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Grapevine cell early activation of specific responses to DIMEB, a resveratrol elicitor

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Abstract

Background: In response to pathogen attack, grapevine synthesizes phytoalexins belonging to the family of stilbenes. Grapevine cell cultures represent a good model system for studying the basic mechanisms of plant response to biotic and abiotic elicitors. Among these, modified β cyclodextrins seem to act as true elicitors inducing strong production of the stilbene resveratrol.

Results: The transcriptome changes of Vitis riparia × Vitis berlandieri grapevine cells in response to the modified β -cyclodextrin, DIMEB, were analyzed 2 and 6 h after treatment using a suppression subtractive hybridization experiment and a microarray analysis respectively. At both time points, we identified a specific set of induced genes belonging to the general phenylpropanoid metabolism, including stilbenes and hydroxycinnamates, and to defence proteins such as PR proteins and chitinases. At 6 h we also observed a down-regulation of the genes involved in cell division and cellwall loosening.

Conclusions: We report the first large-scale study of the molecular effects of DIMEB, a resveratrol inducer, on grapevine cell cultures. This molecule seems to mimic a defence elicitor which enhances the physical barriers of the cell, stops cell division and induces phytoalexin synthesis.

Background

Plants respond to pathogens through constitutive and inducible mechanisms [1]. Structural barriers represent preformed constitutive defences, while the accumulation of pathogenesis-related proteins (PR), phytoalexins and reactive oxygen species is part of an active mechanism stimulated by the pathogen [2]. Grapevine also responds to fungal infection via PR-protein synthesis and phyto-

alexin accumulation [3]. Plant phytoalexins are lowmolecular-weight secondary metabolites with antimicrobial properties and they show wide chemical diversity among different plant species [4]. In grapevine they mainly belong to the stilbene family and consist of transresveratrol (3,5,4'-trihydroxystilbene) its oligomers, called viniferins [5-7] and pterostilbene, a dimethylated derivative of resveratrol [8]. Stilbene synthesis in berries [9] and leaves can be elicited by fungal infection [5,10], but also by treatment with UV-irradiation [11], ozone [12] and heavy metals [13].

Plant cell cultures are a useful tool for studying plant cell defence response to biotic and abiotic elicitors [14]. Stilbene accumulation has been reported in grapevine cells treated with different elicitors: fungal cell wall fragments [15], Na-orthovanadate, jasmonic acid and methyljasmonate [16,17] and laminarin, a β -glucan polysaccharide from brown algae [18]. In addition, special attention has been given to the β -cyclodextrin molecular class. These are cyclic oligosaccharides consisting of seven α -D-glucopyranose residues linked by $\alpha \ 1 \rightarrow 4$ glucosidic bonds forming a structure with a hydrophobic central cavity and a hydrophilic external surface [19]. Among β -cyclodextrins, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB), was reported to be the most effective resveratrol elicitor in different Vitis vinifera cultivars [19,20]. The ability of the modified β -cyclodextrins to act as elicitors probably resides in their chemical similarity to the alkyl-derivatized pectic oligosaccharides released from the cell walls during fungal infection [20]. Along with stilbene accumulation these experiments highlighted a more general response involving peroxidase activity as well as inhibition of Botrytis cinerea growth [19,20].

Zamboni et al. [21] further investigated DIMEB activity on additional *Vitis* genotypes and observed that its effect was more pronounced when tested on *Vitis riparia* × *Vitis berlandieri* cell cultures. The kinetics of resveratrol synthesis showed that trans-resveratrol, the induced form, started to accumulate from 6 h after treatment and reached its maximum at 24 h. Moreover, this metabolite was much more localized in the medium than within the cell.

With these results [21] as our starting point, we report here the first large-scale transcriptional characterization of the early response of *Vitis riparia* × *Vitis berlandieri* cells to DIMEB treatment.

After 2 h, 127 positively modulated genes were identified by suppression subtractive hybridization (SSH), whereas after 6 h, 371 genes turned out to be differentially expressed when control and treated cells on the *Vitis vinifera* GeneChip[®] Genome Array (Affymetrix) were compared. These results showed that DIMEB specifically modulates the expression of a small number of genes involved in resveratrol and lignin biosynthesis, PR synthesis, cell division and cell wall modification.

Results and discussion

The ability of DIMEB to elicit defence responses in grapevine cell culture was suggested by previous results showing stilbene accumulation, changes in peroxidase activity, as well as inhibition of *Botrytis cinerea* growth [19,20]. Considerable stilbene accumulation in response to DIMEB treatment was also observed by our group using non-*vinifera* (*Vitis riparia* × *Vitis berlandieri*) liquid cell cultures [21]. In this study we analyzed the changes in gene expression of these cells elicited with DIMEB after 2 h and 6 h using SSH and microarray experiments, respectively.

The rationale behind the two approaches was that after 2 h of treatment, a small number of genes are expected to be modulated, and only to a limited extent, whereas after 6 h an increase in the number of genes and in their expression level is envisaged. The SSH technique appeared then the right choice for identifying the low abundance differential transcripts at 2 h, while the Affymetrix GeneChip[®] microarray was used to measure the expression of a larger number of genes (~14,500 unigenes) after 6 h of treatment [22].

Starting with 384 clones from the constructed cDNA subtractive library and then performing a hybridization screening to eliminate clones which were not really differentially expressed (false positives), we obtained 168 highquality sequences which clustered in 127 tentative consensuses (Additional File 1). The microarray experiments instead identified 371 (223 upregulated and 148 downregulated) significantly modulated probe sets in the treated cells compared with the control ones (Additional File 2). Sequence annotation and classification according to Gene Ontology categories [23], revealed that at both time points primary (mainly signal transduction related genes) and secondary metabolisms, together with response to the stimulus, were the most affected categories (Additional Files 3 and 4). At 6 h, the analysis also highlighted downregulation of the cellular component organization and the biogenesis category (Additional file 4).

In general, the two experiments showed modulation of specific mechanisms had already occurred at 2 h and continued more extensively at 6 h after DIMEB treatment. The data summarized in Table 1 suggest that the grapevine cell responds to the elicitor by the activation of a signal transduction cascade which leads to the induction of specific classes of transcription factors. The downstream effect of this process is, on the one hand, the induction of some branches of the secondary metabolism and defence response, and, on the other hand, the blockage of cell duplication (Figure 1).

At 2 h the treatment caused positive transcriptional regulation of a grapevine gene (CLU090) encoding a protein with homology to an Arabidopsis kinase-associated protein phosphatase (KAPP) (Table 1). KAPP protein may function as a signalling component in the pathway involving the serine-threonine receptor-like kinase, RLK5

Table I: List of transcripts modulated by DIMEB and reported in the Discussion

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1621405_at Plastidic 3-decxyD-arabino-heprulosonate 7-phosphate synthase 2 Q2407 TC51974 × 1609646_at 3-Dehydroquinate synthase-like protein Q9FKX0 TC56864 × 1603046_at Prephenate dehydratase Q6[]29 TC53641 × 161315_at Prephenate dehydratase Q6[]29 TC53641 × 1613165_at Phorylatine ammonia lyase Q6[D55 TC60180 CLU024 Trans-cinnamate 4-monoxygenase Q48240 TC71512 × 1610821_at Coumarate CoA ligase Q94858 TC70715 × 1616191_s_at Cinnamic acid 4-hydroxylase Q94858 TC70715 × 161930_at 4-Coumarate-CoA ligase 2 P31687 TC66943 × 161930_at 4-Coumarate-CoA ligase 2 Q86AU9 TC689632 × CLU002 Stilbene synthase Q85982 TC67071 × CLU023 Stilbene synthase Q81974 × 1608009_s_at Stilbene synthase Q81974 × 1608020 Stilbene synthase Q948973 TC687020 ×	1619357_at	3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase	O24046	TC57642		х		
160946_at 3-Dehydroquinate synthase-like protein QPIX0 TC58654 × 1609932_at Prophenate dehydratase QI[29 TC53641 × 1611895_at Putative chorismate mutase QI[19 TC53641 × 1611813_at Phonylapanoid metabolism TC62307 × 1613113_at Phonylapanoid metabolism CG1026 TC60180 1613113_at Cinnamic acid 4-hydroxylase Q44588 TC70715 × 1616191_s_at Cinnamic acid 4-hydroxylase Q55017 TC66743 × 1615801_at 4-Coumarate-CoA ligase 2 Q15807 TC687701 × 1615801_at 4-Coumarate-CoA ligase 2 Q55017 TC66743 × CLU022 Silbene synthase Q68AU9 TC68701 × CLU023 Silbene synthase Q89874 × CLU049 × CLU049 Silbene synthase Q89843 × × CLU049 × × 160809.4_s.at Silbene synthase Q91447 × × CLU049 × × 16080965_s.at Silbene synthase <td>1621405_at</td> <td>Plastidic 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 2</td> <td>O22407</td> <td>TC51974</td> <td></td> <td>х</td>	1621405_at	Plastidic 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 2	O22407	TC51974		х		
1609932_at Prephenate dehydratase Q6 29 TC53641 × 1611895_at Prephenate dehydratase Q6 19 TC53641 × 1611895_at Prephenate dehydratase Q6UD65 TC60180 CLU024 Trans-cinnamate 4-monoxygenase Q43240 TC71512 × 1611813_at Cinnamic acid 4-hydroxylase Q94858 TC70715 × 161801_at 4-Coumarate:-CoA ligase Q55017 TC60943 × 161801_at 4-Coumarate:-CoA ligase 2 P31687 TC67015 × Stilbene synthase Q95W2 TC69731 × CLU002 Stilbene synthase Q95W2 TC89701 × CLU022 Stilbene synthase Q818P4 TC78210 × 1608005_at Stilbene synthase Q95982 TC64974 × CLU023 Stilbene synthase Q95982 TC64974 × 1608069_at Stilbene synthase Q95982 TC67020 × 1609696_x_at Stilbene synthase Q944W7 <td< td=""><td>1609646_at</td><td>3-Dehydroquinate synthase-like protein</td><td>Q9FKX0</td><td>TC56854</td><td></td><td>x</td></td<>	1609646_at	3-Dehydroquinate synthase-like protein	Q9FKX0	TC56854		x		
1621307_at Prephenate delydratase Q6 29 TC53641 x 1611855_at Putative chorismate mutase Q5 N19 TC5207 x 1613113_at Phenylalanine ammonia lyase Q6UD65 TC60180 CLU024 Trans-cinnamate 4-monooxygenase Q43240 TC71512 x 161891_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 161891_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 161891_at 4-Coumarate-CoA ligase 2 Q91687 TC66743 x 161930_at 4-Coumarate-CoA ligase 2 Q65017 TC60943 x 161930_at 4-Coumarate-CoA ligase 2 Q68409 TC87632 x CLU002 Stilbene synthase Q68409 TC87632 x CLU023 Stilbene synthase Q81PP4 TC78210 x CLU043 Stilbene synthase Q68A12 TC67020 x 1606750_at Stilbene synthase Q94458 TC67020 x 160809_a_st Stilbene synthase Q94474 x X 1609696_x_st	1609932_at	Prephenate dehydratase	Q6JJ29	TC53641		х		
1611895_at Putative chorismate mutase Q5 N19 TC62307 x 1613113_at Phenylalanine ammonia lyase Q6UD65 TC60180 CLU024 Trans-cinnamate 4-monooxygenase Q43240 TC71512 x 1610821_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 1610821_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 161930_at 4-Coumarate:-CoA ligase 2 P31687 TC66743 x Stilbene biosynthesis CLU002 Stilbene synthase Q959W2 TC89701 x CLU033 Stilbene synthase Q81494 TC89632 x CLU047 Stilbene synthase Q81494 x CLU044 x 1606750_at Stilbene synthase Q95982 TC84974 x CLU047 Stilbene synthase Q648L2 TC67020 x 1606750_at Stilbene synthase Q9343 TC88944 x 1606695_at Stilbene synthase Q9343 TC67020 x 1609669_x_at Stilbene synthase Q93444W7 TC60746 x </td <td>1621307_at</td> <td>Prephenate dehydratase</td> <td>Q6JJ29</td> <td>TC53641</td> <td></td> <td>x</td>	1621307_at	Prephenate dehydratase	Q6JJ29	TC53641		x		
General phenylproponid metabolism [613113,at Phenylakinie ammonia lyase Q6UD65 TC60180 CLU024 Trans-cinnamate 4-monooxygenase Q43240 TC71512 x 1618113,at Chinnamic acid 4-hydroxylase Q44858 TC70715 x 1618113,at Chinnamic acid 4-hydroxylase Q44858 TC70715 x 1618131,at 4-Coumarate-CoA ligase 2 Q55017 TC66743 x 161820_at 4-Coumarate-CoA ligase 2 Q55017 TC66743 x 2018000 Stilbene synthase Q68AU9 TC89701 x CLU0021 Stilbene synthase Q68AU9 TC89701 x CLU022 Stilbene synthase Q81294 TC77210 x CLU031 Stilbene synthase Q95992 TC84974 x CLU049 Stilbene synthase Q25982 TC84974 x CLU049 Stilbene synthase Q28433 TC67020 x 1608096_x_at Stilbene synthase Q344V7 TC67020 x	1611895_at	Putative chorismate mutase	Q5JN19	TC62307		х		
161311_at Phenylalanine ammonia lyase Q4UD55 TC60180 CLU024 Trans-cinnamate 4-monocxygenase Q43240 TC71512 x 1610821_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 1611801_at 4-Coumarate-CoA ligase 2 Q91858 TC70715 x 1611801_at 4-Coumarate-CoA ligase 2 P31687 TC60743 x Stilbene biosynthesis CLU002 Stilbene synthase Q95PW2 TC89701 x CLU021 Stilbene synthase Q46AU9 TC89632 x CLU022 Stilbene synthase Q8LPP4 TC78210 x CLU035 Stilbene synthase Q8LP4 TC78210 x CLU049 Stilbene synthase Q8LP4 TC78210 x CLU037 Stilbene synthase Q48A12 TC67020 x 1606750_at Stilbene synthase P28343 TC67020 x 1609695_x_at Stilbene synthase P28434 TC67020 x 1609695_x_at Stilbene synthase P28433 TC67020 x 1609695_x_at </td <td>General phenylpro</td> <td>opanoid metabolism</td> <td></td> <td></td> <td></td> <td></td>	General phenylpro	opanoid metabolism						
CLU024 Trans-cinnamate 4-monooxygenase Q43240 TC7/15/2 x 1610821_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 161681_s_at Cinnamic acid 4-hydroxylase Q94858 TC70715 x 161680_at 4-Coumrate:-CoA ligase Q55017 TC66043 x 1619320_at 4-Coumrate:-CoA ligase Q35W12 TC89701 x CLU009 Stilbene synthase Q95PW2 TC89701 x CLU023 Stilbene synthase Q86BAU9 TC89742 x CLU03 Stilbene synthase Q81PP4 TC78210 x CLU049 Stilbene synthase Q68AL2 TC67020 x 1606750_at Stilbene synthase Q944W7 TC67020 x 1608009_s_at Stilbene synthase Q94583 TC67020 x 160850_at Stilbene synthase Q94W7 TC67020 x 160850_at Stilbene synthase Q94W7 TC67020 x 1610824_s_at Stilbene synthase <td>1613113_at</td> <td>Phenylalanine ammonia lyase</td> <td>Q6UD65</td> <td>TC60180</td> <td></td> <td></td>	1613113_at	Phenylalanine ammonia lyase	Q6UD65	TC60180				
1610821_at Cinnamic acid 4-hydroxylase Q94858 TC70715 × 1616191_s_at Cinnamic acid 4-hydroxylase Q94858 TC70715 × 1615801_at 4-Coumarate-CoA ligase 2 P31697 TC66743 × Stibene biosynthesis Q95PW2 TC89701 × CLU002 Stibene synthase Q68AU9 TC89632 × CLU023 Stibene synthase Q84874 × CLU047 × CLU049 Stibene synthase Q84840 TC76210 × CLU053 Stibene synthase Q84874 × CLU047 × CLU075 Stibene synthase Q84842 TC67020 × × CLU03 Stibene synthase Q68AL2 TC67020 × × 1606750_at Stibene synthase Q937X5 TC52746 × 1609697_at Stibene synthase Q94333 TC67020 × 1609697_at Stibene synthase Q94333 TC67020 × 1609697_at Stibene synthase Q944583 TC67020 × <td>CLU024</td> <td>Trans-cinnamate 4-monooxygenase</td> <td>Q43240</td> <td>TC71512</td> <td>х</td> <td></td>	CLU024	Trans-cinnamate 4-monooxygenase	Q43240	TC71512	х			
1616191_s_at Cinnamic acid 4-hydroxylase Q94858 TC70715 × 1615801_at 4-Coumarate:CoA ligase 2 Q55017 TC60943 × 1619320_at 4-Coumarate:CoA ligase 2 P31687 TC66743 × Stilbene bisynthesis CLU009 Stilbene synthase Q6BAU9 TC89701 × CLU021 Stilbene synthase Q8LPP4 TC7210 × CLU023 Stilbene synthase Q8LP4 TC7210 × CLU049 Stilbene synthase Q8LP4 TC7210 × CLU047 Stilbene synthase Q8LP4 TC7210 × CLU049 Stilbene synthase Q8LP4 TC72010 × CLU047 Stilbene synthase Q8B433 TC67020 × 1606750_at Stilbene synthase Q93447 TC60946 × 1609667_x_at Stilbene synthase Q94477 TC60946 × 1609667_at Stilbene synthase Q94458 TC67020 × 1609667_at Stilbene synthase Q9458 TC67020 × 1609667_at St	1610821_at	Cinnamic acid 4-hydroxylase	Q948S8	TC70715		х		
161380 _at 4-CoumarateCoA ligase 2 P31687 TC66743 x Stilbene biosynthesis QSPW2 TC89701 x CLU009 Stilbene synthase Q6BAU9 TC89632 x CLU021 Stilbene synthase Q6BAU9 TC89632 x CLU022 Stilbene synthase Q8BAU9 TC89632 x CLU049 Stilbene synthase Q8LPP4 TC78210 x CLU049 Stilbene synthase Q9S982 TC84974 x 16068009_s_at Stilbene synthase Q9S982 TC84974 x 16068009_s_at Stilbene synthase Q9S982 TC84974 x 16068009_s_at Stilbene synthase Q9S982 TC87020 x 1609696_s_at Stilbene synthase Q93YX5 TC5726 x 1610824_s_at Stilbene synthase Q94G58 TC67020 </td <td>1616191_s_at</td> <td>Cinnamic acid 4-hydroxylase</td> <td>Q948S8</td> <td>TC70715</td> <td></td> <td>х</td>	1616191_s_at	Cinnamic acid 4-hydroxylase	Q948S8	TC70715		х		
16193/D_at 4-Coumarate-CoA ligase 2 P3168/ 1C667/43 x Stilbene biosynthesis Q95PW2 TC89701 x CLU021 Stilbene synthase Q68AU9 TC89632 x CLU023 Stilbene synthase Q281P4 TC78210 x CLU049 Stilbene synthase Q81P4 TC78210 x CLU097 Stilbene synthase Q95982 TC84974 x I606750_at Stilbene synthase Q95982 TC84974 x I609697_at Stilbene synthase Q96BAL2 TC67020 x I60850_at Stilbene synthase Q944W7 TC60946 x I610850_at Stilbene synthase Q9458 TC67020 x I610850_at Stilbene synthase Q944W7 TCS2746 x I612804_at Stilbene synthase Q959W2 TCS2746	1615801_at	4-Coumarate:CoA ligase	Q5S017	1C60943		x		
Sublene biosynthesisCLU009Silbene synthaseQ95PW2TC89701xCLU021Stilbene synthaseQ86AU9TC89632xCLU023Stilbene synthaseQ8LPP4TC78210xCLU049Stilbene synthaseQ8LPP4TC78210xCLU03Stilbene synthaseQ8LPP4TC78210xCLU03Stilbene synthaseQ95982TC84974xCLU03Stilbene synthaseQ68AL2TC67020x1606750_atStilbene synthaseQ68AL2TC67020x1609696_x_atStilbene synthaseQ94W7TC60946x1609696_x_atStilbene synthaseQ944W7TC60946x1609696_x_atStilbene synthaseQ9433TC67020x1609696_x_atStilbene synthaseQ9433TC67020x1610820_atStilbene synthaseQ94588TC67020x161190_s_atResveratrol synthaseQ94583TC67020x1616264_atStilbene synthaseQ95PW2TC52746x16162657_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ95PW2TC52746x1613763_atABC transporterQ52958TC81892x161363_at <td>1619320_at</td> <td>4-CoumarateCoA ligase 2</td> <td>P31687</td> <td>1C66/43</td> <td></td> <td>x</td>	1619320_at	4-CoumarateCoA ligase 2	P31687	1C66/43		x		
CLU009Stilbene synthaseQ95/W2IC89/01xCLU021Stilbene synthaseQ6BAU9TC89632xCLU023Stilbene synthaseP28343TC84974xCLU049Stilbene synthaseQ95982TC84974xCLU03Stilbene synthaseQ95982TC84974xCLU03Stilbene synthaseQ95982TC68634xI606750_atStilbene synthaseQ6BAL2TC67020xI60809_5_atStilbene synthaseP28343TC67020xI60969_7_atStilbene synthaseQ944W7TC60946xI60867_atStilbene synthaseQ944W7TC67020xI608630_atStilbene synthaseQ944W7TC50746xI611802_asResveratrol synthaseQ94658TC67020xI611804_atStilbene synthaseQ944W7TC52746xI612804_atStilbene synthaseQ944W7TC52746xI614621_atStilbene synthaseQ944W7TC52746xI616255_atStilbene synthaseQ95PW2TC52746xI612804_s_atStilbene synthaseQ95PW2TC52746xI614621_atStilbene synthaseQ95PW2TC52746xI614624_atStilbene synthaseQ95PW2TC52746xI6146455_atStilbene synthaseQ95PW2TC52746xI614641_atStilbene synthaseQ95PW2TC52746xI614641_atStilbene synthaseQ95PW2 <td>Stilbene biosynthe</td> <td>S/S</td> <td>0000004/0</td> <td>TC00701</td> <td></td> <td></td>	Stilbene biosynthe	S/S	0000004/0	TC00701				
CLU022Stilbene synthaseQBBAU9TCB9622xCLU033Stilbene synthaseQBLP44TC78210xCLU049Stilbene synthaseQ95982TC84974xCLU03Stilbene synthaseQ95982TC84974xCLU049Stilbene synthaseQ95982TC84974x1608009_s_atStilbene synthaseQ95107x1609696_x_atStilbene synthaseP28343TC67020x1609696_x_atStilbene synthaseP28343TC67020x1609697_atStilbene synthaseQ944W7TC60946x1610820_atStilbene synthaseQ93YX5TC52746x1610850_atStilbene synthaseQ94G58TC67020x1610850_atStilbene synthaseQ94G58TC67020x1612804_atStilbene synthaseQ94G58TC67020x1614621_atStilbene synthaseQ944W7TC52746x1612804_atStilbene synthaseQ944W7TC52746x1612804_atStilbene synthaseQ944W7TC52746x1612632_x_atStilbene synthaseQ95PV2TC52746x1612634_x_atStilbene synthaseQ95PV2TC52746x1612634_x_atStilbene synthaseQ95PV2TC52746x1612634_x_atStilbene synthaseQ95PV2TC52746x1612634_x_atStilbene synthaseQ95PV2TC52746x161275_atStilbene synthaseQ95PV2<	CLU009	Stilbene synthase	Q9SPVV2	1C89/01	х			
CLU023 Stilbene synthase P28343 TC8497/4 x CLU049 Stilbene synthase Q8LPP4 TC78210 x CLU07 Stilbene synthase Q9S982 TC84974 x CLU03 Stilbene synthase Q9S982 TC84974 x CLU03 Stilbene synthase P28343 TC67020 x 1609605_at Stilbene synthase P51070 x 1609606_x_at Stilbene synthase P28343 TC67020 x 1609606_x_at Stilbene synthase P28343 TC67020 x 1609607_at Stilbene synthase Q944W7 TC60946 x 1610850_at Stilbene synthase Q941W7 TC60946 x 1610850_at Stilbene synthase Q94558 TC67020 x 1610850_at Stilbene synthase Q94G58 TC67020 x 1612804_at Stilbene synthase Q944W7 TC52746 x 1612804_s_at Stilbene synthase Q95PW2 TC52746 x 1612064_s_at Stilbene synthase Q95PW2 TC52746 <td>CLU022</td> <td>Stilbene synthase</td> <td>Q6BAU9</td> <td>1C89632</td> <td>x</td> <td></td>	CLU022	Stilbene synthase	Q6BAU9	1C89632	x			
CLU047 Stilbene synthase Q8LP44 1C78210 X CLU097 Stilbene synthase Q9582 TC84974 X CLU013 Stilbene synthase Q6BAL2 TC67020 X 1608009_s_at Stilbene synthase Q6BAL2 TC67020 X 160966_x_at Stilbene synthase P28343 TC60946 X 160969_at Stilbene synthase Q944W/7 TC60946 X 1609697_at Stilbene synthase Q93YX5 TC52746 X 1610850_at Stilbene synthase Q944W/7 TC67020 X 1610850_at Stilbene synthase Q944W/7 TC52746 X 161190_s_at Resveratrol synthase Q95W2 TC52746 X 1614621_at Stilbene synthase Q95W2 TC52746 X 162064_s_at Stilbene synthase Q98FW2 TC52746 X 162054_s_at Stilbene synthase Q98FW2 TC52746 X 162064_s_at Stilbene synthase Q98FW2	CLU023	Stilbene synthase	P28343	TC84974	х			
CLU077Silbene synthaseQ35721C84974XCLU103Silbene synthaseP28343TC68894X1606750_atSilbene synthaseQ6BAL2TC67020X1609696_x_atSilbene synthaseP28343TC67020X1609697_atSilbene synthaseQ944W7TC60946X1609697_atSilbene synthaseQ937X5TC52746X1610824_s_atSilbene synthaseQ937X5TC52746X1610825_atSilbene synthaseQ94658TC67020X161880_atSilbene synthaseQ94658TC67020X161820_atSilbene synthaseQ95PW2TC52746X161820_atSilbene synthaseQ95PW2TC52746X1616575_atSilbene synthaseQ9284W7TC67020X1620964_s_atSilbene synthaseQ9284W7TC52746X1620964_s_atSilbene synthaseQ928343XX1620964_s_atSilbene synthaseQ9289W2TC52746X1620964_s_atSilbene synthaseQ9289W2TC52746X1612638_x_atSilbene synthaseQ9289W2TC52746X1612638_x_atSilbene synthaseQ9289W2TC52746X161363_atABC transporterQ8GU88TC6318X161363_atABC transporter-like proteinQ91YS2TC64710X1613863_atABC transporter-like proteinQ91YS2TC64210X1613863_atGIMDR1<	CLU049	Stilbene synthase	Q8LPP4	TC/8210	×			
LCU103Stilbene synthaseP2343TC68054X1606750_atStilbene synthaseQ6BAL2TC67020x1609696_x_atStilbene synthaseP28143TC67020x1609696_x_atStilbene synthaseQ944W7TC60946x1609697_atStilbene synthaseQ93YX5TC52746x1610824_s_atStilbene synthaseQ9458TC67020x1610850_atStilbene synthaseQ9458TC67020x161190_s_atResveratrol synthaseQ9458TC67020x1611804_atStilbene synthaseQ9458TC67020x1618642_atStilbene synthaseQ9458TC67020x16186375_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ944W7TC52746x1622638_x_atStilbene synthaseQ989PW2TC52746x1622638_x_atStilbene synthaseQ989PW2TC52746x1622638_x_atStilbene synthaseQ989PW2TC52746x1622638_x_atStilbene synthaseQ99SPW2TC52746x1622638_x_atStilbene synthaseQ91YS2TC60768x161376_atABC transporterQ91YS2TC60768x161363_atCjMDR1Q91YS2TC64210x161363_atCjMDR1Q94H6TC69843x160930_atGlutathione S-transferaseQ91Y50TC52364x1611890_atGlutathione S-transf	CLUU97	Stilbene synthase	Q73782	TC84974	×			
160735_atStilbene synthaseP51070x1608099_s_atStilbene synthaseP28343TC67020x1609697_atStilbene synthaseQ944W7TC60946x1610824_s_atStilbene synthaseQ93YX5TC52746x1610824_s_atStilbene synthaseQ93YX5TC52746x1610824_s_atStilbene synthaseQ94G58TC67020x16118050_atStilbene synthaseQ94G58TC67020x1612804_atStilbene synthaseQ95PW2TC52746x1614621_atStilbene synthaseQ944W7TC52746x1616575_atStilbene synthaseQ944W7TC52746x1622638_x_atStilbene synthaseQ95PW2TC52746x1622638_x_atStilbene synthaseQ92SPW2TC52746x1613763_atABC transporterQ8GU88TC76318xCLU106PDR-like ABC transporter-like proteinQ92SPS2TC60768x161363_atCijMDR1Q94H6TC69843x1610363_atCijMDR1Q94H6TC69843x161930_atGlutathione S-transferaseQ9450TC52364x161980_x_atGlutathione S-transferaseQ9450TC52364x1619682_x_atCaffeic acid O-methyltransferase IQ00763TC64352x		Stilbene synthase	P28343	TC68894	x			
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	1620342_at	Caffeic acid 3-O-methyltransferase I	Q00763	TC64352		x		

Table 1: List of transcripts modulated by DIMEB and reported in the Discussion (Continued)

Lignin biosynthesis						
1611897_s_at	Caffeoyl-CoA O-methyltransferase	Q8H9B6	TC63685		х	
1614643_at	Caffeoyl-CoA O-methyltransferase	Q43237	TC51729		х	
1613900_at	Cinnamyl alcohol dehydrogenase	Q9ATWI	TC52904		х	
1614045_at	Ferulate 5-hydroxylase	Q6IV45	TC64493		x	
1614502_at	Ferulate 5-hydroxylase	Q6IV45	TC63764		x	
1619065_at	Putative cinnamoyl-CoA reductase	Q8W3H0	TC53437		х	
1622651_at	Polyphenol oxidase	Q68NI4	TC58764		х	
1610806_at	Putative diphenol oxidase	Q6Z8L2	CD007812		х	
CLUI22	Chalcone-flavonone isomerase	P51117	TC78712	x		
CLU048	Flavonol 3-O-glucosyltransferase 6	Q40288	TC85607	х		
1621051_at	Flavonol 3-O-glucosyltransferase 2	Q40285	CN006197			х
Defence response						
CLU088	Chitinase (Class II)	Q43322	TC95665	x		
1613871_at	Class IV chitinase	Q9M2U5	TC57889		x	
1617192 at	Class IV chitinase	Q7XB39	TC63731		x	
1617430 s at	Basic endochitinase precursor	P51613	TC51704		x	
CLU001	Pathogenesis-related protein 10	O9FS42	TC72098	x		
1610011 s at	Pathogenesis-related protein 10	O9FS42			x	
1618568 s at	Pathogenesis-related protein I0	09FS42			x	
	Pathogenesis-related protein PR-4A precursor	P29062	TC91296	x	~	
CLU036	Merlot proline-rich protein ?	060671	TC85591	v		
1609875 at	Protosso inhibitor	OKYEYK	1000071	^	v	
1607075_at	Protesse inhibitor	QUILIO OKYEYK	TC70006		Ŷ	
1611660_s_at	Proteste minipitor		TC70000		×	
1612552_at	Putative S-adenosyl-L-methonine.salicylic acid carboxyl methyltransierase		TC37170		x	
1620309_at	Putative S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	Q9C9VV8	TC(03451		x	
1622147_at	I-Aminocyclopropane-I-carboxylate oxidase 3	Q08507	1C60326		x	
1616358_at	MLO-like protein 11	Q9FI00	BQ/98612			х
Cell wall metabolis	m	004170	T C/20/5			
16080/4_s_at	Expansin	Q84U10	1C62965			х
1620840_at	Alpha-expansin	Q8LKJ8	TC53122			х
1615995_at	Xyloglucan endotransglycosylase XET2	Q9LLC2	CF212592			х
1620003_at	Xyloglucan endotransglycosylase I	Q9ZRVI	TC63269			х
1608799_at	Pectin methylesterase	Q96497	TC58800			х
1619468_at	Pectin methylesterase PMEI	Q94B16	TC53043			х
1619522_at	Putative beta-galactosidase BGI	Q94B17	TC56838			х
1608756_at	Polygalacturonase-like protein	Q84LI7	TC59719			х
1606763_at	Putative beta-1,3-glucanase	Q8L868	TC67051			х
1609506_at	Putative cellulase CEL2	Q94B13	NP596365			х
1610263_at	Putative beta-1,3-glucanase	Q8L868	TC67051			х
Cell duplication						
1612320_a_at	Tubulin alpha chain	P33629	TC57547			х
1616815_at	Tubulin beta-8 chain	Q41785	TC55048			х
1618413_at	Tubulin alpha chain	P33629	TC63601			х
1619167_at	Tubulin beta-8 chain	Q41785	TC62643			x
1621015_at	Alpha-tubulin I	Q8H6M1	TC65238			х
1622466 at	Tubulin beta-8 chain	Q41785	TC62809			х
1608927 at	Putative histone H2A	O6L500	TC53574			x
	Histone H3	A2Y533	TC56731			x
1613041 at	Histone H4	O76H85	TC61904			x
1613076 at	Histone H4	Q76H85	TC62637			×
1620332 at	Histone H3	A2Y533	TC59489			x
1620002_at	Histone H3	A2Y533	TC64779			Ŷ
1622737 at	Histone H2B	022582	TC64405			Ŷ
1610854 at	Proliferating coll nuclear antigen	P22177	TC54917			Ĵ
161000-1_at	Patallia 6		TC41422			Ŷ
1610422_at	Giol like protein	Q73C01	TC66111			Ŷ
161000/_at	Sipi-like protein		TC55240			x
10133/3_at	Putativa DNA polymorphic alpha catalytic suburit	Q030F0	1C33247			X
1007792_at	rutative DINA polymerase alpha catalytic subunit	048633	1039012			х

^aCluster or Affy ID of transcripts modulated at 2 or 6 h. (+) and (-) refer to up- and down-regulation in the treated sample with respect to the control.

^bUniprotID [73]of the first hit obtained by "Blast" analysis.

cTC: corresponding grapevine Tentative Consensus sequence obtained by a search (BlastN) against the Grape Gene Index database [75].



Figure I Molecular events triggered by DIMEB as deduced by transcriptional profiling.

of Arabidopsis [24]. In rice the RLK XA21 confers resistance to bacterial blight disease [25]. Other genes possibly involved in signal transduction showed overexpression at 6 h: a gene (1620080_at) with homology to a putative receptor-like protein kinase ARK1 of Oryza sativa and a gene (1611172_at) homologous to a Glycine max Salt Overly Sensitive gene encoding a SOS2-like protein kinase (Table 1). In Arabidopsis thaliana ARK genes seem to be involved in plant defence response to wounding and to bacterial infections [26], while SOS2 is a signalling kinase involved in salt tolerance response [27]. Phospholipidderived molecules are emerging as novel second messengers in plant defence signalling and phospholipases are key enzymes for their synthesis [14,28]. In the array experiment we observed the overexpression of a putative phospholipase gene (1608981_at), which may generate lipid messengers for the signalling response (Table 1).

The activation of a signal cascade generally induces the expression of genes encoding for specific transcription factors, which in turn regulate downstream effector genes.

Two genes, upregulated at 6 h, showed homology to a hot pepper WRKY-b (1610775_s_at) and Arabidopsis WRKY11 (1611285_s_at) respectively (Table 1). WRKY proteins are plant-specific transcription factors whose expression is modulated in response to wounding, pathogen infection and abiotic stress [29]. Other classes of transcription factors appeared to take part in regulation of the response of grapevine cells to DIMEB treatment. The grape homologue (1619311_at) of a tomato pathogenesisrelated gene transcriptional activator PTI5 was upregulated at 6 h (Table 1). This transcription factor binds to the GCC-box cis element present in the promoter region of many plant PR genes [30] and its upregulation could explain the observed induction of many PR proteins in this experiment. Another sequence (CLU059), induced at 2 h, which might modulate the expression of PR genes is the homologue of the tobacco bZIP TGA10 factor (Table 1). It has been reported that this protein can bind to the regulatory activation sequence-1 (as-1) [31] identified in the promoter of Arabidopsis PR-1 gene [32].

Our results indicated that one of the final grapevine cell responses to the DIMEB-elicited signal consists in the modulation of phenolic metabolism, especially stilbene and monolignol biosynthesis (Figure 2).

Genes encoding enzymes involved in phenylalanine biosynthesis such as 3-deoxy-d-arabino-heptulosonate 7phosphate synthase (CLU083; 1611211_at; 1614440_at; 1619357_at; 1621405_at), 3-dehydroquinate synthase (1609646_at), prephenate dehydratase (1609932_at; 1621307 at) and chorismate mutase (1611895 at) were positively modulated both at 2 and 6 h after DIMEB treatment (Table 1). These enzymes participate in the synthesis of aromatic amino acids, particularly of phenylalanine, which is the link between primary and secondary metabolism, being a precursor of general phenylpropanoid metabolism. A recent report showed that cyclodextrins stimulates the expression of the structural genes of the general phenylpropanoids metabolism which sustains the synthesis of p-cumaroyl CoA, one of the two precursors of stilbenes [17].

Although we focused on the earlier cell response time, at both time points we also observed upregulation of this pathway's genes, namely phenylalanine ammonia lyase (1613113_at), cinnamic acid 4-hydroxylase (CLU024; 1610821_at; 1616191_s_at) and 4-coumarate-CoA ligase (1615801_at; 1619320_at) (Table 1). Similarly, several stilbene synthase genes were induced at 2 h and 6 h (CLU009, CLU022, CLU023, CLU049, CLU097, CLU103,



Figure 2

Modulation of secondary metabolism at 2 and 6 h after DIMEB treatment. Modulation (+ or -) of genes encoding enzymes of phenylalanine biosynthesis, general phenylpropanoid metabolism, monolignol, stilbene and anthocyanin pathways are reported within a simplified secondary metabolism scheme. Abbreviations: DHAP synthase, 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase; DHQ synthase, 3-dehydroquinate synthase; CM, chorismate mutase; PDT, prephenate dehydratase; PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CAD, cinnamyl alchol dehydrogenase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; COMT, caffeic acid O-methyltransferase; CCR, cinnamoyl-CoA reductase; F5H, ferulate-5-hydroxylase; STS, stilbene synthase; CHI, chalcone isomerase; UFGT, flavonoid-3-Oglucosyltransferase.

1606750_at, 1608009_s_at, 1609696_x_at, 1609697_at, 1610824_s_at, 1610850_at, 1611190_s_at, 1612804_at, 1614621_at, 1616575_at, 1620964_s_at, 1622638_x_at). According to the classification proposed by Richter et al. [33], they correspond to 7 different stilbene synthase genes plus one pseudogene (1606750_at). In particular, the probeset 1616575_at, encoding a stilbene synthase 2, appeared to be the most induced one, being 23 times higher in the DIMEB treated sample with respect to the control. In agreement, the chemical analysis proved stilbene accumulation in the medium already at 2 h and at higher levels after 6 h, as previously reported [21].

The accumulation of stilbenes in the growth medium requires, besides stilbene biosynthesis, the presence of export machinery. In fact, induction of genes encoding putative secondary metabolite transporters, such as those belonging to the ATP-binding cassette (ABC) transporter family, was found. Genes encoding for pleiotropic drug resistance (PDR)-like ABC transporters (CLU106; CLU119), ABC transporter-like proteins (1613763_at; 1618493_s_at) and a CjMDR transporter (1610363_at) were indeed induced (Table 1). The ABC transporters play an important role in some host-pathogen interactions [34]. In some pathogenic fungi they are involved in resist-

ance to plant phytolexins and antifungal compounds, while in plants they seem to take part in plant defence response [34]. The induction of genes encoding glutathione S-transferase (1609330_at; 1611890_at) at 6 h correlates well with the ABC-mediated transport (Table 1). A glutathione moiety seems to function as a "recognition tag" for the transport of phenols [35]. Resveratrol translocation outside the cells has two main objectives: to mediate the defence response against pathogens and to avoid intracellular accumulation of this compound at cytotoxic levels.

Phenylpropanoid metabolism also produces the precursors (p-coumarate and p-coumaroyl-CoA) for the synthesis of monolignols, which are used to reinforce the cell wall during defence response [36]. DIMEB treatment caused a general induction of genes involved in their synthesis at 6 h: the genes for caffeic acid O-methyltransferase (1607475_s_at, 1619682_x_at, 1620342_at), caffeoyl-CoA O-methyltransferase (1611897_s_at; 1614643_at), cinnamyl alcohol dehydrogenase (1613900_at), ferulate 5-hydroxylase (1614045_at; 1614502_at) and cinnamoyl-CoA reductase (1619065_at) were overexpressed (Table 1, Figure 2). Genes coding for enzymes such as polyphenol oxidase and diphenol oxidase, probably responsible for the lignin polymerization process [36], were induced as well (1622651_at; 1610806_at) (Table 1).

The other branches of phenolic metabolism seemed not to be affected by DIMEB. Only two genes of the anthocyanin pathway (a chalcone-flavonone isomerase (CLU122) and a flavonol-3-O-glucosyltransferase (CLU048)) were induced at 2 h but not at 6 h (Table 1, Figure 2). Interestingly, selective induction of the early steps of phenylpropanoid metabolism and of the late steps leading to monolignol biosynthesis was also described in Arabidopsis in the early response to oligogalacturonide treatment [37].

The results strongly suggest that DIMEB acts as an elicitor modifying cell metabolism to promote the accumulation of phytoalexins and cell wall lignification. These two defence responses have been described as typical biochemical responses occurring in vegetal cells after elicitor exposure [14].

The transcriptional profiling results, however, show that the response to DIMEB seems to include other defence mechanisms. Overexpression of sequences for pathogenesis-related proteins such as chitinase (CLU088; 1613871_at; 1617192_at; 1617430_s_at), PR-10 (CLU001; 1610011_s_at; 1618568_s_at) and PR-4 (CLU021), but also for a prolin-rich protein (CLU036) and a protease inhibitor (1609875_at; 1611666_s_at) was observed in both experiments, while upregulation of two genes encoding the S-adenosyl-L-methyonine:salicylic acid carboxyl methyltransferase (1612552_at; 1620309_at) was recorded at 6 h (Table 1). Interestingly, this enzyme mediates the synthesis of gaseous methyl salicylate which was recently demonstrated to be a key mediator in plant systemic acquired resistance [38] in tobacco, as well as an inducer of the expression of PR-1 gene and TMV resistance [39]. This result strengthens the hypothesis that DIMEB acts as a true elicitor. The increase in the expression of a gene encoding for a 1-aminocyclopropane-1-carboxylate oxidase (1622147_at), would suggest the involvement of ethylene as well (Table 1). This hormone is a major regulator of the plant's reaction to pathogen attack [40] and via the action of a group of ethylene responsive factors it modulates the expression of plant defence-related genes such as, for example, phenylalanine ammonia-lyase, hydroxylproline-rich glycoprotein and acid class II chitinase [41,42]. It appears from the finding that a gene (1616358_at) homologous to an MLO-like 11 of Arabidopsis was downregulated at 6 h (Table 1), that the similarities between the cell's responses upon DIMEB treatment and upon pathogen attack are even greater. In barley, downregulation of the Mlo gene is involved in response to powdery mildew caused by the fungus Blumeria graminis f.sp.hordei [43], and in the dicot Arabidopsis thaliana, resistance to powdery mildews also depends on loss-of-function mlo alleles [44].

Our data support another effect of DIMEB on grapevine cells: blockage of the cell-division process. Upon treatment, we measured a lower expression of the genes involved in modification of the cell wall structure, cell division and microtubule organization. At 6 h, downregulation of genes related to cell wall modification [45], such as those encoding expansins (1608074 s at; 1620840_at), xyloglucan endotransglycosylase (1615995_at; 1620003_at), pectin methylesterases 1619468_at), β-galactosidase (1608799_at; а (1619522_at), a polygalacturonase (1608756_at) and endoglucanases (1606763_at; 1609506_at; 1610263_at), was observed (Table 1). The sequence 1609506_at corresponds to the VvCEL2 transcript which encodes a grapevine cellulase. Since in Arabidopsis the expression of the cel1 gene was related to growing tissues [46], downregulation of VvCEL2 could be related to repression of the cell growth. Microtubules play an essential role in cell division and cell elongation too. They set the cellular division planes and axes of elongation and influence the deposition and orientation of cellulose microfibrils [47]. The downregulation of genes coding for α - and β -tubulin (1612320_a_at; 1616815_at; 1618413_at; 1619167_at; 1621015_at; 1622466_at) is indication of a stop in cell expansion and cell division (Table 1). mRNA degradation of a β -tubulin isoform was observed in soybean cells elicited by *Phytophthora sojae*-derived glucan fragments suggesting re-routing of the cellular resources towards the defence-related metabolism and repression of the cellular growth [48].

Further indication of cell division reduction were the lower transcription of genes coding for histones H2A, H3, H4 and H2B (1608927_at; 1612573_at; 1613041_at; 1613076 at; 1620332 at; 1622440 at; 1622737 at), a cyclin (1610854_at), a pattelin protein (1610422_at), a GA-induced-like protein (GIP-like) (1610607_at), a putative formin homology (FH) protein (1613373_at) and a DNA polymerase alpha catalytic subunit gene (1607792_at) (Table 1). All these proteins are either related to DNA organization and synthesis or to the cytokinesis process. The down-regulated grapevine GIP gene is homologous to GIP-5 of Petunia hybrida, which is expressed during the cell division phase in stems and corollas [49]. In Arabidopsis patellin1 plays a role in membrane-trafficking when the cell-plate is formed during cytokinesis [50], and formins are plant cytoskeletonorganizing proteins which take part in cytokinesis and in the establishment and maintenance of cell polarity [51]. Very similar effects on cell growth have been reported upon elicitation of parsley cell cultures with an oligopeptide elicitor. Pep 25 provoked the repression of genes regulating the cell cycle, such as cdc2, cyclin and histones [52].

A likely explanation for the repression of cell division would be the need of the cell to use, almost exclusively, the transcription system as well as the available resources to establish a defence-related metabolism.

Conclusion

The transcriptional profiles measured at 2 h and 6 h after DIMEB treatment highlight the fact that this compound is able to induce an early and specific defence response in grapevine liquid cell cultures, supporting the hypothesis of its role as a true elicitor.

The classes of genes modulated by the treatment reveal that DIMEB triggers a signal transduction cascade which activates different families of transcription factors, in turn modulating the effector genes of specific metabolisms. These results thus suggest that in grapevine cells DIMEB induces a stop in cell division, reinforcement of the cell wall and the production of resveratrol and defence proteins (Figure 3). This response largely resembles that occurring upon pathogen attack.

Methods

Plant material

Liquid cell cultures of a cross between *Vitis riparia* and *Vitis berlandieri* were used to carry out the treatment experi-

ments with DIMEB (50 mM) [21]. Cell cultures were collected 2 h and 6 h after DIMEB treatment from control and treated samples. Cells and medium were separated by centrifugation at 12.000 ×g for 10 min at room temperature.

Total RNA extraction

Total RNA was extracted from control and treated samples using a modified hot-borate method, as described by Moser *et al.* [53]. DNA traces were removed by DNase I treatment (Sigma-Aldrich, St.Louis, MO, USA) according to the manufacturer's procedure. RNA was isolated from one replicate for the SSH experiment (2 h) and from 3 biological replicates for the microarray experiment (6 h).

cDNA synthesis and SSH library construction

Double-stranded cDNA was synthesized from 0.6 µg of total RNA of the control and treated samples (2 h) using the SMART[™] PCR cDNA synthesis kit (Clontech Laboratories, Mountain View, CA) as recommended by the manufacturer.

Suppression subtractive hybridization (SSH) was carried out using the PCR-Select cDNA subtraction Kit (Clontech Laboratories) according to the manufacturer's procedure. The cDNA from the treated sample was used as the "tester" while the cDNA from the control sample was used as the "driver". Following hybridization, the subtracted cDNA molecules were inserted into a pCR^{*} 2.1-TOPO^{*} Vector (Invitrogen, Carlsbad, CA) and then used to transform One Shot^{*} TOP10 Chemically Competent *Escherichia coli* cells (Invitrogen). Positive transformants, based on blue/ white screening, were picked and arrayed in a 384-well plate containing LB medium (Sigma-Aldrich) supplemented with ampicillin (50 μ g mL⁻¹) and glycerol (10% v/ v). The SSH cDNA library was stored at -80°C.

Amplification of cDNA inserts and spotting on filters

The SSH library clones were cultured overnight at 37°C in a 384-well plate with LB medium and ampicillin (50 µg mL-1). A small aliquot (1 µl) of each liquid culture was then transferred into four 96-well plates containing PCR mix and used as template to amplify the corresponding cDNA inserts. PCR reactions (95°C for 15 min, 94°C for 45 sec, 68°C for 45 sec, 72°C for 2 min for 35 cycles, 72°C for 7 min) contained 300 nM Nested Primer PCR 1 and 300 nM Nested Primer PCR 2R (Clontech Laboratories), 0.5 U HotStartTaq DNA polymerase (Qiagen, Shanghai, China), 200 µM dNTPs, 1.5 M betain (Sigma-Aldrich) and 80 µM Cresol Red (Sigma-Aldrich). The 40 µl PCR reactions were then concentrated by overnight incubation at 37°C. The human nebulin cDNA (NM_004543) was PCR amplified in the same way to serve as a positive control. One microliter of each concentrated cDNA insert together with one microliter of a 2 ng/µl solution of



Figure 3

Cellular processes triggered by DIMEB as deduced by transcriptional profiling. Grapevine cell model showing the major genes involved in the cellular processes modulated by DIMEB treatment. Abbreviations: CAD, cinnamyl alchol dehydrogenase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; COMT, caffeic acid O-methyltransferase; CCR, cinnamoyl-CoA reductase; F5H, ferulate-5-hydroxylase; PME, pectin methylesterase; PPO, polyphenol oxidase, PR protein, pathogenesis-related protein; STS, stilbene synthase; XET, xyloglucan endotransglycosylase.

amplified nebulin were transferred onto 8×12 cm Hybond+ nylon membranes (Amersham, GE Healthcare Bio-Sciences AB, Little Chalfont, UK) using a manual 96pin tool. The samples were arrayed in duplicate according to a 4×4 grid pattern. Before and after spotting, membranes were denatured on Whatmann 3 MM paper saturated with denaturation buffer (0.5 M NaOH, 1.5 M NaCl) for 15 min. Membranes were then neutralised on Whatmann 3 MM paper saturated with neutralization buffer (1.5 M NaCl, 0.5 M Tris-HCl, pH 7.2) for 15 min, rinsed in 2× SSC, air dried and crosslinked at 80°C for 2 h.

Target labelling

To assess whether the isolated clones were truly positive, they were hybridized with the same total RNAs used for SSH library construction. The RNAs were DIG-labelled by reverse transcription according to Vernon et al. [54] with the following modifications: 7.5 µl of PCR DNA Labelling MIX 10× (Roche, Basel, Switzerland) and 1.5 µl of 50 µM of $Oligo(dT)_{20}$ were added to 5 µg of total RNA of each sample (tester and driver). After incubation of the two samples at 65°C for 10 min and then on ice for 2 min, a mix of 6 µl of RT Buffer 5× (Invitrogen), 3 µl of 0.1 M DTT (Invitrogen), 1.5 μ l of RNase OUT (40 U/ μ l) (Invitrogen) and 1.5 µl of Superscript II (200 U/µl) (Invitrogen) was added to each sample. Reverse transcription was performed at 42°C for 1 h and then continued for a further hour after addition of another 1.5 µl of Superscript II (200 $U/\mu l$) (Invitrogen). The reaction was stopped by incubation at 70°C for 15 min and was followed by treatment with 1.5 µl of RNase H (2 U/µl) (Invitrogen) at 37°C for 20 min. The digoxigenin-labelled probe of the control target was synthesized by PCR amplification of a portion of human nebulin cDNA cloned in pBluescript II SK/KS (-) (Stratagene) in the presence of PCR DNA Labelling MIX $10 \times$. PCR reaction was carried out in 50 µl using 7 ng/µl

of pBluescript II SK (-) containing human nebulin cDNA as template and the primers nebulin-for 5'-CAGGAGAC-TATTACAGGTTT-3' and nebulin-rev 5'-ACCCATAG-GCAGCTTGAGAA-3', according to the manufacturer's procedure. PCR conditions were 95°C for 15 min, 35 cycles of 94°C for 45 sec, 52°C for 45 sec, 72°C for 1 min, followed by 72°C for 7 min.

Hybridization, washing and detection

Two filters were incubated with 20 ml of pre-hybridization solution (5× SSC, 0.1% (w/v) N-lauroylsarcosine, 0.02% (w/v) SDS, 1% (v/v) blocking solution in 1× acid maleic buffer) at 72°C for 30 min. Two different probes were prepared: the first was obtained by mixing the DIGlabelled "tester" DNA (30 µl) with the DIG-labelled human nebulin $(2 \mu l)$, the second by mixing the DIGlabelled "driver" DNA (30 $\mu l)$ with the DIG-labelled human nebulin (2 μ l). After a short denaturation step (95°C for 3 min) the two probes were incubated separately with one filter each overnight at 68°C in hybridization solution (20 ml, 5× SSC, 0.1% (w/v) Nlauroylsarcosine, 0.02% (w/v) SDS, 1% (v/v) blocking solution in 1× acid maleic buffer). After hybridization, four high-stringency washings at 68°C for 20 min (2× SSC, 0.5% (w/v) SDS) followed by two low-stringency washings (0.2× SSC, 0.5% (w/v) SDS) at 68°C for 20 min, were carried out. Chemiluminescence was detected by 30min exposure to Kodak[®] BioMax Light Film (Kodak, Rochester, NY) after incubation with anti-DIG antibodies and CDP-Star, according to the manufacturer's procedure (Roche).

Sequencing of transcripts identified by SSH

Following the screening procedure, the 289 positive clones were amplified, as described above for filter production, but without betain and Cresol Red in the PCR reaction mix. Five microliters of each PCR reaction were purified from primers and nucleotides using 1.5 µl of ExoSAP-IT[™] (Amersham) at 37°C for 1 h. The reaction was stopped at 75°C for 15 min. Three nanograms for every 100 bp of amplified fragment were used for the sequencing reaction with Nested PCR Primer 1. Sequencing of 243 positively amplified clones was outsourced to the BMR Sequencing Service of C.R.I.B.I. (University of Padua, Padua, Italy) [55]. Electropherograms were analyzed with Phred [56,57] to assign a quality score and with a perl script using the UniVec Database [58] to identify any vector and adaptors sequences. Interspersed repeats and low complexity DNA sequences were identified through analysis with RepeatMasker [59]. The sequences were then organized in transcript consensus sequences (clusters) using the CAP3 DNA sequence program [60].

Affymetrix GeneChip experiments

Total RNA of the control and treated cells after 6 h of DIMEB treatment (3 biological replicates for each type of

sample) were used to hybridize 6 different GeneChip® Vitis vinifera Genome Arrays (Affymetrix, Santa Clara, CA). Ten micrograms of total RNA for each replicate were purified as described above (Total RNA extraction), subjected to further purification using "RNeasy" columns (Qiagen) and sent to an external service (IFOM-IEO Campus for ONCOGENOMICS, Milan, Italy) for labelling and hybridization. RNA samples passed the quality check as determined by electrophoresis run on a Agilent BioAnalyzer (Agilent, Palo Alto, CA, USA). Biotin-labelling, hybridization, washing, staining and scanning procedures were performed according to the Affymetrix technical manual. Analysis of raw data was performed using the open source software of the Bioconductor project [61,62] with the statistical R programming language [63,64]. The quality of the hybridization reactions was checked using the affyPLM package. Intensity distribution of PM for each chip and the quality of the 3 biological replicates of both control and treated conditions were analyzed with the functions and plots (histogram and MA plots) of the affy package [65-67]. Background adjustment, normalization and summarization were performed using gcrma and the affy package. Data, before and after application of the gcrma algorithm [68], were compared through the graphical representation of box-plots and MA plots. Probe sets which were not expressed or were non-differentially expressed between the two conditions considered were eliminated in a filtering step based on the inter-quantile range method (IQR = 0.25) using the genefilter package. A two-class paired SAM analysis ($\Delta = 0.9$; FDR = 13.3%) [69] was performed using the probe sets resulting from the filtering procedure in order to identify differentially expressed probe sets between the control and treated conditions. A fold-change of two was then applied.

Functional annotation of the SSH transcripts and Affymetrix probesets

Protein sequences encoded by the SSH transcripts or by the representative sequence of each probeset as provided by the NetAffx Analysis Center [70] were predicted using a consensus generated by three different CDS predictors [71]. Blastp analyses [72] of the polypeptides obtained from the predicted CDSs were performed by searching against the UniProt database [73]. GO terms (molecular function, biological process and cellular component) [23] were linked at every consensus sequence on the basis of the results of the Blastp analysis (Additional files 1 and 2). The sequences were organized in main functional categories according to the GO term biological process (Additional files 3 and 4). In cases of non significant Blastp results (Evalue <1e-8; sequence alignment length <75% of the query polypeptide length), these were classified as "No hits found".

The SSH transcripts were deposited at the NCBI database [74] under the sequence IDs reported in the Additional

file 1. Both SSH transcripts and probesets were also referred to corresponding Tentative Consensus sequences obtained by a search (BlastN) against the Grape Gene Index database [75] and to the corresponding genomic locus on Pinot Noir clone ENTAV 115 [76] (Additional files 1 and 2).

Real-time reverse transcription (RT)-PCR

To validate the SSH and microarray data, 12 genes and 5 genes identified by SSH and GeneChip array respectively, were also analyzed by quantitative RT-PCR experiments (Additional file 5). Specific primers were designed to generate 100-200 bp PCR products (Additional file 5). The actin gene (TC45156) was used to normalize the data (actin forward: 5'-TCCTTGCCTTGCGTCATCTAT-3'; actin reverse: 5'-CACCAATCACTCTCCTGCTACAA-3') since in preliminary trials it appeared to be constantly expressed in the RNA samples subjected to gene expression analyses. For RT-PCR, total RNA from control and treated samples of the SSH experiment and from 3 biological replicates of control and treated samples of the GeneChip experiments were used. DNA traces were removed with DNase I treatment (Sigma-Aldrich) according to the manufacturer's procedure. Reverse transcription reactions and real-time RT-PCR reactions were performed using the SuperScript[™] III Platinum® Two-Step qRT-PCR Kit with SYBR® Green (Invitrogen) according to the manufacturer's protocols with minor modification (300 nM of each primer in a final volume of 12.5 µl). PCR reactions contained 20 ng of cDNA and were replicated 3 times (technical replicates). Amplification reactions were performed with an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). The following thermal profile was used: 50°C for 2 min; 95°C for 10 min; 40 cycle of 95°C for 15 sec and 55°C for 1 min. Data were analysed with the ABI PRISM® 7000 SDS Software (Applied Biosystems). PCR reaction efficiencies were calculated with the LinRegPCR program [77]. For all the consensus sequences, the differential expression between treated and control samples was expressed as a ratio calculated with the Pfaffl equation [78]. The overall standard error of the mean normalized expression was obtained by applying the error calculation based on Taylor's series as developed for REST[®] software [79].

Data Availability

All microarray expression data are available at EBI ArrayExpress under the series entry E-MEXP-2114.

Abbreviations

DIMEB: (heptakis(2,6-di-O-methyl)-β-cyclodextrin); SSH: Suppression subtractive hybridization; cDNA: Complementary DNA; CDS: Coding Sequence; EST: Expressed Sequence Tag; GO: Gene Ontology; NCBI: National Center for Biotechnology Information; SAM: Significance Analysis of Microarrays; RT-PCR: Real time polymerase chain reaction.

Authors' contributions

AZ made a substantial contribution to conception, data collection and interpretation and manuscript drafting. PG participated in data analysis and manuscript writing. AC contributed to sequence analysis and annotation. SP participated in data analysis and manuscript revision. RV critically revised the manuscript. CM contributed to data interpretation and manuscript writing. RV (Velasco) and FM participated in the project's design and coordination. All authors read and approved the final manuscript.

Additional material

Additional file 1

Functional annotation of the transcripts identified by SSH. Cluster ID, Cluster length, GenBank Accession Number at NCBI [74], NCBI Sequence ID of the corresponding genomic locus on Pinot Noir clone ENTAV 115 [76], reference Tentative Consensus sequence in Grape Gene Index [75], GO terms, Ontology type [23], UniProtID [73], description and E-value are reported for each sequence. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-

[http://www.biomedcentral.com/content/supplementary/14/1-2164-10-363-S1.xls]

Additional file 2

Functional annotation of differentially expressed probe sets. AffyID, Fold change, reference sequence accession numbers, NCBI Sequence ID of the corresponding genomic locus on Pinot Noir clone ENTAV 115 [76], reference Tentative Consensus sequence in Grape Gene Index [75], GO terms, Ontology type [23] and UniProtID [73] description are reported for each probe set.

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Additional file 3

Functional category distribution of 127 transcripts modulated at 2 h. Each transcript is grouped in a single functional category defined by Gene Ontology "Biological process" terms [23]. Number and percentage of transcripts are reported for each main category. "No hits found" refers to transcripts with no significant homology to UniProt proteins. Click here for file

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Additional file 4

Functional category distribution of 223 upregulated and 148 downregulated probe sets. Each probe set is grouped in a single functional category defined by Gene Ontology "Biological process" terms [23]. Number and percentage of probe sets is reported for each main category. "No hits found" refers to probe sets with no significant homology to Uniprot proteins.

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Additional file 5

Real-time RT-PCR validation of a set of genes identified in the SSH experiment or in the microarray experiment. ClusterID or AffyID, description, RT-PCR relative expression value (treated vs. control) and sequences of forward and reverse primers are reported for each experiment. RT-PCR data for SSH validation are expressed as means ± SE of three technical replicates, while RT-PCR data for microarray validation are expressed as means \pm SE of three biological replicates.

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References

- Dixon RA, Harrison MJ: Activation, structure and organization L. of genes involved in microbial defence in plants. Adv Genet 1990, 28:165-234.
- Jeandet P, Douillt-Breuil AC, Bessis R, Debord S, Sbaghi M, Adrian M: 2. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J Agr Food Chem 2002, 50(10):2731-2741.
- Derckel JP, Baillieul F, Manteau S, Audran JC, Haye B, Lambert B, Leg-3. endre L: Differential induction of grapevine defenses by two strains of Botrytis cinerea. Phytopathology 1999, 89(3):197-203.
- Harborne JB: The comparative biochemistry of phytoalexin 4. induction in plants. Biochem Syst Ecol 1999, 27(4):335-367
- Langcake P, Pryce RJ: Production of resveratrol by Vitis Vinifera 5. and other members of Vitaceae as a response to infection or injury. Physiol Plant Pathol 1976, 9(1):77-86.
- Stein U, Hoos G: Induktions- und Nachweismethoden für Stil-6 bene bei Vitaceen. Vitis 1984, 23:179-184.
- 7. Dercks W, Creasy LL: The significance of stilbene phytoalexins in the Plasmopara viticola grapevine interaction. Physiol Mol Plant Pathol 1989, 34(3):189-202.
- Langcake P, Cornford CA, Pryce RJ: Identification of pterostil-8. bene as a phytoalexin from Vitis Vinifera leaves. Phytochemistry 1979, 18:1025-1027.
- Gatto P, Vrhovsek U, Muth J, Segala C, Romualdi C, Fontana P, Pruefer D, Stefanini M, Moser C, Mattivi F, Velasco R: **Ripening and** 9. genotype control stilbene accumulation in healthy grapes.] Agr Food Chem 2008, 56(24):11773-11785
- 10. Adrian M, Jeandet P, Veneau J, Weston LA, Bessis R: Biological activity of resveratrol, a stilbenic compound from grapevines, against Botrytis cinerea, the causal agent for gray mold. J Chem Ecol 1997, 23(7):1689-1702
- 11. Langcake P, Pryce RJ: Production of resveratrol and viniferins by grapevines in response to UV irradiation. Phytochemistry 1977. 6:1193-1196
- 12. Schubert R, Fischer R, Hain R, Schreier PH, Bahnweg G, Ernst D, Sandermann H: An ozone-responsive region of the grapevine resveratrol synthase promoter differs from the basal pathogenresponsive sequence. Plant Mol Biol 1997, 34(3):417-426.
- 13. Adrian M, Jeandet P, Bessis R, Joubert JM: Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with alu-
- minum chloride (AICI3). JAgr Food Chem 1996, 44(8):1979-1981. Radman R, Saez T, Bucke C, Keshavarz T: Elicitation of plants and 14. microbial cell systems. Biotechnol Appl Bioc 2003, 37(Pt 1):91-102.
- 15. Liswidowati , Melchior F, Hohmann F, Schwer B, Kindl H: Induction of stilbene synthase by Botrytis cinerea in cultured grapevine cells. Planta 1991, 183(2):307-314.
- Tassoni A, Fornale S, Franceschetti M, Musiani F, Michael AJ, Perry B, 16. Bagni N: Jasmonates and Na-orthovanadate promote resver-

atrol production in Vitis vinifera cv. Barbera cell cultures. New Phytol 2005, 166(3):895-905.

- 17 Lijavetzky D, Almagro L, Belchi-Navarro S, Martínez-Zapater J, Bru R, Pedreno MA: Synergistic effect of methyljasmonate and cyclodextrin on stilbene biosynthesis pathway gene expression and resveratrol production in Monastrell grapevine cell cultures. BMC Res Notes 2008, 1:132.
- Aziz A, Poinssot B, Daire X, Adrian M, Bezier A, Lambert B, Joubert 18. JM, Pugin A: Laminarin elicits defense responses in grapevine and induces protection against Botrytis cinerea and Plasmopara viticola. Mol Plant Microbe Interact 2003, 16(12):118-1128.
- Morales M, Bru R, Garcia-Carmona F, Barcelo AR, Pedreno MA: 19. Effect of dimethyl-beta-cyclodextrins on resveratrol metabolism in Gamay grapevine cell cultures before and after inoculation with Xylophilus ampelinus. Plant Cell Tiss Org 1998, 53(3):179-187
- Bru R, Selles S, Casado-Vela J, Belchi-Navarro S, Pedreno MA: Mod-20. ified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. J Agr Food Chem 2006, 54(1):65-71.
- Zamboni A, Vrhovsek U, Kassemeyer HH, Mattivi F, Velasco R: Elic-21. itor-induced resveratrol production in cell cultures of different grape genotypes (Vitis spp.). Vitis 2006, 45(2):63-68.
- Cao WX, Epstein C, Liu H, DeLoughery C, Ge NX, Lin JY, Diao R, Cao H, Long F, Zhang X, Chen YD, Wright PS, Busch S, Wenck M, 22 Wong K, Saltzman AG, Tang ZH, Liu L, Zilberstein A: Comparing gene discovery from Affymetrix GeneChip microarrays and Clontech PCR-select cDNA subtraction: a case study. BMC Genomics 2004, 5(1):26.
- 23
- The Gene Ontology [http://www.geneontology.org/] Stone JM, Collinge MA, Smith RD, Horn MA, Walker JC: Interaction 24. of a protein phosphatase with an Arabidopsis serine-threonine receptor kinase. Science 1994, 266(5186):793-795.
- Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P: **A receptor** 25. kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 1995, 270(5243):1804-1806.
- Pastuglia M, Swarup R, Rocher A, Saindrenan P, Roby D, Dumas C, Cock JM: Comparison of the expression patterns of two small gene families of S gene family receptor kinase genes during the defence response in Brassica oleracea and Arabidopsis thaliana. Gene 2002, 282(1-2):215-225
- Halfter U, Ishitani M, Zhu JK: The Arabidopsis SOS2 protein 27. kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci USA 2000, 97(7):3735-3740.
- Laxalt AM, Munnik T: Phospholipid signalling in plant defence. 28. Curr Opin Plant Biol 2002, 5(4):332-338.
- Ulker B, Somssich IE: WRKY transcription factors: from DNA 29 binding towards biological function. Curr Opin Plant Biol 2004, 7(5):491-498.
- Gu YQ, Wildermuth MC, Chakravarthy S, Loh YT, Yang CM, He XH, 30. Han Y, Martin GB: Tomato transcription factors Pti4, Pti5, and Pti6 activate defense responses when expressed in Arabidopsis. Plant Cell 2002, 14(4):817-831.
- Schiermeyer A, Thurow C, Gatz C: Tobacco bZIP factor TGAI0 31. is a novel member of the TGA family of transcription factors. Plant Mol Biol 2003, 51(6):817-829.
- Zhang YL, Fan WH, Kinkema M, Li X, Dong XN: Interaction of 32. NPRI with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. Proc Natl Acad Sci USA 1999, 96(11):6523-6528.
- Richter H, Pezet R, Viret O, Gindro K: Characterization of 3 new partial stilbene synthase genes out of over 20 expressed in Vitis vinifera during the interaction with Plasmopara viticola. Physiol Mol Plant Pathol 2005, 67(3-5):248-260.
- Campbell EJ, Schenk PM, Kazan K, Penninckx IAMA, Anderson JP, 34. Maclean DJ, Cammue BPA, Ebert PR, Manners JM: Pathogenresponsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in Arabidopsis. Plant Physiol 2003, 133(3):1272-1284.
- 35. Yazaki K: Transporters of secondary metabolites. Curr Opin Plant Biol 2005, 8(3):301-307.
- 36. Whetten R, Sederoff R: Lignin biosynthesis. Plant Cell 1995, **7(7):**1001-1013.

- 37. Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel FM, Dewdney]: Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jassignaling but requires PHYTOALEXIN monate DEFICIENT3. Plant Physiol 2007, 144(1):367-379.
- 38. Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF: Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 2007, 318(5847):113-116.
- Shulaev V, Silverman P, Raskin I: Airborne signalling by methyl 39 salicylate in plant pathogen resistance. Nature 1997. 386(6626):738-738.
- 40. Broekaert WF, Delauré SL, De Bolle MF, Cammue BP: The role of ethylene in host-pathogen interactions. Annu Rev Phytopathol 2006, **44:**393-416.
- 41. Ecker JR, Davis RW: Plant defense genes are regulated by ethylene. Proc Natl Acad Sci USA 1987, 84(15):5202-5206.
- 42. Marcos JF, Gonzalez-Candelas L, Zacarias L: Involvement of ethylene biosynthesis and perception in the susceptibility of citrus fruits to Penicillium digitatum infection and the accumulation of defence-related mRNAs. J Exp Bot 2005, 56(418):2183-2193.
- 43. Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, vanDaelen R, vanderLee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F, Schulze-Lefert P: The barley mlo gene: A novel control element of plant pathogen resistance. Cell 1997, 88(5):695-705.
- 44. Consonni C, Humphry ME, Hartmann HA, Livaja M, Durner J, Westphal L, Vogel J, Lipka V, Kemmerling B, Schulze-Lefert P, Somerville SC, Panstruga R: Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. Nat Genet 2006, 38(6):716-720.
- 45. Cosgrove DJ: Enzymes and other agents that enhance cell wall extensibility. Annu Rev Plant Phys 1999, 50:391-417
- 46. Shani Z, Dekel M, Roiz L, Horowitz M, Kolosovski N, Lapidot S, Alkan S, Koltai H, Tsabary G, Goren R, Shoseyov O: Expression of endo-1,4-beta-glucanase (cell) in Arabidopsis thaliana is associated with plant growth, xylem development and cell wall thickening. Plant Cell Rep 2006, 25(10):1067-1074.
- 47. Dixon DC, Seagull RW, Triplett BA: Changes in the accumulation of alpha-tubulin and beta-tubulin isotypes during cotton fiber development. Plant Physiol 1994, 105(4):1347-1353
- Ebel C, Gomez LG, Schmit AC, Neuhaus-Url G, Boller T: Differential mRNA degradation of two beta-tubulin isoforms correlates with cytosolic Ca2+ changes in glucan-elicited soybean cells. Plant Physiol 2001, 126(1):87-96.
- 49. Ben-Nissan G, Lee JY, Borohov A, Weiss D: GIP, a Petunia hybrida GA-induced cysteine-rich protein: a possible role in shoot elongation and transition to flowering. Plant | 2004, 37(2):229-238.
- Peterman TK, Ohol YM, McReynolds LJ, Luna EJ: Patellin I, a novel 50. Sec14-like protein, localizes to the cell plate and binds phosphoinositides. Plant Physiol 2004, 136(2):3080-3094.
- Favery B, Chelysheva LA, Lebris M, Jammes F, Marmagne A, de Alme-51. ida-Engler J, Lecomte P, Vaury C, Arkowitz RA, Abad P: Arabidopsis formin AtFH6 is a plasma membrane-associated protein upregulated in giant cells induced by parasitic nematodes. Plant Cell 2004, 16(9):2529-2540.
- 52. Logemann E, Wu SC, Schroder J, Schmelzer E, Somssich IE, Hahlbrock K: Gene activation by UV light, fungal elicitor or fungal infection in Petroselinum crispum is correlated with repression of cell cycle-related genes. *Plant J* 1995, 8(6):865-876. 53. Moser C, Gatto P, Moser M, Pindo M, Velasco R: Isolation of func-
- tional RNA from small amounts of different grape and apple tissues. Mol Biotechnol 2004, 26(2):95-99.
- 54. Vernon SD, Unger ER, Rajeevan M, Dimulescu IM, Nisenbaum R, Campbell CE: Reproducibility of alternative probe synthesis approaches for gene expression profiling with arrays. J Mol Diagn 2000, 2(3):124-127.
- BMR Genomics [http://bmr.cribi.unipd.it/]
- Ewing B, Hillier L, Wendl MC, Green P: Base-calling of automated 56. sequencer traces using phred. I. Accuracy assessment. Genome Res 1998, 8(3):175-185.
- Ewing B, Green P: Base-calling of automated sequencer traces 57. using phred. II. Error probabilities. Genome Res 1998. 8(3):186-194.
- 58 The UniVec Database [http://www.ncbi.nlm.nih.gov/VecScreen/ UniVec]

- 59.
- RepeatMasker [http://www.repeatmasker.org/] Xuang X, Madan A: CAP3: a DNA sequence assembly program. 60. Genome Res 1999, 9:868-877.
- 61. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, lacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J: **Bioconductor: open** software development for computational biology and bioinformatics. Genome Biol 2004, 5(10):R80.
- 62. The Bioconductor project [http://www.bioconductor.org/]
- Ihaka R, Gentleman RC: R: a language for data analysis and 63. graphics. J Comput Graph Stat 1996, 5(3):299-314.
- The R Project for Statistical Computing 64. [http://www.rproject.org/
- 65. Bolstad BM, Irizarry RA, Astrand M, Speed TP: A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003, 19(2):185-193.
- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, 66. Scherf U, Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003, 4(2):249-264
- Gautier L, Cope L, Bolstad BM, Irizarry RA: affy analysis of 67. Affymetrix GeneChip data at the probe level. Bioinformatics 2004, 20(3):307-315.
- Wu ZJ, Irizarry RA: Preprocessing of oligonucleotide array 68. data. Nat Biotechnol 2004, 22(6):656-658.
- Tusher VG, Tibshirani R, Chu G: Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 2001, 98(9):5116-5121.
- 70 Affymetrix [http://www.affymetrix.com/index.affx]
- Wasmuth JD, Blaxter ML: prot4EST: translating expressed sequence tags from neglected genomes. BMC Bioinformatics 2004. 5:187.
- Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, 72. Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25(17):3389-3402.
- The UniProt Database [http://www.uniprot.org/] 73.
- The NCBI database [http://www.ncbi.nlm.nih.gov/] 74
- 75. The DFCI Grape Gene Index [http://compbio.dfci.harvard.edu/ tgi/cgi-bin/tgi/gimain.pl?gudb=grape]
- Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss 76. D, Pindo M, Fitzgerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma T, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Dematte L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Si-Ammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L, Vandepoele K, Grando SM, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar SK, Fontana P, Gutin A, Peer Y Van de, Salamini F, Viola R: A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 2007, 2(12):e1326
- Ramakers C, Ruijter JM, Deprez RHL, Moorman AFM: Assumption-77. free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett 2003, 339(1):62-66.
- 78. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001, 29(9):e45.
- 79. Pfaffl MW, Horgan GW, Dempfle L: Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002, 30(9):e36.