Characterization of a F-box gene up-regulated by phytohormones and upon biotic and abiotic stresses in grapevine

Sandra Paquis · Florence Mazeyrat-Gourbeyre · Olivier Fernandez · Jérôme Crouzet · Christophe Clément · Fabienne Baillieul · Stéphan Dorey

Abstract F-box proteins are key components of the ubiquitin (Ub)/26S proteasome pathway that mediates selective degradation of regulatory proteins involved in a wide variety of cellular processes affecting eukaryotic cells. In plants, F-box genes form one of the largest multigene superfamilies and control many important biological functions. Among the F-box genes characterized to date only few have been involved in the regulation of plant defense responses. Moreover, no F-box genes have been studied and characterized in grapevine. Using a differential display approach we isolated a F-box gene (BIG-24.1), which is up-regulated during Botrytis cinerea infection of grapevine leaves. BIG-24.1 encodes a polypeptide of 386 amino acids with a conserved F-box domain in the N-terminus region and a kelch domain. By investigating expression profiles of BIG-24.1, we show that the gene expression is strongly stimulated in B. cinerea infected berries and in grapevine cells challenged by MAMP rhamnolipids, a non-host bacterium and an endophytic rhizobacterium. The gene is also strongly induced by abiotic stresses including UV-C and wounding or by salicylic acid, methyl-jasmonate, ethylene and abscisic acid that are known to be involved in defense signalling pathways. In addition, sequence analysis of the BIG-24.1 promoter revealed the presence of several regulatory elements involved in the activation of plant defense responses.

Keywords Abiotic stress · Biotic stress · Defense responses · F-box · Phytohormones · Plant

Introduction

In the course of their life, plants are frequently exposed to pathogenic attacks and they have evolved several defensive strategies to protect themselves from diseases. These strategies include pre-existing physical and chemical barriers, as well as inducible defense responses that become activated upon pathogen perception [17]. The molecular mechanisms underlying activation of plant defense responses are complex. In this process, plants usually activate a large set of genes leading to de novo biosynthesis of numerous novel proteins, including key regulatory components and transcriptional factors [12]. In addition, some negative regulatory proteins, which repress specific defense pathways and also suppressors have to be removed or destroyed in order to activate the defense machinery [8, 21].

Using a differential display approach aimed at characterizing grapevine defense genes that are regulated upon Botrytis cinerea infection, we isolated several candidates named BIG (for Botrytis Induced Grapevine) genes. One of these genes was found to encode a F-box protein with a kelch motif and named BIG-24.1. F-box proteins are involved in the ubiquitin (Ub)/26S proteasome machinery that mediates selective degradation of regulatory proteins and that is essential in the regulation of cellular processes including defense responses and stress tolerance [24, 25, 37]. F-box proteins are part of the E3 ubiquitin ligases complex that is responsible for the final tagging of proteins, thereby conferring specificity to the degradation process.
Different types of E3 ubiquitin ligases have been documented in plants and among them the SCF complex (Skp1/Cullin/F-box) is the largest and best understood [25]. F-box proteins from SCF complex contain a conserved F-box domain at their N-terminus, which interacts with Skp1 [25]. The C-terminus of the proteins generally contains one or several protein-protein interaction domains that interact with the target, like leucine-rich repeat (LRR), kelch repeat and WD40 [18].

Over the past few years, several F-box proteins have been identified in eukaryotes. To date there are 14 F-box protein-encoding genes predicted in yeast, 24 in Drosophila, 337 in Caenorhabditis elegans and 38 in human [18, 47]. F-box proteins are also ubiquitous in plants. They have been shown to be involved in the regulation of various processes. For instance, F-box proteins play important roles in floral development, lateral root formation, leaf senescence circadian rhythms and plant hormone signalling [18, 38]. Evidence is accumulating that the F-box proteins participate in plant defense responses. Arabidopsis COI1 and its tomato homologue JAI1 are key regulators in the jasmonate (JA) signalling pathway [8]. Mutations in COI1 result in insensitivity to JA and a defect in the expression of the JA-responsive defense gene PDF1 [26, 46]. Another F-box protein, SON1, plays a key role as negative regulator in plant defense responses in Arabidopsis [22]. The son1 mutant showed effective resistance against the bacterial pathogen Pseudomonas syringae pv. tomato DC3000 in NahG plants which are unable to accumulate salicylic acid. Recently, ACRE189/ACIF1 F-box protein which is closely related to F-box proteins regulating plant hormone signalling, has been shown to regulate cell death and defense responses activated during pathogen recognition in tobacco and tomato [42].

To date, no F-box genes have been characterized in grapevine. In this paper, we described in silico analysis of BIG-24.1 gene and BIG-24.1 promoter and investigated the expression profile of BIG-24.1 during biotic and abiotic stresses in grapevine. We report for the first time that a F-box/kelch gene is up-regulated during plant defense responses. The gene expression is also induced by different hormones, which are known to be involved in defense signalling pathways. Altogether, our data highlight the potential involvement of BIG-24.1 in response to multiple stresses.

Materials and methods

Plant cultivation

Grapevine plantlets (Vitis vinifera L. cv. Chardonnay 7535) were micropropagated in vitro at 26°C with a 16/8 h photoperiod [43]. Eight-week old plants were used for the experiments. Grapevine cell suspensions [43] were cultured in Murashige-Skoog medium (pH 5.8) containing vitamins (×1.5), sucrose (30 g l⁻¹), 2,4-D (0.2 mg l⁻¹) and BAP (0.5 mg l⁻¹) and were propagated in the dark at 25°C under shaking at 120 rpm. They were subcultured every 7 days to be maintained in exponential phase. For experiments, 30 ml of cells subcultured for 6 days were used. Before any treatment, cells were allowed to adjust to the new condition overnight. Based on BBCH scale [27], grapevine inflorescences (BBCH 57), young berries (BBCH 75), and mature berries (BBCH 89) were collected in the vineyard (experimental station of the CIVC, Plumecq, France). Mature clusters partially infected by B. cinerea were also collected in the same vineyard and berries were sorted according to four different stages of infection as described by Bézier et al. [3].

Micro-organisms and plant assays

Botrytis cinerea strain T4 (kind gift of C. Levis, INRA, Versailles, France) was grown on solid tomato/agar medium (tomato juice 25% (v/v), agar 2.5% (p/v)). Infection procedures of leaves from plantlets were performed according to Bézier et al. [3]. As controls, leaves were mock-inoculated with sterile non-inoculated medium. Pseudomonas syringae pv. pisi and Burkholderia phytofirmans strain PsJN were grown in 100 ml Luria–Bertani liquid medium in 250 ml Erlenmeyer flasks and incubated at 28°C, on a rotary shaker (150 rpm). Overnight cultures were used for the experiments. Bacteria were collected by centrifugation (4,500 g, 10 min) and washed with sterile MgCl₂ (10 mM). The final bacterial concentration in cell cultures was 10⁷ cells per milliliter (OD₆₀₀ = 0.01). Rhamnolipids treatments in cell suspensions were performed according to Varnier et al. [43]. Final rhamnolipid concentration was 0.025 mg ml⁻¹. As controls, cell suspension cultures were inoculated with sterile water or MgCl₂. Microorganisms and elicitor concentrations have been chosen according to previous studies in our plant systems [6, 43].

Phytohormone and abiotic treatments

UV-C irradiation, wounding and cold treatments

Detached leaves from 8-week old plantlets were placed lower face up on wet paper in Petri dishes and irradiated using a UV-C lamp (254 nm, Vilber Lourmat, Model VL-6.C, output 7.1 W cm⁻², 9 cm distant) for 8 min. Controls consist of non-irradiated detached leaves. For wounding, leaves were crushed with a forceps corresponding to 40% of leaf area. Controls consist of non-wounded
detached leaves. Petri dishes were then sealed with parafilm (to maintain high relative humidity) and placed in a 26°C growth chamber (16/8 h photoperiod). For cold treatment, plantlets were placed in a 4°C chamber (16/8 h photoperiod) for 24 h. Controls consist of plantlets placed in a 26°C growth chamber.

**Phytohormones**

All hormones and chemicals were purchased from Sigma-Aldrich-Chimie (Saint-Quentin-Fallavier, France), except for sodium salicylate (SA), which was purchased from Eurobio (Les Ulis, France). SA and 1-aminocyclopropane 1-carboxylic acid (ACC) were dissolved in water and methyl jasmonate (MeJA) was dissolved in 10% (v/v) ethanol prior to be diluted in water. Gibberellic acid (GA), abscisic acid (ABA) and indole-3-acetic acid (IAA, a natural auxin) were dissolved in dimethyl sulphoxide (DMSO), prior to be diluted in water. Chemicals were added at different final concentrations: 1 mM for SA and ACC, 200 μM for MeJA, 20 μM for GA, 10 μM for ABA and 5 μM for IAA. Equivalent volumes of 10% ethanol solution or DMSO were added to control cells to ensure that they did not interfere with the experiments. Final ethanol or DMSO solutions did not exceed 0.1% (v/v).

RNA extraction and real-time quantitative PCR

RNA extraction and real-time quantitative PCR were performed as previously described in Petit et al. [32]. Sequences of STS gene primers used for RT-qPCR were previously described by Bonomelli et al. [5]. **BIG 24.1** primers (5'CGT TCG ATC CGGCAA CTA A-3' and 5'TCC CGA ATC AGT CCA ACC A-3') were submitted to BLAST search (Primer-BLAST, NCBI) against *Vitis vinifera* nr database to confirm their mono-specificity. If not mentioned otherwise, results from 24 hpt time points are illustrated for gene expression. This time point corresponds to a maximum in elicitation in our plant systems [6, 43].

Cloning of **BIG-24.1** and **BIG-24.1** promoter

A 576-bp cDNA was characterized using differential display method comparing changes in mRNA levels between *B. cinerea* infected or non infected grapevine leaves [4]. Based on the sequence of this cDNA, gene specific primers (5'-ctgagacagctccataca-3' and 5'-tcattctacaga cttgacagcaa-3') were designed to amplify the reverse and downstream cDNA by 3'- and 5'-RACE (SMART RACE cDNA Amplification Kit, Clontech). Finally, the complete ORF (accession number GQ861441) was amplified with specific primers (5'-atgggagggctgggga-3' and 5'-ctacgctacacttcgctc-3') derived from sequences of the two partial cDNAs. Promoter region of the **BIG-24.1** gene was cloned from genomic DNA by using the BD GenomeWalkerTM Universal Kit (Clontech) following the manufacturer’s protocol.

**Sequence analysis**

Sequencing of both strands was performed with the dideoxynucleotide chain termination reactions by M. Aliova (IBMP, Strasbourg, France). Blat-search on Grape Genome Browser (http://www.genoscope.cns.fr/blat-server/cgi-bin/vitis/webBlat), Blastn and Blastp (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) and ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/index.html) were used for homology searches. Protein sequence analysis was done using ProtParam tool [16]. Searches for conserved domains were carried out using specialized Blast (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), Pfam (http://pfam.sanger.ac.uk/) and InterPro (http://www.ebi.ac.uk/interpro/) databases. The promoter sequence was analysed with PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) and PlantCARE databases (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

**Results**

**BIG-24.1** cDNA cloning and sequence analysis

A 576-bp cDNA was obtained using differential display method comparing changes in mRNA levels between *B. cinerea* infected or non infected grapevine leaves [4]. Based on the sequence of the fragment, 3'-RACE and 5'-RACE primers were designated and used in RACE to generate two fragments of 330 and 893 bp, respectively. By alignment and assembling of these 3 sequences, the full-length cDNA was deduced and the ORF was amplified by PCR and confirmed by sequencing. The full-length cDNA is 1,375-bp long and it contains an 1,158-bp ORF. Blat-search on Grape Genome Browser revealed that this cDNA designed as **BIG-24.1** gene displays 99.4% identity with the gene annotated GSVIVT01013786001 located on chromosome 1 corresponding to the UniGene Vvi. 14838 on NCBI. This gene is predicted to have 3 orthologs, respectively, in *Arabidopsis thaliana* (At1g23390), *Oryza sativa* (01_06_0416) and *Populus trichocarpa* (jgilP0tr1_1 230616/gw1.X) [48].
The deduced BIG-24.1 protein consisted of 386 amino acids with a calculated molecular weight of 43 kDa and an isoelectric point (pI) of 5.92 (ProtParam). BIG-24.1 shared 53, 48, 44, 31 and 30% identity with proteins from Ricinus communis, Populus trichocarpa, Arabidopsis thaliana, Zea mays and Oryza sativa, respectively (Fig. 1). Searches for conserved domains revealed that BIG-24.1 contains a F-box protein domain at the N-terminus and a kelch motif that could be part of a β-propeller.

Expression of BIG-24.1 in grapevine organs

We first analysed the basal expression of BIG-24.1 gene in different grapevine organs including leaves, roots, stems, inflorescences, young and mature berries. As shown in Fig. 2, we detected basal level of BIG-24.1 transcripts in all organs. Basal expression of the gene was quite similar in leaves, stems, mature berries and inflorescences and was much higher in roots and young berries.
Expression of BIG-24.1 during Botrytis cinerea infection of leaves and berries

In order to confirm that BIG-24.1 is up-regulated during B. cinerea infection, we monitored the expression of the gene over a 48 h time course in infected leaves of plantlets (Fig. 3a). After 48 h, leaf tissues were completely necrotized. For these experiments, we also followed stilbene synthase (STS) gene expression that we used as a typical grapevine defense marker [3, 43]. Stilbene synthase is a key enzyme involved in the synthesis of the main grapevine phytoalexin resveratrol [7]. The level of BIG-24.1 transcripts rose slightly at 15 h in infected leaves compared to control and reached a maximum at 48 h post infection.
infection (hpi). A similar expression profile was obtained with the STS marker. BIG-24.1 expression profile was also monitored in naturally field-infected berries sorted according to four different stages of infection (Fig. 3b). The expression of the gene was clearly up-regulated in the infected material at stages 3 and 4. Induction was maximum in stage 4 corresponding to shrivelled berries. A similar profile of induction was observed at the four different stages with the STS marker in a previous study [3]. No amplification was obtained when real time RT–PCR was performed on RNA extracted from B. cinerea grown in liquid culture or from genomic DNA of the fungus indicating that the primers were specific for grapevine BIG-24.1 (data not shown).

Expression of BIG-24.1 in response to abiotic stresses

To get further information on the regulation of BIG-24.1 during plant defense, its expression level was evaluated under different abiotic stress conditions. We first monitored BIG-24.1 expression over time in leaves exposed to UV-C (Fig. 4a). The level of BIG-24.1 transcripts rose at 15 h after UV-C treatment compared to control and reached a maximum at 24 h post treatment (hpt). STS induction occurred slightly earlier with a maximum at 15 hpt. We also monitored BIG-24.1 expression after wounding and cold treatments of grapevine leaves (Fig. 4b). Only 24 h time points are shown. No increase in the accumulation of BIG-24.1 transcripts was detected after cold treatment over the control. In contrast, induction of gene expression was detected after wounding (Fig. 4b) with similar levels to those measured after UV-C treatment.

Expression of BIG-24.1 in grapevine cell suspensions upon biotic stresses and hormonal treatments

The HR-like inducing bacterium Pseudomonas syringae pv. pisi [35, 36] which causes a typical non-host response in grapevine, the endophytic bacterium Burkholderia phytofirmans strain PsJN [10] and rhamnolipids, which are recognized as microbe-associated molecular pattern (MAMP) in grapevine [43] were used to test the scale of BIG-24.1 response to biotic stresses (Fig. 5). F-box proteins are also key factors involved in plant hormone response pathways [25]. In order to specify the regulation of BIG-24.1 gene expression to plant hormones and signalling molecules, we monitored its expression in response to abscisic acid (ABA), auxin (IAA), gibberellin (GA), methyl-jasmonate (MeJA), salicylic acid (SA) and an ethylene precursor (ACC). Because infiltration procedure in plantlet leaves was too stressful even in control plants and because the spraying method has proved to be ineffective in our plant system, the bacterial challenges, the hormones and MAMP treatments have been performed in cell suspension cultures. We used B. cinerea challenge of grapevine cells as positive control for BIG-24.1 stimulation.

Fig. 4 BIG-24.1 expression in response to abiotic stresses.

a BIG-24.1 expression was monitored over a time course after UV-C treatment (closed circles) or control (open circles) plantlet leaves. STS expression was also monitored as a positive control of defense marker.

b BIG-24.1 expression analysis was also performed after plantlet treatments with UV-C (UV), cold and wounding. Transcript accumulation of the gene was monitored 24 h post treatment and expressed as fold increase over each specific controls (×1 expression level). Analyses were performed by real-time quantitative polymerase chain reaction as described in Fig. 3. Results shown are the mean ± standard deviation of three independent experiments.
A characteristic TATA box was identified at the promoter region of BIG-24.1 after challenge with B. cinerea. The promoter region was obtained and the presence of potential cis-regulatory elements, including: (i) four W-box motifs which are binding sites for WRKY transcription factors (C/TTGACT/C, positions −1435/−1325/−1144/−218) [31] and which are well characterized for their role in salicylic acid, wounding and pathogen-induced pathways, (ii) three activation sequence-1 (as-1) elements which can be stimulated by MeJA or after biotic and abiotic stresses (TGACGT, positions −1434/−1226/−1176) [15, 34], (iii) four Ethylene Responsive Elements (EREs; ATTTCAA positions −823/−731/−430/−269) [33], (iv) two Gibberellic Acid Responsive Elements (GAREs; TAACAAAG, positions −809/−280) [29] and (v) two TC-rich repeats (ATT TCTTC/AC/A, positions −488/−113) [23] which are related to stress and plant defense.

**Discussion**

In the present study we describe for the first time the isolation of a grapevine F-box gene regulated during biotic and abiotic stresses and in response to hormonal treatments. F-box proteins represent a large family in plants [25]. Recent studies revealed approximately 660, 320 and 780 different F-box genes in Arabidopsis, poplar and rice genomes, respectively [20, 47, 48]. Approximately 156 F-box predicted genes were identified in the newly sequenced Vitis vinifera [19, 44, 48]. Only a few members of F-box genes have been studied in model plants [20] and so far, no grapevine F-box gene has been characterized. Analyses in databases revealed different homologues of BIG-24.1 protein in A. thaliana, Ricinus communis, Populus trichocarpa, Zea mays and Oryza sativa with significant level of identity. Not surprisingly, the highest level of identity was found in the woody species P. trichocarpa and R. communis [48]. The similar domain organization between these F-box proteins could suggest that they may function to interact with the same or similar substrates [47]. In addition, Yang et al. [48] hypothesized that the smaller number of F-box genes on P. communis suggest that some F-box proteins may have evolved in such way that they can recognize multiple substrates.

We found that BIG-24.1 contains a kelch motif that could be part of a kelch β-propeller structure. Typically, individual kelch repeats form four stranded β-sheets that assemble together to create a β-propeller tertiary structure that interact with the specific targets [2]. In Arabidopsis, poplar and rice, F-box proteins with kelch protein–protein interaction domains represent one of the largest family [47]. In Arabidopsis, most of the Kelch domain-containing F-box proteins characterized have been involved in light signalling and circadian clock regulation [18]. Recently, the F-box kelch JFK has been shown to link the SCF complex to p53 regulation in human cells [40]. Interestingly, p53 is a tumor suppressor that plays a central role in integrating cellular responses to various stresses.

**BIG-24.1** has been isolated by differential display method using a screen based on grapevine leaves infected by the fungus B. cinerea [4]. Using quantitative RT–PCR, we confirmed that the gene is up-regulated in B. cinerea...
infected leaves. Other defense genes like STS and those coding for a phenylalanine ammonia-lyase, two chitinases and a polygalacturonase inhibitor protein are also induced in \textit{B. cinerea} infected leaves [3]. The late expression profile of \textit{BIG-24.1} is quite surprising given the potential role of F-boxes in signalling but could be explained by the complexity of the infection process or by a potential role in targeting proteins in the later stages of plant defense responses. We also found that \textit{BIG-24.1} expression is strongly induced during the infection of grapevine berries. Interestingly, berries are the main target of \textit{Botrytis} infection in the vineyard. There is only few data on the expression profiles of F-box genes in the literature especially during plant stress responses. \textit{SON1} that regulates defense response in \textit{Arabidopsis} is constitutively expressed in wild type plants and is not up-regulated after pathogen infection [22]. Based on mutant analysis, it has been shown that \textit{Arabidopsis COI1} and its soybean and tomato homologs GmCOI1 and JAI1 are involved in jasmonate-signaled defense responses [8, 45]. Unfortunately no consistent data have been published on the expression of these genes following pathogen infection in wild type plants. Recently, it has been demonstrated that \textit{ACIF1} was quickly induced (30 min) in tobacco and tomato cell cultures and leaves.
challenged with Avr9 elicitor or after wounding but there is no data about its expression during pathogen infection [42]. To our knowledge, our data are the first to provide a comprehensive picture of the expression profile of an F-box gene under biotic stress conditions in plants.

The expression profile of BIG-24.1 after B. cinerea infection raised the question of its link with general stress response. To address this question, we analysed BIG-24.1 expression after challenge of grapevine cells or leaves by various biotic and abiotic stresses. UV-C, cold treatment and wounding have been reported to be effective inducers of defense responses in grapevine [1, 5, 11]. Pseudomonas syringae pv. pisi is a non-host pathogen of grapevine known to induce a characteristic hypersensitive-like response [35, 36]. Burkholderia phytofirmans strain PsJN is an endophytic bacterium that promotes growth and induces resistance in grapevine [10]. We also monitored BIG-24.1 expression in response to rhamnolipids. Rhamnolipids were recently characterized as MAMPs recognized by grapevine cells [43]. Interestingly, all treatments except cold significantly increased BIG-24.1 expression suggesting that BIG-24.1 regulation could be linked to non-specific stress signalling. We can hypothesized that BIG-24.1 could be a general component involved in several defense pathways and thus could be a point of convergence in stress signalling networks in grapevine. In agreement with this hypothesis, we identified some putative defense elements in the BIG-24.1 promoter region like as-1 motifs and W-box elements. As-1 region is known to be stimulated differentially by biotic and abiotic stresses and is involved in transcriptional activation of several genes by auxin and/or SA and MeJA [28, 34]. W-boxes are involved in SA, wounding and pathogen-induced pathways [9, 31]. The presence of two TC-rich repeats, related to stress and plant or SA and MeJA [28, 34]. W-boxes are involved in SA, wounding and pathogen-induced pathways [9, 31]. The presence of two TC-rich repeats, related to stress and plant defense [23] reinforces the idea of a strong regulation of BIG-24.1 by multiple stress stimuli. Interestingly, transcriptome analysis revealed that the Arabidopsis gene ortholog to BIG-24.1, At1g23390 also seems to be involved in general stress responses. At1g23390 is induced during rehydration process following dehydration [30] or following abiotic stress in plants overexpressing the stress-tolerance-related transcription factor AtbZIP60 [14]. Transcriptome data available on PathoPlant® databases (http://www.pathoplant.de/) showed that At1g23390 is also up-regulated after Phytophthora infestans, Pseudomonas syringae pv. phaseolicola, Xanthomonas campestris or tobacco mosaic virus infections. Surprisingly, the gene seems to be down-regulated following B. cinerea infection. Pseudomonas syringae pv. tomato also repress At1g23390 expression. F-box proteins are key factors involved in phytohormones signalling [41]. In grapevine cells we found that ABA, SA, MeJA, GA and ACC exogenous treatments up-regulated BIG-24.1 expression. Moreover, in addition to W-box and as-1 promoters elements, which are known to be activated by SA and MeJA, we found some GA and ethylene responsive elements in the BIG-24.1 promoter. Altogether our data suggest that BIG-24.1 expression is not specific to the perception of a given phytohormone but rather to many signal molecules which are key factors in plant defense signalling and which are involved in the crosstalk between abiotic and biotic stresses [13].

It is also plausible that BIG-24.1 role might be wider than its potential involvement in plant response to stress and could also be related to development and growth. In agreement with this hypothesis, a recent report showed that the variations of the Arabidopsis ortholog At1g23390 transcripts in 94 accessions correlate with biomass variations and carbon status perturbations [39]. Moreover, phylogenetic analysis revealed that the 3 predicted orthologs of BIG-24.1 in A. thaliana, P. trichocarpa and O. sativa belongs to the AOP clade which contains groups of genes involved in basic biological processes required for plant growth and development [48]. Whether or not BIG-24.1 F-box protein function is related to signalling regulation through protein degradation remains to be demonstrated and functional analysis will be the next step in the understanding of BIG-24.1 gene involvement in defense responses or other physiological processes in grapevine.

Acknowledgments We thank Cathy Hachet and Fanja Rabenoelina for technical support. This work was supported by a fund from Europél’Agro.

References

induced by the endophytic plant growth-promoting rhizobacteria BNyB homology to phytoalexin elicitors. Am J Enol Vitic 55:60–64


