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Original article

The effects of drought stress on leaf gene expression during flowering in blackcurrant (*Ribes nigrum* L.)

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Summary

This study provides genome expression analyses from the blackcurrant cultivar 'Ben Gairn' after five days of drought stress. RNA Sequencing (RNA-Seq) data was utilized to generate a non-redundant set of 40,225 predicted transcripts used to design a custom *Ribes* microarray. A set of 2,115 differentially expressed genes were identified during drought treatment; 429 of these genes were up-regulated, with 263 showing homology to unique *Arabidopsis thaliana* (At) accessions, and 1,686 genes were down-regulated, with 675 unique At numbers. The *Arabidopsis* homologs were analysed for enrichment of GO (gene ontology) terms using the Term Enrichment Tool. This showed a number of GO terms highly enriched in the drought up-regulated and down-regulated gene lists in GO categories associated with molecular function, biological process and cellular component. The identification of several hormone metabolism, cell wall, cell cycle, and transcription factor genes indicated that they could play an important role in the drought stress tolerance response. The results provide relevant information for focusing future studies with the aim to develop drought tolerant cultivars for sustainable production.

Keywords
cell wall and cell cycle, GO term enrichment analyses, hormone metabolism genes, *Ribes* microarray, transcription factors

Significance of this study

What is already known on this subject?

- Drought is a major limitation for crop productivity worldwide and in future periods of water stress are more likely to occur. Molecular responses to drought stress are very complex, but our understanding has rapidly progressed with the identification of thousands of genes involved in acclimatization and adaptation.

What are the new findings?

- Genome expression analyses from blackcurrant 'Ben Gairn' after five days of drought stress. Volcano filtering identified 2,115 differentially expressed microarray probes; 429 were up-regulated, with 263 showing homology to unique *Arabidopsis thaliana* (At) accessions, and 1,686 were down-regulated, with 675 unique At numbers.

What is the expected impact on horticulture?

- Putative candidate genes involved in drought stress tolerance of blackcurrant were identified, but require further, more detailed, studies to confirm their role. The results provide relevant information for focusing future studies with the aim to develop drought tolerant cultivars for sustainable production.

Introduction

Blackcurrant (*Ribes nigrum* L.) is a woody fruit shrub grown mostly in temperate climates for juice processing, as the berries have a very high nutritional value in terms of antioxidants (Brennan and Graham, 2009). During recent growing seasons, more extreme weather conditions have had a negative impact on the productivity and sustainability of the crop in the North Sea Region. For example, extended periods of droughts, as in 2013, or heavy rainfall during the production season, as in 2012, and lack of winter chill during several recent winters (Kahu et al., 2009; Anon., 2013) threaten the production of blackcurrants.

Drought conditions are one of the main limiting factors for crop productivity that may result in reduced yields (Hsiao, 1973; Blum, 2011). The response of plants to water deficit varies with genotypes and usually involves a mixture of tolerance and stress avoidance mechanisms (Chaves et al., 2003). The majority of studies on the molecular responses to drought stress were previously carried out using the model plant *Arabidopsis thaliana*, and many genes were characterized (Huang et al., 2008). Recently, genome and transcriptome sequence information of other, non-model plant species have become available, which has led to research on the molecular responses to drought stress in perennial plants such as *Populus simonii* (Chen et al., 2013), *Malus domestica* (Wisniewski et al., 2008), and *Citrus reticulata* (Gimeno et al., 2009). However, the specific functions of many genes remain unknown in perennial species due to inconsistency of both experimental growth conditions and species-specific genomic resources. Therefore, it is now imperative to understand the mechanism of specific responses to drought conditions in blackcurrant (Čereković et al., 2013, 2014). This information can subsequently be used to select cultivars with an increased ability to respond to and recover from stressful conditions.

Drought stress has a great similarity with other abiotic stresses at a physical and molecular level (Chaves et

al., 2003). In addition, when plants are exposed to drought stress it is common that several abiotic stresses act concurrently on the plant, where effects of drought stress are often compounded by associated pressures such as heat, salinity, oxidative, and nutrient stress (Jewell et al., 2010). All these abiotic stresses induce morphological, physiological, biochemical, and molecular changes which affect plant growth and productivity (Zhu, 2001). They often activate similar cell signalling pathways and cellular responses (Zhu, 2001; Shinozaki and Yamaguchi, 2007), such as the production of stress proteins, up-regulation of antioxidants and accumulation of compatible solutes (Wang et al., 2003). This overlap in multiple stress responses has been termed 'cross-talk' (Chinnusamy et al., 2007).

It has been recognized that many drought genes are commonly responsive to several hormones (Huang et al., 2008). Cell wall-related genes and protein kinases also have regulatory roles in stress response and signal transduction (Qiang et al., 2000). As many biological processes are regulated at the level of transcription, understanding the roles of transcription factors (TFs) is also important to increase our understanding of the molecular regulation of drought stress (Zhang, 2003).

Microarray technology is an important method used for the identification and characterization of drought stress response genes in plants (Huang et al., 2008; Lorenz et al., 2011). Gene expression profiling using this technology is a powerful tool to identify genes of interest in different tissues and stages of development (Slonim and Yanai, 2009), and has also been used in the study of berry crops during different abiotic stress conditions (Hedley et al., 2010).

There is large diversity in the genetic material available in blackcurrants, and many different cultivars are grown in different countries to fit the global production (Brennan, 2008). Traditionally, cultivar selections are made based on productivity and disease resistance. However, with changing climatic conditions and reduced productivity, breeding material needs to be re-evaluated for increased adaptive and phenotypic plasticity (Chaves, 2003; Blum, 2011). Exploitation of the available germplasm to identify the genetic diversity in response to drought stress has not been investigated in blackcurrants, and there is significant potential to select cultivars with an increased ability to respond to and recover from stressful abiotic conditions (Hedley et al., 2010).

The aim of the present study was to investigate the effect of drought stress during flowering in the Scottish blackcurrant cultivar 'Ben Gairn'. Our hypotheses were: i) drought stress induces alterations in gene expression in leaves of 'Ben Gairn' and, therefore, ii) some of the candidate genes involved in drought stress tolerance in blackcurrant can be identified. Using a *Ribes* microarray we identified some of the candidate genes involved in drought stress responses in blackcurrant which should assist in the selection of drought stress tolerant cultivars for blackcurrant breeding programs.

Materials and methods

Plant material and growing conditions

A pot experiment was conducted under greenhouse conditions at Aarhus University, Aarslev, Denmark, under natural light conditions and ambient photoperiod. In March one year old cold-stored plants propagated from cuttings of blackcurrant (*Ribes nigrum* L.) cultivar 'Ben Gairn' were planted in 3 L-pots (VCD 19, Pöppelmann GmbH & Co, Germany) in a sandy loam topsoil with 67% coarse sand, 16% fine sand, 15% clay and 2% silt and containing 1.2% organic matter. From the day of potting, all plants were ebb/flood irrigated daily with a nutrient solution containing (in mg L^{-1}): 181 N, 30 P, 200 K, 32 Mg, 138 Ca, 18 Na, 41 Cl, 37 SO_4, 2 Fe, 1 Mn, 0.22 B, 0.1 Cu, 0.14 Zn and 0.08 Mo and with a pH of 6.0 and an EC of 1.99 dS m^{-1}.

Experimental design and treatments

Plants were grown in a completely randomized layout. At the beginning of flowering, one month after planting, two different water availabilities were established: 1) Fully irrigated (FI) and 2) Non-irrigated (NI) for 5 days. Water loss (evapotranspiration) was recorded on a daily basis using an electronic scale for each pot individually. The FI plants were given water individually every day in the late afternoon according to the weight loss since the last irrigation. For the plants exposed to drought (NI treatment), the water loss was recorded every day. The youngest fully expanded leaves were harvested after five days of treatment and flash frozen in liquid nitrogen, prior to RNA extraction. A total number of 8 plants were used in the experiment; 4 replicates per irrigation treatment.

RNA extraction

Total RNA was extracted from frozen blackcurrant leaf material (100 mg) using the Plant RNeasy Mini Extraction Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's recommendations (substituting buffer RLT for RLC and including 10% v/v RNA Isolation Aid (Ambion) and 1% v/v β-mercaptoethanol). All RNA samples extracted from blackcurrant leaf material had A_{260}/A_{280} ratios in the range of 1.8–2.0 as analyzed by a NanoDrop® ND-1000 Full-spectrum UV-Visible Spectrophotometer (ThermoFischer Scientific, Epsom, UK). The objective RNA integrity indicator (RIN) values for each sample were determined using the Agilent Bio-analyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and were usually >7.0, indicative of a high quality RNA sample. The RNA samples were aliquoted in batches and stored at -80°C.

Microarray processing

A custom Agilent microarray was designed in a 8 × 60k probe format to represent 40,225 predicted transcripts derived from Illumina RNA-Seq data generated from *Ribes* developing leaf bud tissue (A-MEXP-2372 - JHI *Ribes nigrum* 60k v1). A single-channel microarray experimental design was used to process total RNA according to the manufacturer's recommendations (Low Input Quick Amp Labelling, v. 6.5; Agilent Technologies). Data were extracted from scanned microarray images using Feature Extraction software (v. 10.7.3; Agilent Technologies) and imported into Genespring software (v. 7.3; Agilent Technologies) for pre-processing and analysis.

Principal components analysis identified outlying replicates (one from each sample set) which were subsequently removed from the dataset. This ensured the most reliable and significant gene expression changes were determined in downstream analysis. The default settings were: (i) any raw signal <5.0 was set to 5.0; (ii) each signal was divided by the median signal of all probes for that microarray; (iii) each signal was divided by the median of its signal across all microarrays. Data were filtered to remove probes with unreliable signal, selected as absent in >10 of 12 samples,

leaving 31,159 probes. Volcano filtering was used to identify 2,115 probes with significant gene expression changes between watering regimes (*P*-value <0.05; fold-change >2x or <0.5x).

Analysis of Gene Ontology terms

Of the 2,115 significantly and differentially expressed array probes identified from volcano filtering, 938 represented genes with a high degree of homology to annotated *Arabidopsis thaliana* sequences. These genes were subsequently used for GO (Gene Ontology) term enrichment analysis to identify overrepresented pathways and processes. This set of significant genes were split into up- and down-regulated groups based on the ratio (r) of expression between NI and FI treatments (r=NI/FI) with cut off values of 2 and -0.5. Up- and down-regulated sets of *Arabidopsis* homologs were imported into the AgriGO agricultural GO analysis tool (http://bioinfo.cau.edu.cn/agriGO/). A Fisher statistical test with Bonferroni multi-test adjustment was used with a significance level cut-off of 0.05 and a minimum number of mapping entries of 5. Output lists of GO terms with false discovery rates (FDR) <5% were created.

Results and discussion

Identification of differentially expressed genes in response to drought stress

Using volcano filtering, 2,115 probes with minimum a two-fold change in gene expression between NI and FI 'Ben Gairn' were selected for further analysis (Figure 1, Supplemental Information - Table S1). There were 429 up-regulated genes with 263 unique *Arabidopsis thaliana* (At) homologues, and a total of 1,686 down-regulated genes with 675 unique At numbers. Although further homologies could be identified in some perennial plants such as peach, grape or apple, genes from those plants are not as extensively described as for *Arabidopsis* (http://www.arabidopsis.org/), so only the At numbers were taken forward for further analysis (Supplemental Information - Table S1).

Gene Ontology terms

The *Arabidopsis* homologs were subsequently analyzed for enrichment of GO terms using the Term Enrichment Tool AgriGO. A number of GO terms associated with molecular function, biological process and cellular component were highly enriched in the drought up-regulated and down-regulated genes in blackcurrant (Supplemental Information - Table S2).

Within the cellular component the terms 'cell wall', 'endomembrane system', 'external encapsulating structure' and 'anchored to membrane' were highly enriched among homologs up-regulated under drought stress, whereas highly enriched down-regulated homologs were mostly related to 'cell' and 'intracellular part' terms. Many of the significantly enriched up- or down-regulated cellular components detected in blackcurrant were also up- or down-regulated in drought stressed *Populus* (Cohen et al., 2010).

Regarding molecular function, the most significantly enriched terms for up-regulated genes were: 'catalytic activity', 'transferase activity', 'lyase activity', 'oxidoreductase activity', 'carboxylesterase activity' and 'hydrolase activity'. For down-regulated genes the most highly enriched terms were 'catalytic activity', 'transferase activity' and 'hydrolase activity', together with 'transporter activity', 'kinase activity', 'phosphotransferase activity', 'oxidoreductase activity' and 'ATPase activity'. This analysis showed that some terms were enriched for both up- and down-regulated genes concurrently in drought stressed blackcurrant. The results concerning cellular components and molecular processes are consistent with other stud-

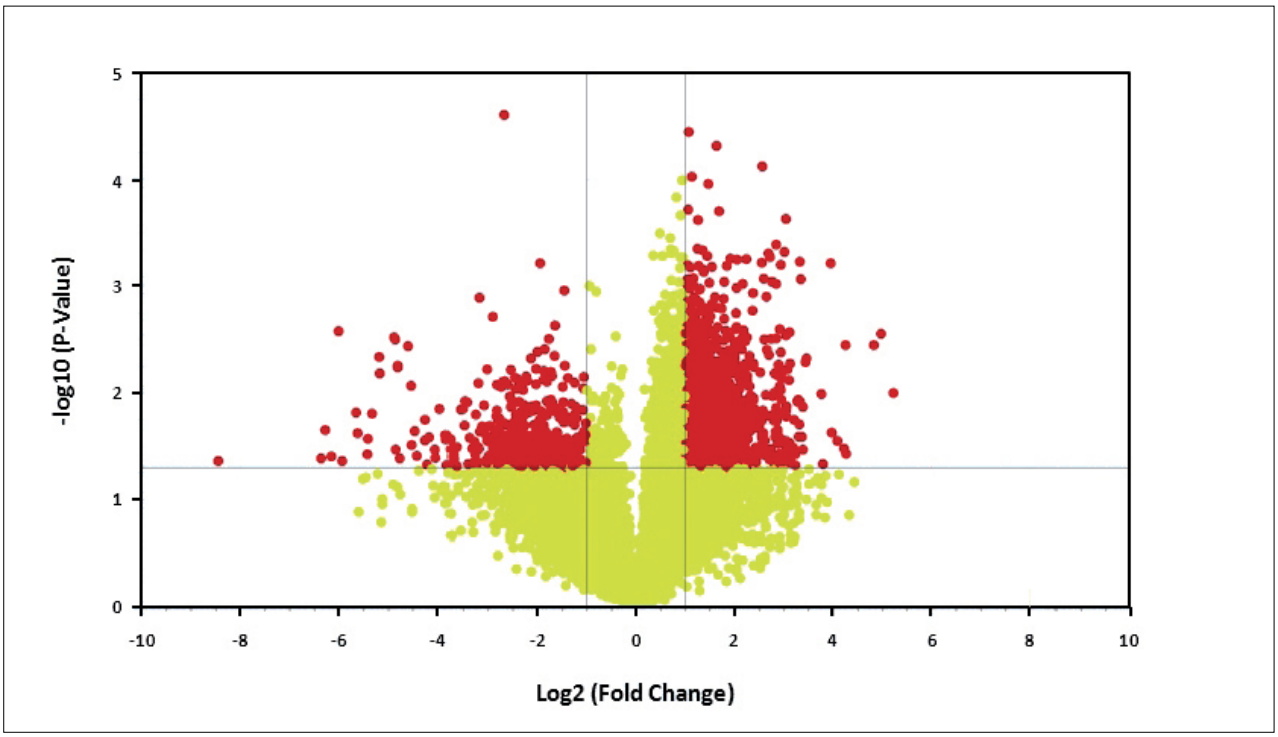

Figure 1. The differential gene expression profiles between fully irrigated (FI) and non-irrigated (NI) leaf samples in blackcurrant leaves of cultivar 'Ben Gairn'. The analysis indicates genes of interest that display large-magnitude fold-changes (log2 of fold change, x-axis) and high statistical significance (-log10 of p-value, y-axis) shown as red dots.

Table 1. Blackcurrant probes (Gene ID) with homology to *Arabidopsis* hormone related genes (At ID) and their At annotation. The ratio between expression in non-irrigated (NI) and fully irrigated (FI) plants is given.

Gene ID	At ID	At annotation	Ratio NI/FI
		Up-regulated probes	
CUST_8573_PI427822113	AT2G29090	cytochrome P450, family 707, subfamily A, polypeptide 2	50.26526
CUST_3948_PI427822113	AT2G45970	cytochrome P450, family 86, subfamily A, polypeptide 8	2.784312
CUST_14545_PI427822113	AT5G04660	cytochrome P450, family 77, subfamily A, polypeptide 4	2.051875
CUST_2781_PI427822113	AT2G42820	HVA22-like protein F	4.409667
CUST_2907_PI427822113	AT1G74670	Gibberellin-regulated family protein	77.65397
CUST_14590_PI427822113	AT5G14920	Gibberellin-regulated family protein	42.83840
CUST_10433_PI427822113	AT1G77330	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	10.35410
CUST_13834_PI427822113	AT1G77110	Auxin efflux carrier family protein	10.63710
		Down-regulated probes	
CUST_3764_PI427822113	AT3G26300	cytochrome P450, family 71, subfamily B, polypeptide 34	0.457096
CUST_12351_PI427822113	AT3G52970	cytochrome P450, family 76, subfamily G, polypeptide 1	0.361130
CUST_8505_PI427822113	AT5G36110	cytochrome P450, family 716, subfamily A, polypeptide 1	0.119911
CUST_16323_PI427822113	AT2G45570	cytochrome P450, family 76, subfamily C, polypeptide 2	0.141938
CUST_17080_PI427822113	AT1G05010	ethylene-forming enzyme	0.469501
CUST_18920_PI427822113	AT3G62550	Adenine nucleotide alpha hydrolases-like superfamily protein	0.400607
CUST_7012_PI427822113	AT2G20880	Integrase-type DNA-binding superfamily protein	0.208874
CUST_15155_PI427822113	AT1G25560	AP2/B3 transcription factor family protein	0.234244
CUST_2903_PI427822113	AT1G78080	related to AP2 4	0.404808
CUST_3616_PI427822113	AT1G17870	ethylene-dependent gravitropism-deficient and yellow-green-like 3	0.334529
CUST_6707_PI427822113	AT2G30840	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	0.147052
CUST_2185_PI427822113	AT1G75580	SAUR-like auxin-responsive protein family	0.496254
CUST_5151_PI427822113	AT2G26170	cytochrome P450, family 711, subfamily A, polypeptide 1	0.489605
CUST_1660_PI427822113	AT4G03400	Auxin-responsive GH3 family protein	0.480483
CUST_13748_PI427822113	AT2G36210	SAUR-like auxin-responsive protein family	0.192268
CUST_15190_PI427822113	AT2G46370	Auxin-responsive GH3 family protein	0.359351

ies on drought stress in different plant species such as *Arabidopsis*, rice, chickpea, and poplar (Huang et al., 2008; Deokar et al., 2011; Chen et al., 2013; Shaik and Ramakrishna, 2013).

GO terms for up-regulated homologs involved in biological processes were related to 'lipid metabolic and biosynthetic process', 'carbohydrate metabolic process', 'cellular ketone metabolic process', as well as different 'acid metabolic processes' (including fatty-, monocarboxylic-, oxoacid-, carboxylic- and organic acid metabolic processes) and 'biosynthetic acid processes' (including organic- and carboxylic acid biosynthetic processes). Plant acclimatization to drought stress modulates membrane activity and levels of oleic and linoleic acid, using lipase for facilitating proper functioning of critical integral proteins (Upchurch, 2008). Up-regulation of homologs associated with 'fatty acid metabolism', 'lipid biosynthetic processes' and 'metabolic processes' indicates altered membrane lipid composition in blackcurrant leaves under drought stress, which could help to maintain membrane integrity and preserve

Table 2. Up-regulated blackcurrant probes (Gene ID) with homology to *Arabidopsis* cell wall and cell cycle genes (At ID) and their At annotation.

Gene ID	At ID	At annotation	Ratio NI/FI
		Up-regulated probes	
CUST_8601_PI427822113	AT1G48100	Pectin lyase-like superfamily protein	27.92830
CUST_15239_PI427822113	AT1G60590	Pectin lyase-like superfamily protein	28.09135
CUST_7808_PI427822113	AT2G22620	Rhamnogalacturonate lyase family protein	12.47054
CUST_18697_PI427822113	AT1G70370	polygalacturonase 2	6.157895
CUST_16576_PI427822113	AT1G23760	BURP domain-containing protein	16.54134
CUST_4173_PI427822113	AT5G04310	Pectin lyase-like superfamily protein	4.493876
CUST_13533_PI427822113	AT1G09890	Rhamnogalacturonate lyase family protein	3.854894
CUST_17622_PI427822113	AT4G23500	Pectin lyase-like superfamily protein	2.319327
CUST_2202_PI427822113	AT4G03270	Cyclin D6;1	8.149630
CUST_11524_PI427822113	AT1G20930	Cyclin-dependent kinase B	6.880954
CUST_15749_PI427822113	AT1G20610	Cyclin B2;3	6.696747

cell compartmentation. This was shown in *Arabidopsis* where leaf membrane resistance to drought stress was due to the capacity to maintain lipid contents and the stability of lipid composition (Gigon et al., 2004). Similar to the present study, genes involved in 'fatty acid metabolism' were up-regulated in *Arabidopsis* and rice under drought stress (Shaik and Ramakrishna, 2013). The enrichment of down-regulated homologs related to biological processes included abiotic stress responses such as 'salt stress', 'osmotic stress' and 'light stimulus'. The down-regulated homologs were also highly enriched for 'abscisic acid (ABA) stimulus', 'hormone stimulus', 'transport and localization', 'phosphorylation', 'protein amino acid phosphorylation' and 'phosphate- and phosphorus metabolic processes'. In other plant species, such as chickpea (Deokar et al., 2011), it was also shown that the same groups of genes responded to drought stress. However in the majority of cases, transcripts were up-regulated in this study. This may indicate the clear biological differences between perennial and annual plant species and the need for specific targeted transcriptome studies.

Hormone related genes

A number of hormone metabolism genes were up- or down-regulated in our study (Table 1), including several genes involved in ABA metabolism. A set of cytochrome P450 genes (at2g29090, at2g45970, at5g04660) was up-regulated during drought, whereas another set (at3g26300, at3g52970, at5g36110, at2g45570) was down-regulated. At2g29090, which belongs to the CYP707A gene family, encodes a hydrolase involved in ABA catabolism. In *Populus simonii* a number of CYP family related genes were induced under drought stress (Chen et al., 2013). Moreover, the transcript of a HVA22F homolog (at2g42820), an ABA synthesis-degradation protein, was up-regulated in blackcurrant leaves under drought. HVA22F was previously identified in *Populus euphratica* exposed to severe drought (Yan et al., 2012). It is well known that plant responses to drought stress include accumulation of ABA in leaves, which induces stomata closure. We have previously shown that 5 days of water withholding induced stomatal closure in blackcurrant cultivar 'Ben Gairn' (Čereković et al., 2013). Two gibberellin synthesis genes (at1g74670 and at5g14920) were up-regulated in blackcurrant under drought stress. The same two genes were up-regulated in *Arabidopsis* under drought stress (Huang et al., 2008). It has previously been reported that gibberellin is involved in different abiotic stress responses (Magome et al., 2004).

One up-regulated homolog (at1g77330) and a number of down-regulated homologs (at1g05010, at3g62550, at2g20880, at1g25560, at1g78080, at1g17870, at2g30840) are involved in ethylene metabolism in *Arabidopsis* and may potentially be involved in reduced leaf growth and accelerated leaf senescence in blackcurrant. It was previously shown that 12 days of drought stress reduced leaf growth in cultivar 'Ben Gairn' (Čereković et al., 2013). This is consistent with other studies which have shown that ethylene is involved in controlling vegetative growth under drought stress (Chaves et al., 2003).

Finally, one up-regulated homolog (at1g77110) and a number of down-regulated homologs (at1g75580, at2g26170, at4g03400, at2g36210, at2g46370) were re-

Table 3. Blackcurrant probes (Gene ID) with homology to *Arabidopsis* transcription factors (At ID) and their At annotation.

Gene ID	At ID	At annotation	Ratio NI/FI
		Up–regulated probes	
CUST_1805_PI427822113	AT1G46264	Heat shock transcription factor (HSFB4)	15.56851
CUST_11719_PI427822113	AT3G58120	Basic-leucine zipper (bZIP) transcription factor family protein	13.30453
CUST_5687_PI427822113	AT3G50060	Myb domain protein 77	3.760343
CUST_7183_PI427822113	AT5G14750	Myb domain protein 66	4.390207
		Down-regulated probes	
CUST_3710_PI427822113	AT5G24800	basic leucine zipper 9	0.465487
CUST_3362_PI427822113	AT4G35900	Basic-leucine zipper (bZIP) transcription factor family protein	0.375457
CUST_4350_PI427822113	AT1G19510	RAD-like 5	0.408883
CUST_16651_PI427822113	AT5G52660	Homeodomain-like superfamily protein	0.478948
CUST_15870_PI427822113	AT1G01060	Homeodomain-like superfamily protein	0.434549
CUST_228_PI427822113	AT3G13540	myb domain protein 5	0.498871
CUST_5769_PI427822113	AT5G04760	Duplicated homeodomain-like superfamily protein	0.450529
CUST_9329_PI427822113	AT4G23810	WRKY family transcription factor	0.235489
CUST_7877_PI427822113	AT2G38470	WRKY DNA-binding protein 33	0.408204
CUST_16830_PI427822113	AT2G23320	WRKY DNA-binding protein 15	0.430263
CUST_3567_PI427822113	AT1G69310	WRKY DNA-binding protein 57	0.495974
CUST_3559_PI427822113	AT4G01720	WRKY family transcription factor	0.399359
CUST_2658_PI427822113	AT5G57660	CONSTANS-like 5	0.463567
CUST_3604_PI427822113	AT5G54470	B-box type zinc finger family protein	0.474767
CUST_1489_PI427822113	AT1G51700	DOF zinc finger protein 1	0.421443
CUST_6589_PI427822113	AT4G27310	B-box type zinc finger family protein	0.326022
CUST_11583_PI427822113	AT2G47890	B-box type zinc finger protein with CCT domain	0.487311
CUST_583_PI427822113	AT4G38960	B-box type zinc finger family protein	0.483137
CUST_7721_PI427822113	AT3G47500	Cycling DOF factor 3	0.363357

lated to auxin metabolism. Transcripts encoding auxin were decreased under drought stress in *Populus simonii*, and the authors speculated that decreased indole-3-acetic acid (IAA) content could be the cause of repressed cell enlargement and plant growth during drought stress in this species (Chen et al., 2013).

Cell wall and cell cycle related genes

All the cell wall homologs identified were up-regulated during drought stress (at1g48100, at1g60590, at2g22620, at1g70370, at1g23760, at5g04310, at1g09890, at4g23500), and this was also the case for three homologs of the cyclin-dependent protein kinases CYCD6;1 (at4g03270), CDKB2;2 (at1g20930) and CYCB2;3 (at1g20610) (Table 2). Contrary, the expression of genes involved in cell wall metabolism were repressed by drought stress in *Arabidopsis* indicating that leaf growth inhibition could be the result of reduced cell number and/or reduced cell expansion (Bray, 2004). Cyclin-dependent protein kinases are activated by ABA-dependent and ABA-independent signaling pathways, regulate stress responsive gene expression and are involved in plant responses to drought stress; for example, in *Arabidopsis* some protein kinases (CYCD) reduce stomata aperture and regulate drought stress responses through an ABA-independent pathway and enhance drought tolerance (Zhou et al., 2013). Hence, the decrease in leaf growth observed in drought stressed 'Ben Gairn' (Čereković et al., 2013) could be partly mediated by cell wall genes, whereas, increase in stomatal closure may be partly mediated by cyclin-dependent protein kinases.

Transcription factors (TF)

Analyses of inducible TFs could increase our understanding and provide more information on the regulatory gene networks involved in drought stress responses in perennial crops (Yan et al., 2012; Chen et al., 2013). A number of TF families were regulated in response to drought stress in blackcurrant (Table 3).

The heat-shock transcription factor family (HSF) homolog HSFB4 (at1g46264) was up-regulated. The expression of HSFs is related to various abiotic stresses including early heat stress (Swindell et al., 2007), and since heat stress is often associated with drought it is likely that the up-regulation of HSFs was an indirect response to heat stress and poor leaf cooling. HSFs regulate the expression of Heat Shock Proteins (HSPs) which act as chaperones to ensure the correct folding of proteins (Timperio et al., 2008). In *Populus euphratica*, *Populus simonii* (Yan et al., 2012; Chen et al., 2013), *Malus domestica* (Wisniewski et al., 2008) and *Citrus reticulata* (Gimeno et al., 2009), HSPs were also up-regulated under drought stress.

In blackcurrant, one homolog of bZIP transcription factors was up-regulated (at3g58120), while others were down-regulated (at5g24800, at4g35900). The up-regulated at3g58120 homolog has also been demonstrated to be modulated under drought stress in *Arabidopsis* (Huang et al., 2008). Members of the bZIP TF family are known to bind to (ABA)-responsive element (ABRE) sequences which in turn activate ABA-dependent gene expression (Chen et al., 2013). The down-regulated homolog at4g35900 encodes a bZIP TF which is involved in drought, salinity and cold responses in *Arabidopsis* (Jacoby et al., 2002). Two (at3g50060 and at5g14750) and five (at1g19510, at5g52660, at1g01060, at3g13540, at5g04760) homologs belonging to the MYB domain TF family were up- and down-regulated, respectively. Many MYB TFs are ABA inducible and have been shown to be involved in drought stress responses in *Arabidopsis* via an ABA-mediated signaling pathway (Abe et al., 2003).

Five homologs belonging to the WRKY family of TFs (at4g23810, at2g38470, at2g23320, at1g69310, at4g01720) were down-regulated. The WRKY TF family is one of the largest TF families identified in various tissues (root, leaf, seed, inflorescence, abscission zone, and vascular tissue) of drought-stressed, salt-stressed or pathogen-infected plants (Eulgem et al., 2000). The at2g38470 homolog identified in blackcurrant, was also reported to be down-regulated in drought stressed *Arabidopsis* (Huang et al., 2008).

The TF homologs also included seven members of the zinc finger family (at5g57660, at5g54470, at1g51700, at4g27310, at2g47890, at4g38960, at3g47500) which were all down-regulated. In *Arabidopsis*, the zinc finger TF family was identified as an RNA-binding protein participating in flower development and abiotic stresses (Li et al., 2001). This indicates that these families of TFs may play an important role in the response of blackcurrant to drought stress.

Conclusion

Transcriptional analysis of leaves harvested from the drought stressed cultivar 'Ben Gairn' indicated an important role for the plant hormones ABA, ethylene and auxin in mediating downstream gene expression and metabolic responses in blackcurrant. Cell wall and cell cycle genes also appeared to be involved in drought stress responses of 'Ben Gairn'. In addition, results suggested that members of the bZIP, HSF, WRKY and the zinc finger TF families play an important role in such gene regulation. However, further investigations of blackcurrant drought responsive genes are necessary across a range of diverse germplasm, in order to confirm the role of key genes and increase our overall understanding of the drought response at different developmental stages.

This study is the first indication of the putative genes and processes involved in drought responses in blackcurrant, and confirms that drought stress induces changes in gene expression in leaves of the blackcurrant cultivar 'Ben Gairn'. Some putative candidate genes involved in drought stress tolerance of blackcurrant are identified, and require further more detailed studies to confirm their role. From this, mapping of key genes onto the *Ribes* linkage map developed by Russell et al. (2014) can eventually be taken forward to the development of molecular markers for targeted breeding approaches for the production of new blackcurrant cultivars with resilience to drought. Such resilient plant material is likely to be of increasing importance for sustainable crop production in future climate scenarios.

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