factor myb12-like	**-4.82**	**0.01**	1.76	0.51	1.36	0.96
1	Contig12421	transcription factor bhlh63-like	**-4.09**	**0.04**	-1.22	0.85	-1.44	0.75
1	Contig14511	homeobox-leucine zipper protein hat5-like	-1.61	0.46	**-4.42**	**0.00**	**-3.80**	**0.04**
1	Contig15411	transcription factor bhlh68	**-5.23**	**0.02**	1.14	0.94	-1.37	0.84
2	Contig00787	nac domain-containing protein 2-like	**10.24**	**0.01**	**2.30**	**0.03**	1.80	0.29
2	Contig01913	transcription factor myb44-like	**3.60**	**0.05**	-1.84	0.12	-1.10	1.06
2	Contig02808	probable wrky transcription factor 31	**17.90**	**0.00**	1.57	0.41	1.09	0.83
2	Contig02956	probable wrky transcription factor 7	**4.01**	**0.03**	1.29	0.73	1.62	0.56
2	Contig04787	protein tify 6b-like	**3.54**	**0.04**	1.15	0.99	1.13	1.04
2	Contig05465	nac domain-containing protein 2-like	**6.42**	**0.01**	1.63	0.20	1.72	0.54
2	Contig05634	nac domain-containing protein 2-like	**35.61**	**0.00**	**4.56**	**0.00**	1.16	1.04
2	Contig05923	transcription factor bhlh35	**3.59**	**0.04**	1.71	0.14	1.75	0.41
2	Contig06152	exordium like 2	**55.72**	**0.00**	3.21	0.06	-1.20	0.77
2	Contig06230	protein tify 10a-like	**22.88**	**0.00**	2.56	0.12	1.39	0.93
2	Contig12753	myb-related protein 308-like	**6.51**	**0.01**	-0.33	1.00	0.38	1.06
2	Contig13870	platz transcription factor family protein	1.69	0.38	1.66	0.33	**3.92**	**0.02**
2	Contig13895	nac transcription factor 29-like	**24.73**	**0.00**	1.57	0.44	1.50	0.89
2	Contig15806	probable wrky transcription factor 75-like	**8.60**	**0.01**	-1.12	0.94	-1.14	1.01
2	Contig17882	protein tify 10a-like	**22.53**	**0.00**	**3.72**	**0.00**	1.96	0.26
2	Contig18538	nac domain-containing protein 2-like	**11.99**	**0.00**	2.33	0.13	1.77	0.58
2	Contig20304	homeobox-leucine zipper protein athb-13-like	3.61	0.06	1.74	0.15	**2.83**	**0.04**
2	Contig20472	probable wrky transcription factor 2	**3.59**	**0.05**	1.23	0.94	-1.05	0.99
2	Contig20599	protein tify 10a-like	**26.56**	**0.00**	2.12	0.14	1.55	0.72
2	Contig20931	protein tify 10a-like	**3.98**	**0.04**	1.51	0.75	1.24	1.03
2	Contig24637	platz transcription factor family protein	-1.67	0.41	1.37	0.54	**3.06**	**0.04**
2	Contig25517	protein tify 10a	**9.46**	**0.01**	1.87	0.18	1.56	0.75
2	Contig30708	low quality protein: protein tify 10b-like	**9.06**	**0.01**	**2.01**	**0.05**	1.88	0.43
2	Ppnisotig00498	nac domain-containing protein 2-like	**4.02**	**0.03**	1.59	0.22	1.52	0.52
2	Ppnisotig05388	protein exordium-like 2	**14.03**	**0.00**	1.26	0.84	1.11	1.03

Cluster	ID	Seq. Description	R1		R2		R3	
			FC	FDR	FC	FDR	FC	FDR
2	Ppnisotig06078	protein exordium-like 2	**4.36**	**0.03**	1.33	0.70	0.41	1.04
2	Ppnisotig07889	platz transcription factor family protein	-1.46	0.60	1.20	0.83	**3.56**	**0.02**
2	Ppnisotig12838	probable indole-3-pyruvate monooxygenase yucca4	**-3.15**	**0.04**	1.65	0.17	**3.50**	**0.01**
3	Contig06361	protein tify 10a-like	**64.22**	**0.00**	**8.34**	**0.00**	**2.86**	**0.04**
4	Contig05551	probable wrky transcription factor 51-like	-2.66	0.13	**-3.91**	**0.00**	**-3.69**	**0.04**
4	Contig09007	exordium like 2	-1.15	1.10	**-2.35**	**0.03**	1.10	1.11
4	Contig20476	exordium like 2	1.78	0.34	**-3.07**	**0.01**	1.41	0.72
5	Contig03423	floricaula leafy homolog	-2.25	0.20	1.61	0.21	**5.78**	**0.00**
5	Contig13239	axial regulator yabby 5-like	**-3.82**	**0.05**	**2.44**	**0.03**	1.20	1.01
5	Contig23326	transcription factor myb12-like	-2.89	0.09	1.36	0.71	**3.06**	**0.03**
6	Contig03401	flowering-promoting factor 1-like protein 3-like	1.79	0.43	**2.59**	**0.03**	**9.31**	**0.00**
6	Ppnisotig04954	growth-regulating factor 1-like	-1.15	1.11	**2.68**	**0.02**	**18.01**	**0.00**
6	Ppnisotig05462	homeobox-leucine zipper protein meristem l1-like	-0.33	1.15	**3.16**	**0.01**	**16.06**	**0.00**
6	Ppnisotig07853	homeobox-leucine zipper protein hdg11-like	-0.41	1.15	1.94	0.10	**11.42**	**0.00**
6	Ppnisotig08430	protodermal factor 1-like	-1.22	1.06	**8.48**	**0.00**	**73.43**	**0.00**
Hormone signalling								
1	Contig12053	ethylene-responsive transcription factor rap2-4-like	-1.28	0.85	**-2.96**	**0.01**	**-3.74**	**0.04**
2	Contig24730	auxin-responsive protein iaa26-like	**3.51**	**0.05**	-0.02	0.91	1.82	0.36
2	Contig39240	ethylene-responsive transcription factor rap2-12-like	1.47	0.58	**2.65**	**0.02**	**3.80**	**0.01**
2	Ppnisotig06916	auxin-responsive protein iaa26-like	**3.48**	**0.05**	1.07	1.01	1.68	0.50
4	Contig15238	auxin-responsive protein iaa13-like	1.42	0.65	1.26	0.73	**-3.35**	**0.05**
6	Contig14132	non-specific lipid-transfer protein 2-like	-3.02	0.15	**3.66**	**0.01**	**106.88**	**0.00**
Non annotated genes and unknown functions								
1	Contig08604	—NA—	-0.46	0.96	**-16.10**	**0.00**	**-7.68**	**0.01**
1	Contig13781	—NA—	**-15.86**	**0.00**	-1.39	0.55	-2.35	0.15
1	Contig19504	—NA—	**-15.59**	**0.00**	-1.38	0.60	-2.16	0.21
2	Contig11518	—NA—	**28.49**	**0.00**	-0.37	0.14	1.27	0.80
2	Contig22230	—NA—	**33.54**	**0.00**	**3.91**	**0.03**	-1.24	0.97
2	Contig22397	—NA—	**30.95**	**0.00**	1.89	0.17	0.44	1.07
2	Contig23569	—NA—	**79.79**	**0.00**	2.66	0.09	1.05	1.01
2	Contig43786	—NA—	**28.79**	**0.00**	1.61	0.46	1.86	0.32
3	Contig03506	hypothetical protein SELMODRAFT_115352	**129.97**	**0.00**	**85.55**	**0.00**	**15.08**	**0.00**
5	Contig03452	—NA—	**-11.39**	**0.00**	**5.01**	**0.00**	**6.61**	**0.00**
5	Contig13782	—NA—	**-15.68**	**0.00**	1.31	0.69	2.20	0.13

Moreover, we selected 11 DEGs for validation of the expression profiles resulted from microarrays (Table 1), including 4 genes with key role in cell wall formation and lignin deposition (pectinesterase-like, CeSA-like, CCoAOMT-like and PAL-like proteins), coding for transcription

factors involved in developmental processes (MYB46-like, ATHB13-like, NAC2-like, WRKY51-like, and YAB5-like), and encoding proteins related to stress response (EXORDIUM-like, and Major Allergen Pru AR1-like proteins). According to the correlation coefficients between both qPCR and microarray hybridization, the general profiles obtained in this study were validated (Fig. 4).

Some of DEGs overexpressed at R0 (clusters 2 and 3), and regardless their significance level at R1/R2, presented the highest expression values, with FC values ranging from 32.41 to over 450. Among them, we found genes coding for hydrolytic enzymes (f.i., Contig18804 and Ppnisotig13431 coding for PI206), and putative pathogenesis-related PR-4-like proteins (Contig19053, -22375, and Ppnisotig13133). Furthermore, another important group of induced genes at R0 were annotated as chitinases- and endochitinases-like proteins (f.i., Contig00126, -23442 or Ppnisotig08058 in cluster 2, or Contig10307, -21216 and Ppnisotig00751 in cluster 3), antimicrobial peptide 1-like (Ppnisotig12265), defensin-like protein (Contig00602 and – 17617), and a major allergen pru ar1 homologous (Contig22185). These two clusters also included induced DEGs at R0 coding for proteins related to oxidative stress, such as peroxidases (f.i., Contig06170, -08118 and Ppnisotig01747), or glutathione-S-transferase-like proteins (Ppnisotig02901 and – 06171). We also found three transcripts coding for PAL-like proteins (Contig16597, -20555 and – 20817) showing overexpression at R0 in cluster 2. Among transcriptional factors, genes coding for NACs (f.i., Contig00787, -05634 and – 13895), and WRKYs (f.i. Contig02808 and – 15806), together with two MYB-like proteins (Contig01913 and – 12753), were found in cluster 2 to be overexpressed at R0. Moreover, several TIFY-like transcription factors were also induced at R0, including one transcript coding for a TIFY 10a-like protein (Contig06361) found in cluster 3 highly overexpressed.

Conversely, some genes showed repression at R0, as those included in clusters 1 and 5. In this regard, we found genes coding for catalytic subunits of the cellulose synthase (f.i., Contig00654, -20721, and – 41981), members of the CAZymes superfamily (f.i., Contig05066, -09025, -11436, -13778 or Ppnisotig01389), a COBRA-like protein (Contig01405) or genes involved in phenylpropanoid biosynthesis (Contig02447 and – 06476). In addition, transcription factors of the MYB (Contig02588, -12050, and – 12416) and bHLH families (Contig12421 and – 15411) were also found in cluster 1 to be repressed at R0, together with the homologous of *Arabidopsis* ATHB14 (Contig01739). Also in cluster 1 we found a homologous of auxin efflux carrier component 1c-like (PIN1C), encoded by Contig19183, that was repressed in R0.

A total of 436 genes were differentially expressed R1 and R2, when the new epicormic shoots arose from stem, significantly up- or downregulated. From the latter, 244 DEGs showed a significant repression during both R1 and R2, or at least at one of these stages. On the other hand, cluster 6 included 21 DEGs overexpressed at R1 and, to a greater extent, R2, with high FC values ranging from 8 to over 100. Among these DEGs, we should mention those transcription factors belonging to the HD Zip Class IV family, such as PROTODERMAL FACTOR1-like (PDF1; Ppnisotig08430) and MERISTEM LAYER1-like proteins (ML1; Ppnisotig05462), or the homologous of the HOMEODOMAIN GLABROUS 11-like protein (HDG11; Ppnisotig07853). Moreover, the transcript Ppnisotig04954, encoding for a *P. canariensis* orthologous member of the GROWTH-REGULATING FACTOR (GRF) family, showed increasing FC values in R1 and especially in R2. Additionally, a FLOWERING-PROMOTING FACTOR1-like (FPF1) protein, encoded by Contig03401, had a similar expression profile than GFR during R1 and R2.

Among the transcription factors found in cluster 5, Contig13239, encoding for a YABBY5-like protein, was significantly overexpressed in phase R1, while a homologous of LEAFY (LFY; Contig03423) was induced at R2, as well as a PHD (plant homeodomain) finger protein (Contig03217). Also in cluster 5, repression at R0 followed by overexpression at R1 and R2 was detected for the sequence Ppnisotig05961, which is coding for an auxin-responsive protein IAA33. Finally, in cluster 2 we also found a gene encoding for a ATHB13-like protein (Contig20304), a YUCCA4-like, and three members of the PLATZ-like transcription factor family (Contig13870, -24637, and Ppnisotig07889), all of them induced at R2.

The Venn diagram in Fig. 5 illustrates a comparative analysis by groups between DEGs in the resprouting process and DEGs found during the seasonal apical growth in the Canary Island pine. From the 1164 resprouting-DEGs, 571 were shared with apical growth (49.05%), of which 295 DEGs were underexpressed during the whole response and 276 overexpressed at some point of the process. From the latter, 108 DEGs were induced during the resprouting phases R1 and/or R2. Moreover, 170 DEGs were shared between R1, R2 and apical development, but not with R0. Correspondingly, 6599 DEGs were exclusive for apical growth, while another set of 593 genes were resprouting-exclusive DEGs (included as DEGs in R0, R1 and R2, but not in apical growth). From them, 407 were uniquely expressed at R0 (34.97% of total), including 188 induced and 219 repressed genes. On the other hand, 95 resprouting exclusive DEGs (16.84% of total) were expressed also during R1/R2 (including 48 overexpressed DEGs in at least one sampling date), and 91 were expressed just in R1/R2 (where 32 DEGs were induced at some point).

Discussion

Microarray hybridizations of RNA samples covering the resprouting response to wounding yielded 903 DEGs in R0, the largest set, while R1 and R2 included 278 and 261 DEGs, respectively. Considering these significant differences, two main periods were covered by the sampling

design, i) R0 as the immediate response to wounding, including 728 DEGs (62.54% of total) that were exclusive for this time, and ii) R1 and R2 involved in the emerging and *de novo* development of epicormic shoots. Additionally, even though GO enrichment analysis yielded no statistically enriched terms, we were able to identify terms related to resprouting or outgrowth processes that were associated to R1/R2 DEGs (Online Resource Fig. S2). Thus, we found remarkable GO terms at levels 2 and 3 related with developmental activity, such as "growth", "anatomical structure development" or "biosynthetic process", or more specific terms such as "shoot system development" and "flower development" in higher levels (6 and 8, respectively), indicatives of *de novo* lateral organogenesis.

In addition, these 1164 DEGs were grouped in 6 clusters according to their expression profiles. Induced genes during the immediate response at R0 included genes related to pathogenic and stress response, as described for the immediate response during the wound-healing process (Chano et al. 2017) This is the case, for instance, of the disease resistance response protein PI206, which was described in *Pisum sativum* L. to participate during the infection by *Fusarium solani* (Mart.) Sacc. (Culley et al. 1995), or putative PR-4-like proteins, which are coded by a gene firstly named as win-1 and win-2, for "wound-inducible genes", in *Solanum tuberosum* Poepp. Ex Walp. (Stanford et al. 1989). We also described the overexpression of chitinases and endochitinases-like proteins at R0, with important roles in plant defense against fungi and insects (Seo et al. 2008), PAL-like proteins, involved in the phenylpropanoid biosynthesis pathway but also with important roles in plant defense (Ohl et al. 1990; Kervinen et al. 1998; Kim & Hwang 2014), antimicrobial peptide 1-like with antifungal and antibacterial properties (Castro & Fontes 2005), defensin-like protein involved in defense-related processes and plant development (Tam et al. 2015), and a major allergen pru ar1 homologous which is a pathogenesis-related protein involved in the response to ripening in *Prunus armeniaca* (Scop.) Turcz. (Mbéguié-A-Mbéguié et al. 1997). Moreover, the peroxidases and glutathione-S-transferase-like proteins found in clusters 2 and 3 are involved in ROS (Reactive Oxygen Species) detoxification during the response to pathogenesis (Gunnar Fossdal et al. 2001), and in the anti-oxidative plant defense (Meyer et al. 2008). Consistently, transcription factors with active roles in response to stress were also overexpressed during R0, like NACs and WRKYs (Zhang & Wang 2005; Hu et al. 2010).

On the other hand, several genes involved in developmental processes during xylogenesis, like CesA or CAZymes, or in the phenylpropanoid biosynthesis, showed repression at R0. In addition, transcription factors related to cell wall biosynthesis like MYB and bHLH were also found repressed at this time together with ATHB14/PHABULOSA (PHB). The latter, which belongs to the HD Zip Class III family of transcription factors (Ariel et al. 2007), was described as mediator during adaxial/abaxial polarization in ovule primordia (Sieber et al. 2004), the establishment of the SAM and apical bilateral symmetry (Prigge et al. 2005), and the perception of radial positional information in leaf primordia (McConnell et al. 2001). Interestingly, several of these genes were related to earlywood formation during xylogenesis in the Canary Island pine (Chano, López de Heredia, et al. 2017), and its repression seems to be a major trend during the rearrangement of the xylogenesis transcriptional program in the immediate response to wounding, as described also in Chano et al., 2017 (Chano, Collada, et al. 2017).

Furthermore, some of the DEGs that were induced at R1 and/or R2, like those included in clusters 2, 5 and 6, were involved in developmental processes and *de novo* organogenesis. From cluster 2, YUCCA4-like transcription factor was found to be overexpressed in R2. Several members compose the YUCCA family, with overlapping roles in growth under hormone signaling. Particularly, YUCCA4 has been described to play an important role in auxin increase during floral organogenesis in *Arabidopsis thaliana* Schur (Cheng et al. 2006; Munguía-Rodríguez et al. 2020). In *P. canariensis*, this gene was reported to participate during primary growth in the early stages of apical shoot development (Chano et al. 2021). We also found two remarkable transcription factors that may play specific roles during resprouting in cluster 5. One of them is YABBY5-like protein, which was significantly induced at R1. The YABBY family is implicated in the regulation of lateral organ growth in seed plants (Yamada et al. 2011), therefore it is proposed to be a relevant candidate in the initiation of resprouting. Also in cluster 5, we found LEAFY-like transcription factor to be induced at R2. LEAFY is a plant-specific transcription factor controlling flower development from axillary meristems (Schultz & Haughn 1991; Weigel et al. 1992), and, therefore, another suitable candidate gene to be involved in resprouting. As exposed, these two genes differ in the moment of their activity, noticing an implication of YAB5 at R1 (FC = 2.44) and LFY at R2 (FC = 5.78). YAB5 was reported to participate in the fate and maintenance of meristem activity during lateral organogenesis in rice (Tanaka et al. 2012). As well, YAB5 acts redundantly in *Arabidopsis* with FIL/YAB3 and YAB2 in the polar establishment of abaxial surface, activating laminar programs, repressing SAM programs and forming the marginal domain in leaves (Sarojam et al. 2010). Moreover, the homologous YAB5 in *Cabomba coraliniana* was expressed in the abaxial tissues of the distal leaf primordium, playing an important role during the proliferating cell process, as well in the floral bud development. In vegetative shoots, YAB5 displays a similar expression pattern than FIL/YAB3, expressed in marginal and abaxial tissues in lobe primordial and procambial strands (Yamada et al. 2011). Regarding LEAFY, it has been found recently in the lycopsid genus *Isoetes* to be involved in the development of both reproductive and vegetative tissues during lateral organogenesis (Yang et al. 2017). These two genes were found to be expressed during the initiation of apical growth in the Canary Island pine but repressed at later stages (Chano et al. 2021). We also found in this cluster PIN1C, repressed at R0 but followed by a significant induction at R1 and R2. PIN1 is responsible for basipetal transport of auxin from shoot to root and has been found to be expressed during outgrowth from axillary buds in *C. morifolium* (Dierck et al. 2018). This gene was also significantly overexpressed at the initiation of apical shoot development in *P. canariensis* but repressed during the rest of the seasonal growth (Chano et al. 2021).

From genes found in cluster 6 to be induced at R1 and/or R2, PDF1 is a proline-rich cell wall protein expressed exclusively in the L1 layer of shoot meristems in *Arabidopsis*, controlling cell differentiation in the epidermis of new buds (Abe et al. 2003). In *Gossypium* spp. it was found that the GbPDF1 protein has an important role during fiber initiation (Deng et al. 2012). Moreover, the functional role of PDF1 was found under regulation of two HD Zip Class IV members in *Arabidopsis* (Abe et al. 1999, 2003). One of them is PROTODERMAL FACTOR2 (PDF2), which has not been identified as DEG in this work, while the other is the *Arabidopsis thaliana* ATML1 gene, found to be co-expressed together with PDF1, both in cluster 6. This gene was first suggested to be expressed only in the first layer (L1) of the SAM (Lu et al. 1996). In addition, HDG11, which is another member of this gene family that has been also found in cluster 6, was reported to be implicated in the repression of trichome outgrowth in *Arabidopsis* (Nakamura et al. 2006). In addition to this, these three co-expressed HD Zip class IV members (PDF1-, ML1-, and HDG11-like) were downregulated over seasonal growth in the apex in the Canary Island pine (Chano et al. 2021). These HD Zip class IV members may be involved in the development of an *indumentum* and epithelium covering the surface of emerging buds.

As well, other DEGs found in cluster 6 (overexpressed at R1 and R2) that also showed upregulation during the initiation of apical development were FPF1 and GRF1. The former, which in *Arabidopsis* promotes flowering (Kania et al. 1997), has been described to cause a strong effect on wood formation under constitutive overexpression in transgenic lines of poplar but no effects in early flowering (Hoenicka et al. 2012). The latter belongs to a plant specific transcription factor family implicated in multiple developmental processes in different tissues and organs, such as leaves, stems, roots, seeds and flowers (Omidbakhshfard et al. 2015). Recently, GRFs and part of the microRNA regulator machinery of GRFs were reported to be involved in floral organ development, determining sepal/petal identity (Pajoro et al. 2014).

Finally, from those genes that were considered to be exclusive for resprouting (i.e., differentially expressed in R1 and/or R2, but not in apical growth), it is worth to remark 32 DEGs showing overexpression at one or both sampling points, of which 8 genes (25%) were not annotated or annotated as uncharacterized proteins. From annotated genes, we found the overexpression of ATHB13-like protein in R2, which has been suggested to be involved in regulation of cotyledon and leaf development in *Arabidopsis* (Mattsson et al. 2003; Henriksson et al. 2005). This gene was also reported during the immediate response to wounding in the Canary Island pine (Chano, Collada, et al. 2017), but not included among apical shoot elongation as a DEG (Chano et al. 2021). In *A. thaliana* this gene was reported to have a negative role in stem elongation, while it is usually expressed in tapetum development during pollen formation (Ribone et al. 2015), and upregulated in response to abiotic stress conditions and pathogenesis (Cabello & Chan 2012; Cabello et al. 2012). We also found in cluster 5 a plant homeodomain (PHD) finger induced at R2. This gene is involved in the epigenetic regulation of gene expression by the sequence-specific recognition of histone H3 and reading the modification state (Sanchez & Zhou 2011). Interestingly, several of these resprouting-exclusive genes have been reported to participate in cell signaling and transport, including two isoforms of the vacuolar amino acid transporter YPQ1, a nitrite transporter, an amino acid permease 6-like, and WAT1-related protein.

Concluding Remarks

Resprouting is an important and rare trait in conifers that is induced after wounding in the Canary Island pine. The examination of transcriptional changes during this process allowed us to determine genes required for cell signaling, cell identity and cell proliferation sub-processes. In this work, we reported several gene families that are implicated in the resprouting process itself, including indumentum development, laminar outgrowth, and bud emerging. Our data provide evidence in favor of the resprouting activity of YABBY and LEAFY transcription factors, and members of the class IV of the Homeodomain Leucine Zipper such as PDF1, ML1 and HDG11. As well, other transcription factors involved were GRF1 and FPF1. The implication of such transcription factor families, with important roles in leaf and inflorescence development also in flowering plants, suggests underlying homologies between cell proliferation and tissue differentiation patterns in many lateral organogenesis processes.

This work provides the first insights into the transcriptomic basis of resprouting in conifers. Many of the genes reported here showed significant overexpression during resprouting as well as intense transcriptomic activity at the beginning of apical growth, suggesting non-exclusive mechanisms for resprouting in *P. canariensis*, at very least during development of epicormic shoots. Nevertheless, we cannot exclude an exclusive genetic control of resprouting initiation: genes such as ATHB13 or PHD-finger, together with some non-annotated sequences, were not found during seasonal shoot development. Thus, further research is needed to disentangle the functionalities of these candidate genes for comprehensive analysis of the resprouting capability in conifer species.

Declarations

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Authors contribution

AS and CC designed the experiment. AS and VC carried out the experiment and sample collection. VC performed lab work, data analysis, and wrote the first version of the manuscript. AS and OG edited and reviewed the final version. All authors have read and approved the final manuscript.

Competing interests

The authors have no competing interests to disclose.

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Figures

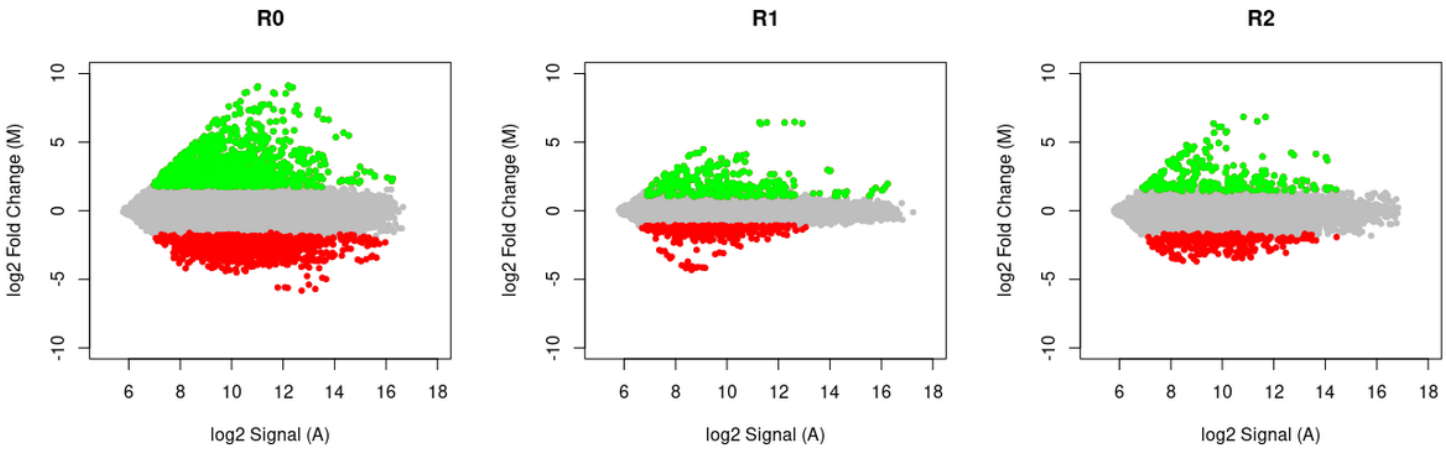

Figure 1

MA plot of microarray normalized data during resprouting process. X-axis: Log2 of microarray signals; Y-axis: Log2 of Fold Change values; Green dots: probes selected as overexpressed (FC > 2, FDR < 0.05, between experimental and control RNA samples); Red dots: probes selected as underexpressed (FC < -2, FDR < 0.05 between experimental and control RNA samples)

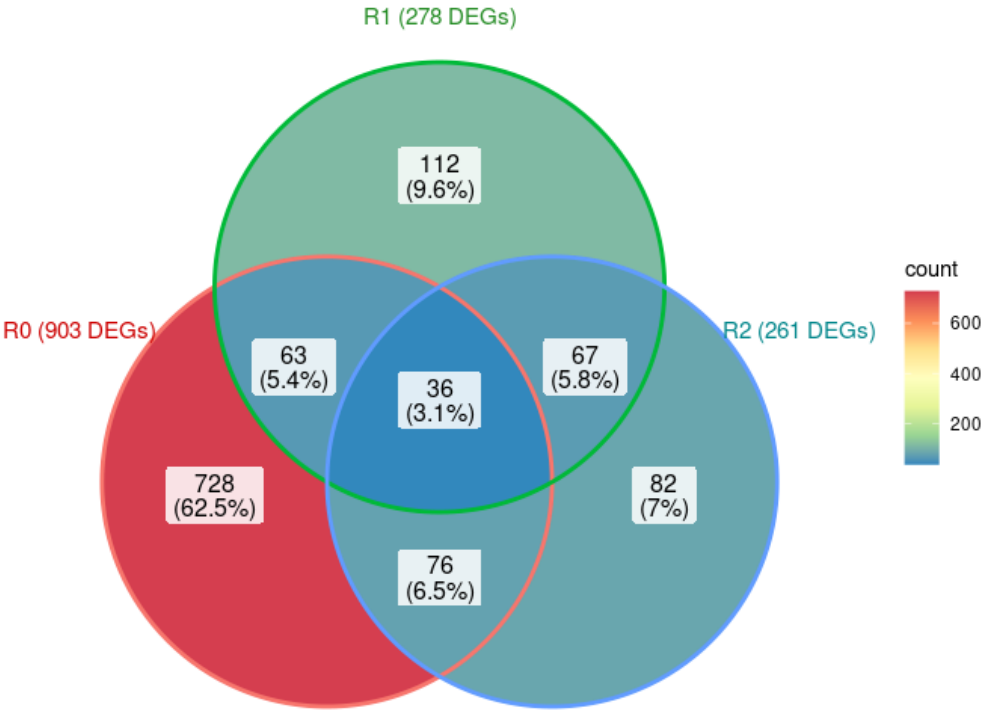

Figure 2

Differentially Expressed Genes during three stages of resprouting in response to wounding (R0: immediate response to wounding; R1: resprouting initiation; R2: resprouting elongation)

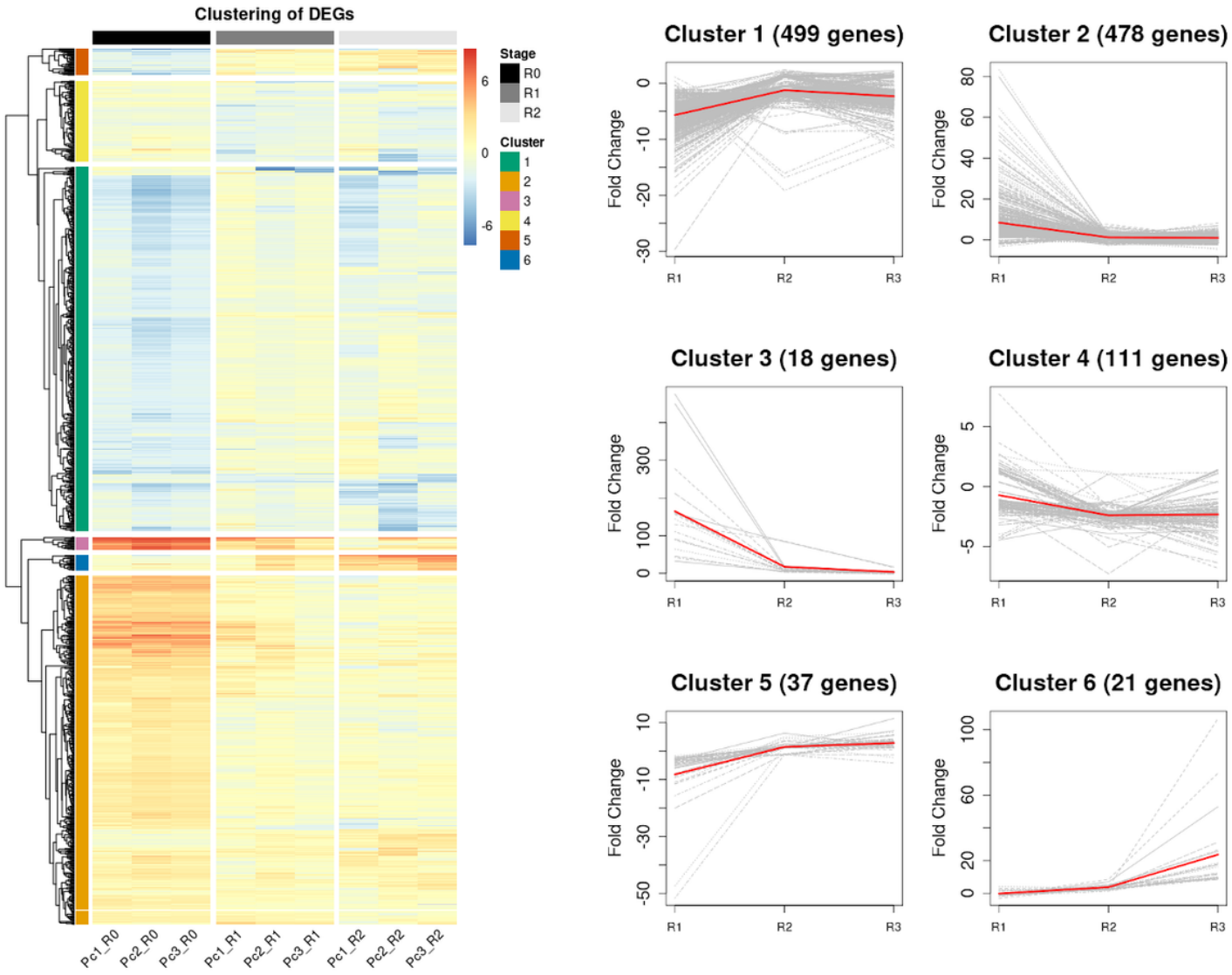

Figure 3

Hierarchical clustering of 1064 DEGs for three biological replicates (PC1, PC2 and PC3) and three resprouting stages (R0, R1, and R2), identifying 6 clusters showing gene expression profiles by Fold Change variations along sampling dates

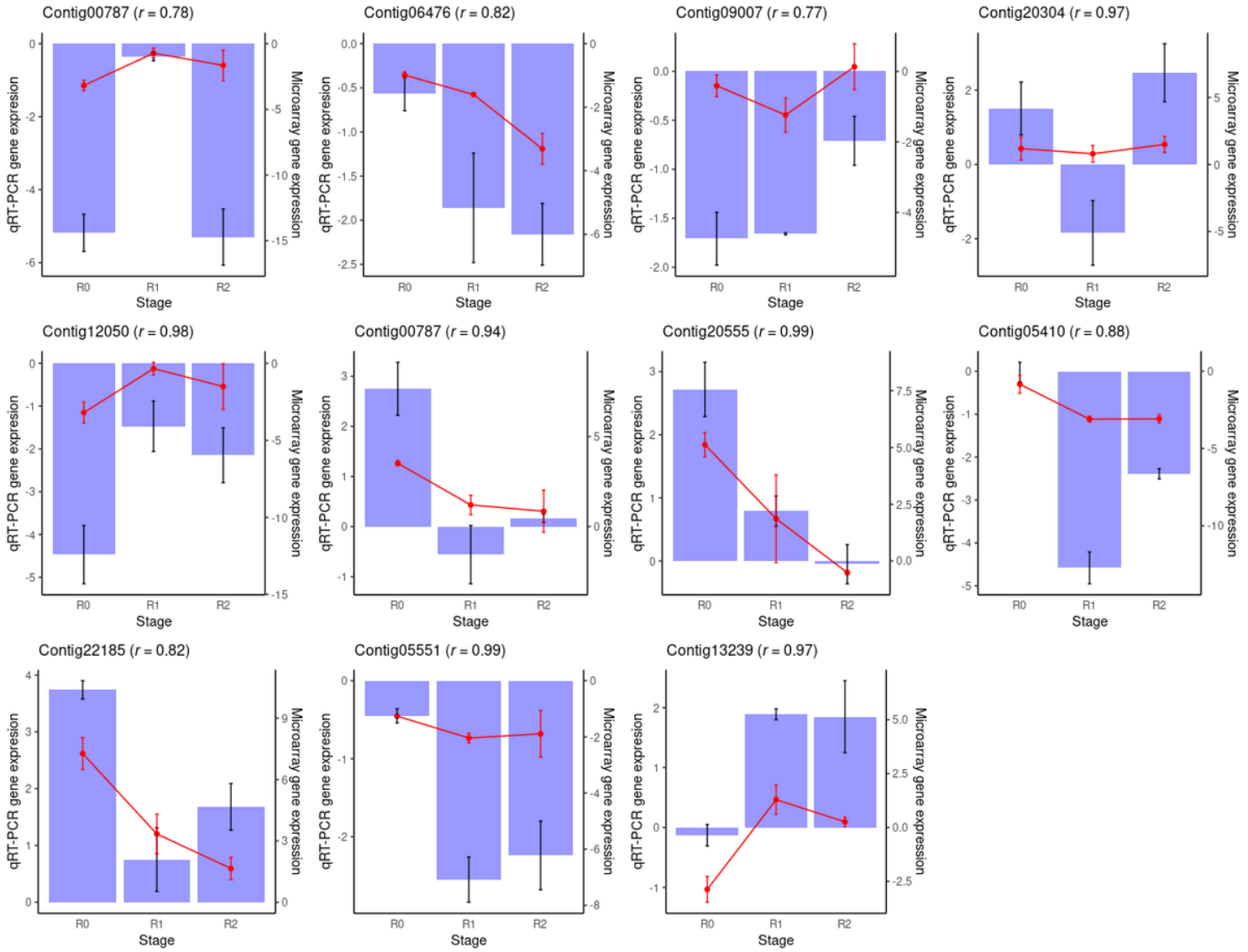

Figure 4

Real-time PCR validation of microarray expression values in *Pinus canariensis* of 12 genes coding for pectinesterase-like (Contig05410), CeSA-like (Contig00654), CCoAOMT-like (Contig06476), PAL-like proteins (Contig20555), MYB46-like (Contig12050), ATHB13-like (Contig20304), NAC2-like (Contig00787), WRKY51-like (Contig05551), YAB5-like (Contig13239), EXORDIUM-like (Contig09007), and Major Allergen Pru AR1-like proteins (Contig22185). x-axis: developmental stages of resprouting; y-axis left: normalized gene expression values for qRT-PCR (bars); y-axis right: absolute expression values for microarray experiments (continuous line). Pearson's correlation coefficients *r* between qRT-PCR and microarray expression values are also indicated for each gene

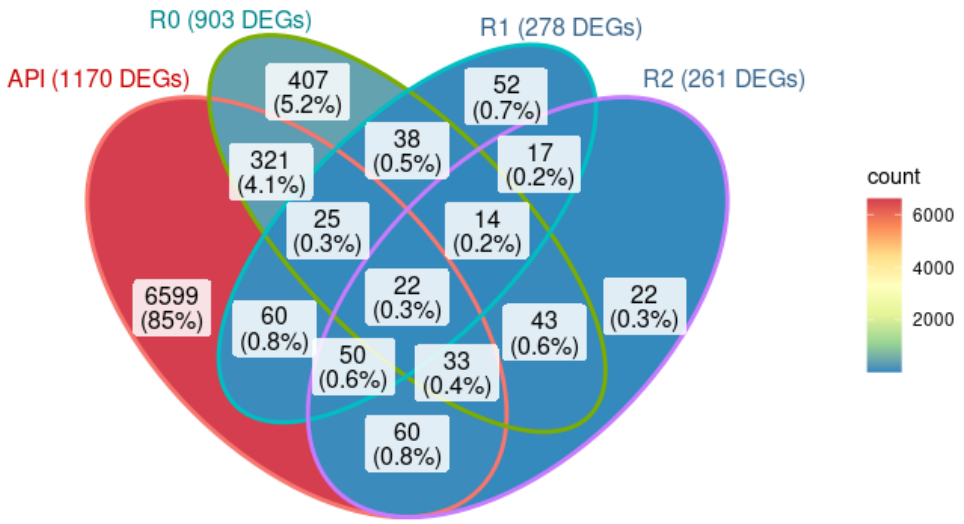

Figure 5

Venn's diagram of apical growth genes expressed during resprouting in response to wounding in P. canariensis

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