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Research article

Characterization and differential expression analysis of complete coding sequences of *Vitis vinifera* L. sirtuin genes

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ABSTRACT

The sirtuin/Sir2 (Silent information regulator 2) family of NAD$^+$-dependent deacetylases and mono-ADP-ribosyltransferases plays an important role in several cellular processes including gene silencing, cell cycle regulation and life span extension in yeast and animals. Compared to other eukaryotes, plants have relatively fewer *SIR2* related genes encoding only two putative SIR2 family proteins. Recently, two putative sirtuin genes were identified also in the grapevine genome. Starting from the predicted coding sequences present in the database, we have been able to obtain two truly expressed coding sequences from the start to the stop codon for both sirtuin genes that were named *VvSRT1* and *VvSRT2*. The search for the expressed coding sequences was performed by comparing the predicted sequences with the recently available grape RNA seq database with the aim to develop the primers to be used in reverse transcriptase PCR reactions to amplify the genes of interest. Finally, in order to better understand the physiological role of both sirtuins, we investigated the expression of these genes in young leaves, mature leaves, and berries sampled at different growing stages. In leaves, usually it has been observed that *VvSRT1* is less expresses than *VvSRT2*, moreover in young leaves *VvSRT2* showed the higher expression during setting while in mature leaves during the flowering time. No particular variations have been observed concerning *VvSRT1*. In berries the two genes showed more similar expression level, and they showed the highest expression during the flowering time. Finally, the expression of *VvSRT2* in berries is smaller than in leaves.

1. Introduction

Sir2p, (silent mating-type information regulation 2) is a NAD$^+$-dependent deacetylase enzymes that was discovered in yeast during a genetic screen for genes required for transcriptional silencing of the mating-type loci [1,2]. Further studies demonstrated that Sir2p is a member of a family of enzymes that are ubiquitous and highly conserved throughout all kingdom of life. Analyses of the structure of several sirtuins from different organisms showed the presence of a highly conserved catalytic domain (designated pfam02146) that is composed of a NAD$^+$-binding domain, a Zn$^+$-binding domain, and the catalytic site. Sirtuins transfer the acetyl moiety from acetylated lysine to NAD$^+$ through the cleavage of nicotinamide glycosidic bond and the formation of O-acetyl-ADP-ribose that is a metabolite uniquely generated by sirtuins deacetylases [3,4].

The number of sirtuins varies in different organisms. It seems that this number increases with the complexity of the organism and it ranges from one in bacteria to five in fungi and seven in vertebrates [5,6]. Phylogenetic analyses show that sirtuin family proteins can be divided into five major classes defined by conserved domain aminoacid sequences (i.e., Class I, II, III, IV, and U groups). Classes from I to IV comprise sirtuins from eukaryotic and prokaryotic organisms. In addition, the ClassU comprises sirtuins from prokaryotes [1,5–7].

The functions of sirtuins have been investigated mainly in fungi and vertebrates where they resulted to be connected with several processes such as transcriptional silencing and changes in gene expression levels [8–10], counter ageing [11–13], metabolic regulation and pathogenesis [6,14,15] Nevertheless there are still many substantial gaps to the full understanding of the sirtuin functions [5,6,16]. NAD$^+$ is crucial for the catalytic reaction of sirtuin, and this suggests that they are strictly regulated in relation to the metabolic state of the cell. Thus, changes in the NAD$^+$/NADH ratio in response to metabolic changes can modify sirtuin activity.

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In animal, sirtuins have been implicated in a wide variety of physiological processes and multiple substrates have been identified, including numerous regulatory proteins that trigger resistance to metabolic, oxidative, heat, and hypoxic stress [17]. Taken together all these data suggests that sirtuins can act as a genome stabilizer. Recent observations show the capacity of sirtuins to move to sites of double-strand and single-strand DNA breaks, promoting DNA repair and hence genomic stability [18,19]. Although there are 5 different classes, situins share a conserved catalytic domain, and they exhibit variable amino- and carboxi-terminal extensions that contribute to their unique sub-cellular localization and may also define their substrate specificity, cellular function, and catalytic activity [6,16,20].

Despite the recent explosion in the number of reports on sirtuins from fungi and animals, only few studies can be retrieved from literature that are dealing with plant sirtuins and not much is known about plant sirtuin functions. Compared to other eukaryotes, plants have relatively fewer sirtuin related genes. The analysis of available genomic DNAs (i.e., *Arabidopsis thaliana*, *Oryza sativa*, *Populus trichocarpa*, and so on) allowed identifying only two putative sirtuin family proteins: SRT1, a protein SIRT6/SIRT7-like clustered in class IV, and SRT2, a protein SIRT4-like clustered in class II [5,21–25]. Considering that sirtuin genes could have been selectively and extensively lost during evolution of plants, it appears likely that the loss of individual sirtuins might have been compensated through the acquisition of redundant functions by the remaining sirtuin family members and that plant sirtuins may have a larger spectrum of functions compared to their yeast and animal counterparts. In Arabidopsis, the presence of alternative splicing patterns of *AtSRT2* gene increases the possibility that alternative splicing has provided added diversity of sirtuin functions [25]. During their evolution, plants have acquired a family of deacetylase proteins unique to plants, the HD2 family, which is not found in animals or fungi, so it cannot be excluded that the HD2 family has taken over some of the sirtuin functions [23,25].

In a study carried out with the aim to analyze the role of histone deacetylase genes in Arabidopsis development, it was observed that the two sirtuin family members exhibit very different expression profiles, in contrast with the deacetylases of the other families, and may act in different tissues, stages and processes. Specifically, *AtSRT1* results to be highly expressed in reproductive tissues, while *AtSRT2* results to be globally expressed in all tissues [23,26].

Treatment with sirtinol (a specific inhibitor of sirtuins) inhibits body-axis formation and vascularization in Arabidopsis seedlings and these phenotypes resemble those of some mutants defective in auxin signalling. Therefore SRT1 and SRT2 might have a role in auxin signalling [23,27]. Other evidences suggest that *AtSRT2* negatively regulates plant basal defence against the pathogen *Pseudomonas syringae* and its expression is down-regulated in response to the infection. Further, plants having *AtSRT2* silenced show an enhanced resistance to the infection and have a higher expression of gene *PR1* (Pathogenesis Related gene 1). In contrast, plants having an over-expression of *AtSRT2* result hyper-susceptible to the pathogen [28]. Recently Bond et al. [29] showed that a mutation in AtSRT2 gene affects the Arabidopsis vernalization response.

In rice (*O. sativa*) the *OsSRT1* gene is widely expressed in active cell dividing organs and tissues. Down-regulation of *OsSRT1* by RNAi induces lesions mimicking cell death and early senescence, while overexpression induces tolerance to oxidative stress. Genes involved in control apoptosis and transposons shuffle are deregulated. These data suggest that *OsSRT1* is involved in the safeguard against genome instability and oxidative stress required for plant cell growth [24].

The National Centre for Biotechnology Information (NCBI) Entrez nucleotide database contains two GNOMON-predicted grapevine sirtuin gene sequences (i.e., *SRT1* (XM_002265801) and *SRT2* (XM_002274418 and CBI21603)) [30,31].

Direct evidence of transcription of these genes can be obtained by screening the recently available grape RNA-Seq database for berry development in three different developmental stages [32]. Further evidences are present in other studies [21,33]. Considering that the function of Sir2 family in plants are poorly understood, the aims of this study were both the first experimental characterization of the full length coding sequence for the two genes and the analysis of their expression levels in several leaf and berry developmental stages.

2. Results

2.1. Experimental evidence of Vitis vinifera L. sirtuin coding sequences

Previously, we amplified and sequenced a fragment of the two genes using the primers developed by Aquea et al. [21]. A high correspondence (i.e., almost a perfect match) was obtained with the predicted sequences. Starting from these results, we considered the possibility to screen short region, from 15 to 20 bases in length, of the predicted sequences, against the RNA-Seq database, in order to find proofs of expression for the truly spliced sirtuin mRNAs. Several positive reads were found in the different experiments available online and some of them were then considered for the development of primers useful to amplify the experimental coding sequence. Using the positive reads we reconstructed, for each gene, two really expressed 5′ and 3′ ends containing the start and the stop codons (Fig. 1). The experiments of Reverse Transcriptase PCR were carried out on a *V. vinifera* RNA extracted from samples taken during different developmental stages.

The results of the Reverse Transcriptase PCR have been reported (Fig. 2). The fragments have been recovered from the gel, cloned and sequenced. Fragments of the expected size have been obtained in leaves and berries for all the growing stage considered.

2.1.1. VvSRT1 (A.N. JN252254)

The length of the experimental sequence was 1467 bp (1404 bp of coding sequence and 63 bp of untranslated regions). In addition, the nucleotide sequence and the intron/exon structure was in agreement with the computationally predicted coding sequence. The two sequences differed only in 4 nucleotides. These differences are very likely varietal polymorphisms. In fact, we used the DNA of a “Merlot” cultivar while the already mentioned PN40024 reference genome sequence is from DNA extracted from “Pinot noir” cultivar. These codon changes are translated in two different amino acids out of the 467 amino acids of the full predicted protein. The first change was inside the sirtuin conserved domain in close proximity to the NAD+ and substrate binding sites while the second change was outside the conserved domain at the C-terminal end of the protein.

The gene maps on chromosome 19 and it is structured in 14 exons and 13 introns spanning a region of circa 22 kb (Fig. 3). The coding sequence is on the positive strand. The resulting protein is of 467 aa. An analysis of the protein sequence allow to highlight the presence of the typical features of sirtuins superfamily (Fig. 3). The 3′end of the gene has been confirmed also by means of 3′RACE. A WolfPSORT prediction of the sub-cellular localization showed that the protein had the main probabilities to be localized inside the nucleus (score of 5.0) followed by chloroplast (score of 3.0).

2.1.2. VvSRT2 (A.N. JN252255)

The length of the experimental sequence was 1273 bp (1149 bp of coding sequence and 124 bp of Untranslated Regions). The experimental sequence was in agreement with the computational