



Research article

## *In silico* analysis of sirtuin-type histone deacetylase genes in sugar beet (*Beta vulgaris* L.)

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### Abstract

Histone deacetylase (HDAC) enzymes catalyze the removal of an acetyl group from the lysine residues of histone N-terminal tails, and they repress gene transcription through condensation of chromatin. In plants, the sirtuins/silent information regulator 2 (SIR2) proteins which are NAD<sup>+</sup>-dependent deacetylases, have been identified in distinct plant species such as *Arabidopsis*, rice, tomato, soybean, maize, etc., but little is known about their functions in plants. They are mainly investigated in *Arabidopsis* and rice and found to be involved in H3K9 acetylation, metabolic pathways, repression of genes associated with stress response, and energy metabolism. A total of eight *RPD3/HDA1* family HDAC genes have been recently identified in the sugar beet (*Beta vulgaris* L.) genome. However, *B. vulgaris* SIR2-type HDACs have not yet been identified and characterized. In this work, an *in silico* analysis of SIR2 family members was performed in sugar beet. Three SIR2 family HDACs were identified from the sugar beet genome, named BvSRT1, BvSRT2, and BvSRT3. The beet SIR2 gene family is found to be located on chromosomes 4, and 9. The phylogenetic tree building with *B. vulgaris*, *Arabidopsis*, tomato, soybean, *Vitis vinifera*, pepper, rice, maize, and *Sorghum bicolor* showed that 3 sugar beet SRTs were divided into two classes: Class II (BvSRT2) and IV (BvSRT1 and BvSRT3). SIR2 family proteins consisted of SIR2 domain (PF02146). The conserved motifs ranged from 6 to 50 amino acids, while the intron-exon numbers of genes ranged from 10 to 14. *BvSRT1* and *BvSRT3* exhibited similar motif distributions and exon/intron structures. Moreover, nuclear, and cytoplasmic localization of BvSRT1 and BvSRT3 has been predicted. BvSRT2 protein was located on the mitochondrion. Analysis of *cis*-elements revealed the involvement of *BvSRT* genes in hormone regulation, light response, abiotic stress response, and meristem expression. This study may shed light on the potential role of SIR2-type HDACs in beets.

**Keywords:** *Beta vulgaris*; histone deacetylase (HDAC); *in silico* analysis; sirtuins; SIR2; sugar beet

### 1. Introduction

Epigenetic modifications including histone acetylation, methylation, phosphorylation, DNA methylation, and RNA interference (RNAi) in eukaryotes regulate numerous cellular processes through gene activation or repression (Salgotra and Gupta, 2019). Deacetylation of the N<sup>ε</sup>-acyl-lysine residues converts neutral acetylated lysine residues to positively charged lysine residues, resulting in a strong interaction with the DNA, and inactivation of gene transcription (Zhao et al., 2018; Perrella et al., 2024). This process is catalyzed by two types of

deacetylases: Zn<sup>2+</sup>-containing deacetylases (Zhao et al., 2018), and sirtuins/silent information regulator 2 (SIR2) (Chen et al., 2015; Zhao et al., 2018). The sirtuins/SIR2 are β-NAD<sup>+</sup>-dependent deacetylase enzymes for histone or non-histone proteins that was discovered in yeast for the first time (Imai et al., 2000). Sirtuin family members have been found in different organisms such as fungi, mammals, human parasites, and plants (Greiss and Gartner, 2009). Yeast and mouse SIR2 proteins catalyze deacetylation of lysine 9 and 14 at histone H3, and H4 lysine 16. Seven sirtuins (SIRT1-7) in mammals have NAD<sup>+</sup>-binding catalytic domain and differ in subcellular localization.

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Their functions are associated with metabolism, senescence, antioxidant protection, apoptosis, tumor suppression, and regulation of cell cycle (Houtkooper et al., 2012; Carafa et al., 2016; Ziętara et al., 2023). Mammal SIRT1, SIRT6, and SIRT7 exist in the nucleus, while SIRT2 is located in the cytoplasm and nucleus. SIRT3, SIRT4, and SIRT5 are present in mitochondria (Carafa et al., 2016). The SIRT4 controls glutamine metabolism and therefore inhibits cell proliferation (Jeong et al., 2013). Similarly, in a very recent work, *Arabidopsis* sirtuins were found to function in cell proliferation by a decline in NAD-glutamate dehydrogenase (GDH) activity (Bruscalupi et al., 2023). Although the functions of animal sirtuins are known, which are involved in energy metabolism, lifetime regulation, apoptosis, proliferation, DNA repair, and stress tolerance, little is known about the roles of plant sirtuins (Houtkooper et al., 2012; Bruscalupi et al., 2023). In plants, they have been mostly studied in *Arabidopsis* and rice (*Oryza sativa*) (König et al., 2014; Zhang et al., 2017; Zheng, 2020). Up to date, SIR2 proteins have been identified in distinct plant species including *Arabidopsis* (Pandey et al., 2002), *O. sativa* (Huang et al., 2007; Zhang et al., 2016; Zhang et al., 2017), *Solanum lycopersicum* (Zhao et al., 2014), *Glycine max* (Yang et al., 2018), *Litchi chinensis* (Peng et al., 2017), *Zea mays* (Zhang et al., 2020), *Triticum aestivum* (Shu et al., 2021), *Capsicum annuum* (Cai et al., 2022), *Vitis vinifera* (Busconi et al., 2009; Aquea et al., 2010), *Fagopyrum tataricum* (Yan et al., 2023), *Sorghum bicolor* (Du et al., 2022), and *Camellia sinensis* (tea plant) (Yuan et al., 2020). Most of these plant sirtuins have been recently identified, and their functions are not well-studied. Phylogenetic analysis revealed that the mammalian sirtuin proteins are divided into four classes (class I-IV), and they have additional functions along with Lys acetylation. For instance, mammalian class III sirtuin, SIRT5 has Lys succinylase and demalonylase activities (Du et al., 2011). *Arabidopsis* genome includes only class IV and class II sirtuins, Silent Information Regulator1 homolog SRT1 (At5g55760) and SRT2 (At5g09230), respectively (Pandey et al., 2002). SRT2 in *Arabidopsis* is a mitochondrial protein (König et al., 2014). Similar to mammals, the subcellular localization of SIR2s in plants was found in the nucleus, mitochondria, chloroplast, and cytosol (Pandey et al., 2002; Martínez-Redondo and Vaquero, 2013; Zheng, 2020). *Arabidopsis* SRT1 (AtSRT1) was reported to interact with cMyc-Binding Protein 1 (AtMBP-1), a truncated version of the cytosolic glycolytic enolase gene *LOS2/ENO2*. Moreover, plant sirtuins use non-histone proteins as substrates, such as AtMBP-1, and OsGAPDH1, suggesting that SIR2 proteins play a key role in different cellular processes at various organs, tissues, and developmental stages. The *AtSRT1* declined the levels of H3K9 acetylation in the *LOS2/ENO2* and *STZ/ZAT10* that both encode stress regulators (Peng et al., 2017). Yeast SIR2 homolog, *Arabidopsis* SRT2 (AtSRT2) which is localized at the inner mitochondrial membrane, is associated with some proteins related to energy metabolism and metabolite transport including the ATP/ADP carriers and ATP synthase, suggesting the involvement of SRT2 protein in mitochondrial energy metabolism. The *srt2* mutants exhibited alterations in the amounts of sugar, amino acid, and ADP (König et al., 2014). Furthermore, AtSRT2 was found to regulate basal defense in response to a bacterial pathogen, *Pseudomonas syringae* pv. *tomato* DC3000 (Chunzheng et al., 2010). In *Arabidopsis*, *srt2* knock-out lines showed increments in the expression of *salicylic acid-related* (*PAD4*, *EDS5*, *SID2*), and *pathogenesis-related 1* (*PR1*) genes in contrast to *AtSRT2* overexpressor lines. In *O.*

*sativa*, OsSRT1 is not only involved in the deacetylation of histone H3 lysine 9 (H3K9) but also inhibits transcription of *glyceraldehyde-3-phosphatedehydrogenase* (*GAPDH*), indicating that the OsSRT1 decreases glycolysis (Zhang et al., 2017). In a recent study, in an ornamental woody plant (*Prunus mume*), 2 SIR2 family members (*PmSRT1*, and *PmSRT2*) were found among 13 *PmHDAC* genes, and they significantly responded to cold stress after 12 hours, and their expression levels varied according to geographical environmental factors or cold-resistance levels in individuals (Meng et al., 2022).

Sugar beet (*Beta vulgaris* L.), a member of the Amaranthaceae family, is a diploid (2n=18) crop grown in temperate and subtropical climates (Dohm et al., 2014). It is used for the production of different materials, such as sugar, bioethanol, animal feed, health-promoting foods, and raw materials for health (Hoffmann, 2010; Yu et al., 2020; Yolcu et al., 2022). Sugar beet is an economically important crop species that is tolerant to salt and drought (Wedeking et al., 2016; Fotouhi et al., 2017). Therefore, sugar beet growth in lands unsuitable for agriculture is possible if plant breeders develop highly stress-tolerant sugar beet varieties (Zhang et al., 2021). However, sugar production and beet development are adversely affected by climate change (Zhang et al., 2021). Identification of *B-box* (*BBX*) genes, *BRASSINAZOLE-RESISTANT* (*BZR*) family genes, and *high-affinity K<sup>+</sup>-transporter* (*HAKs*) genes in sugar beet has been performed at the genome level through *in silico* methods (Wang et al., 2019; Yang et al., 2022; Song et al., 2023). Eight RPD3/HDA1 family members of histone deacetylase-encoding genes (*HDACs*) and seven histone acetyltransferase-encoding genes (*HATs*) in *B. vulgaris* have been recently identified and characterized through bioinformatics tools and databases (Yu et al., 2023; Yolcu et al., 2024). However, there are no reports on the *sirtuin-type HDAC* gene family in sugar beet. Importantly, there are few research articles related to the effects of epigenetic modifications on gene expression in *B. vulgaris* under stress applications (Yolcu et al., 2016; Skorupa et al., 2021). Thus, epigenetic regulation of biological processes and the roles of histone modifiers remain elusive in sugar beet.

This study performed an *in silico* analysis of *B. vulgaris* SIR2 family members by using bioinformatics tools and databases and examined their physicochemical properties, gene structure, distribution of motifs, prediction of subcellular localization, phylogenetic analysis, promoter *cis*-acting regulatory elements, and protein 3D structure.

## 2. Materials and methods

### 2.1. Identification of SIR2 subfamily members in *B. vulgaris*

A total of 2 SIR2 protein sequences from *Arabidopsis* and 2 sequences from rice were retrieved from the TAIR (Reiser et al., 2024), and Ensembl Plants (Bolser et al., 2017; Ensembl, 2017) databases, and then used to search SIR2s with the BLASTP tool using the sugar beet genome (*Beta vulgaris* ssp. *vulgaris* EL10.2\_2, Phytozome genome ID: 782, NCBI taxonomy ID: 3555) in Phytozome version 13 (Goodstein et al., 2012). All homologous protein sequences of the BvSRT candidates are accepted if they have the sequence identity with *Arabidopsis* and rice SRT proteins of more than 50% and  $e < 10^{-10}$ . SIR2 domain (PF02146) of *B. vulgaris* candidate proteins were confirmed by SMART (Letunic et al., 2012; Letunic and Bork, 2020) and NCBI CDD (Wang et al., 2022) databases.

Physicochemical properties of three BvSRT proteins such as isoelectric point (pI), theoretical molecular weight (MW), and GRAVY (grand average of hydrophathy) were predicted online (Gasteiger et al., 2005).

## 2.2. Prediction of BvSRT subcellular localization

Subcellular localization predictions of beet SRT proteins were carried out using two online predictors including CELLO server (Yu et al., 2006), and WoLFPSORT (Nakai and Horton, 1999).

## 2.3. Conserved protein motifs and phylogenetic analysis

The motifs of the BvSRT proteins were analyzed by MEME Version 5.5.2 (meme-suite.org/tools/meme) (Bailey and Elkan, 1994), with the maximum number of motifs set to 10. To understand the evolutionary relationship of BvSRTs with other SRTs, a total of 21 SRT protein sequences in a variety of plant species such as *A. thaliana* (*At*), *O. sativa* (*Os*), *S. lycopersicum* (*Sl*), *G. max* (*Gm*), *Z. mays*, *C. annuum* (*Ca*), *V. vinifera*, and *S. bicolor* (*Sb*) (Table 1) were obtained from National Center for Biotechnology Information (NCBI) and Ensembl Plants to perform a phylogenetic analysis. A phylogenetic tree was constructed by MEGA11 using the maximum likelihood method, with 1000 bootstrap replicates (Tamura et al., 2021).

## 2.4. 3D structures of HAT proteins in B. vulgaris

Three-dimensional structure models have been constructed using the Protein Homology/Analogy Recognition Engine V 2.0 (Phyre2) server (Kelley et al., 2015). The amino acid sequences were used to visualize predicted 3D structures of BvSRT proteins using intensive mood.

## 2.5. Chromosomal locations, and gene structure

The physical locations of the *BvSRT* genes along each chromosome have been retrieved from the sugar beet genome (Phytozome 13) and the chromosomal distribution graph was drawn by Mapgene2chrom 2.1 (MG2C v2.1) online tool (Jiangtao et al., 2015; Chao et al., 2021).

Gene Structure Display Server (GSDS) (Hu et al., 2015) was used to analyze the exon-intron structures of the *BvSRTs*.

## 2.6. Genomic synteny analysis

Genomic synteny was comparatively done to examine the relationship between sugar beet, rice, tomato, and *Arabidopsis* using the Circoletto program (Circos) (Krzywinski et al., 2009). Score/max ratio was used coloring with blue $\leq$ 0.25, green $\leq$ 0.50, orange $\leq$ 0.75, red $>$ 0.75. Three SIR2 proteins from *B. vulgaris* and six SRTs from *Arabidopsis*, tomato, and rice in FASTA format were used as query and database files, respectively.

## 2.7. Analysis of cis-acting regulatory elements and heatmap construction

The sequences 1500 bp upstream of the transcription start site (TSS) were extracted from the sugar beet genome using by Phytozome database. The numbers and types of *cis*-elements were predicted by PlantCARE software (Lescot et al., 2002).

TBtools was used to construct heatmap depending on functional classification of *cis*-elements (Chen et al., 2020).

## 3. Results

### 3.1. Identification of SIR2 genes in B. vulgaris

The protein sequences of *A. thaliana* and *O. sativa* SRTs were obtained from TAIR, and Phytozome, and then these queries were used to search SRT proteins in *B. vulgaris* genome through BLASTP in Phytozome 13. A total of 3 SRTs have been identified in *B. vulgaris* and have been named according to their positions on the 9 chromosomes of the beet. The physicochemical properties of SIR2s such as chromosome location, strand, CDS (bp), amino acid length (aa), molecular weight (MW), isoelectric points (pI), and grand average of hydrophaticity (GRAVY) were indicated in Table 1. The BvSRT1, BvSRT2, BvSRT3 protein lengths were 497, 390, and 458 aa, respectively. The predicted MWs were 55.57, 43.31, and 51.46 kDa, while the pI was 9.24, 8.78, and 8.31. The highest MW, CDS, and amino acid lengths were reported in BvSRT1. Previously known sirtuin proteins in different plants such as *Arabidopsis*, *O. sativa*, *S. lycopersicum*, *G. max*, *Z. mays*, *C. annuum*, *T. aestivum*, *V. vinifera*, *S. bicolor*, *C. sinensis*, *F. tataricum* and *L. chinensis* were presented in Table 2.

### 3.2. Subcellular localization

Bioinformatics tools have been extensively used to predict subcellular localization of proteins. Present study includes only *in silico* approach. Two online predictors (cello-life, and WoLFPSORT) were used to predict subcellular localization of BvSRTs, which are presented in Table 3. WoLFPSORT is a subcellular location predictor based on known sorting signal motifs and amino acid contents (Nakai and Horton, 1999). Except for BvSRT2, two SRT proteins were assumed to be localized in nucleus or cytoplasm according to cello-life tool. Consistent with the *Arabidopsis* SRT2 protein, BvSRT2 was predicted to be present in the mitochondrion (Pandey et al., 2002). Consistent with cello-life results, WoLFPSORT also showed the cytoplasmic and nuclear localization of BvSRT3 (Table 3). The subcellular localizations of BvSRT1 and BvSRT2 were found in the peroxisome and mitochondrion with high frequencies, respectively (Table 3).

### 3.3. Phylogenetic relationships and determination of conserved motifs in SRT proteins

The phylogenetic relationships between BvSRTs and other SRTs from various plant species, such as *A. thaliana* (*At*), *O. sativa* (*Os*), *S. bicolor* (*Sb*), *V. vinifera*, *S. lycopersicum* (*Sl*), *G. max* (*Gm*), *Z. mays*, *C. annuum* (*Ca*) were shown in Fig. 1. According to the phylogenetic analysis, 3 SRT proteins in *B. vulgaris* were divided into two classes: Class II (BvSRT2) and IV (BvSRT1 and BvSRT3). The BvSRT1 protein was closely related to AtSRT1, and clustered together with SRT6901 (*V. vinifera* SRT1), GmSRT3, and GmSRT4. Another class IV member, BvSRT3 was present at the same group with OsSRT1, GRMZM2G058573 (*Z. mays* SRT), and SbSRT1. BvSRT2 was grouped in Class II, and clustered with GmSRT1, GmSRT2, SRT6902 (*V. vinifera* SRT2), AtSRT2, CaSRT2, and SISRT2.

MEME analysis which was carried out to study the diversity of SIR2 protein structures, identified 10 motifs in

**Table 1**

The physicochemical properties of SIR2-type HDAC genes and SIR2 proteins in *Beta vulgaris*.

Phytozome Sequence ID	Gene name	Chromosome location	Strand	CDS (bp)	Length (aa)	MW (kDa)	pI	GRAVY
Bevul.4G007100	<i>BvSRT1</i>	Chr4: 802701-812273	Reverse	1491	497	55.57	9.24	-0.220
Bevul.4G180100	<i>BvSRT2</i>	Chr4: 56999487-57004409	Reverse	1170	390	43.31	8.78	-0.272
Bevul.9G035800	<i>BvSRT3</i>	Chr9: 6905704-6919466	Forward	1374	458	51.46	8.31	-0.149

**Table 2**

Previously identified sirtuin proteins in different plants, such as *A. thaliana*, *O. sativa*, *S. lycopersicum*, *G. max*, *Z. mays*, *C. annuum*, *T. aestivum*, *V. vinifera*, *S. bicolor*, *C. sinensis*, *F. tataricum* and *L. chinensis*.

Plant species	SIR2 proteins	Reference(s)
<i>A. thaliana</i>	SRT1, SRT2	(Pandey et al., 2002) (Huang et al., 2007;
<i>O. sativa</i>	SRT1, SRT2	Zhang et al., 2016; Zhang et al., 2017)
<i>S. lycopersicum</i>	SRT1, SRT2	(Zhao et al., 2014)
<i>G. max</i>	SRT1, SRT2, SRT3, SRT4	(Yang et al., 2018)
<i>Z. mays</i>	GRMZM2G058573, GRMZM5G807054	(Zhang et al., 2020)
<i>C. annuum</i>	CaSRT1, CaSRT2	(Cai et al., 2022)
<i>T. aestivum</i>	TaSRT1A, TaSRT1B, TaSRT1D, TaSRT2A, TaSRT2D, TaSRT2U	(Shu et al., 2021)
<i>V. vinifera</i>	SRT6901, SRT6902	(Aquea et al., 2010)
<i>S. bicolor</i>	SbSRT1, SbSRT2	(Du et al., 2022)
<i>C. sinensis</i>	CsSRT1, CsSRT2, CsSRT3, CsSRT4	(Yuan et al., 2020)
<i>F. tataricum</i>	FtSRT1, FtSRT2	(Yan et al., 2023)
<i>L. chinensis</i>	LcSRT1, LcSRT2	(Peng et al., 2017)

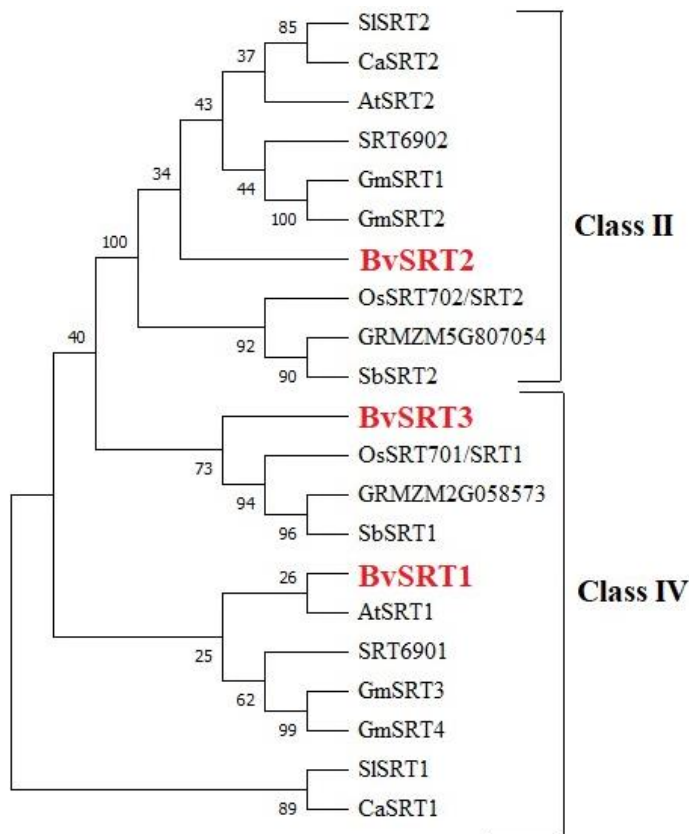
**Table 3**

Predicted subcellular localization of *B. vulgaris* SRT proteins. Two online prediction tools such as cello-life, and WoLFPSORT were used to investigate the possible subcellular localization of BvSRTs.

Protein	Subcellular localization	
	cello-life	WoLF PSORT
<b>BvSRT1</b>	Nuclear/cytoplasmic	pero(11), cyto(2)
<b>BvSRT2</b>	Mitochondrial	mito(7), chlo(3)
<b>BvSRT3</b>	Cytoplasmic/nuclear	cyto(7), nucl(2)

\*cyto: cytosol, nucl: nucleus, chlo: chloroplast, mito: mitochondrium, pero: peroxisome

BvSRT proteins (Fig. 2). Amino acid lengths of conserved domains ranged from 6 to 50. Identical motifs including motifs 1-10 (except for motif 9) were found in Class IV proteins, BvSRT1 and BvSRT3, suggesting that these proteins may possess similar functions. Only BvSRT2 and BvSRT3 contained motif 9. There are fewer motifs in BvSRT2 as compared to BvSRT1 and BvSRT3 proteins. The BvSRT2 comprised three motifs: 1, 2 and 9 (Fig. 2). All SRT proteins contained motif 1

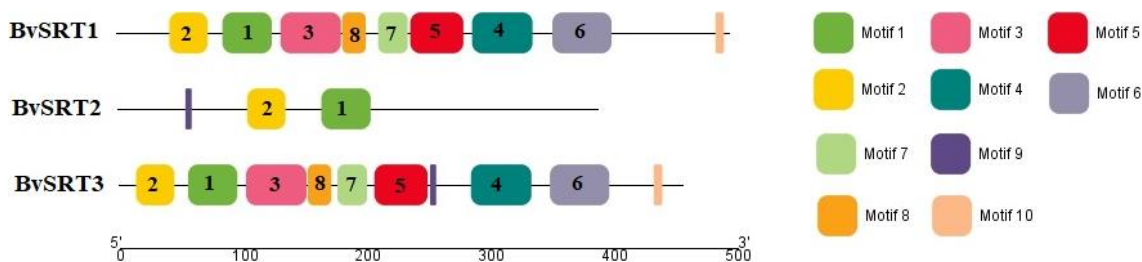


**Fig. 1.** Phylogenetic tree of SIR2 proteins in different plant species. Maximum Likelihood method and Poisson correction model were used to generate the phylogenetic tree (1000 bootstrap replicates) based on multiple alignments with ClustalW. The analysis contains 21 amino acid sequences from *B. vulgaris* (*Bv*), *A. thaliana* (*At*), *O. sativa* (*Os*), *S. bicolor* (*Sb*), *G. max*, *V. vinifera*, *C. annuum* (*Ca*), *Z. mays* and *S. lycopersicum* (*Sl*).

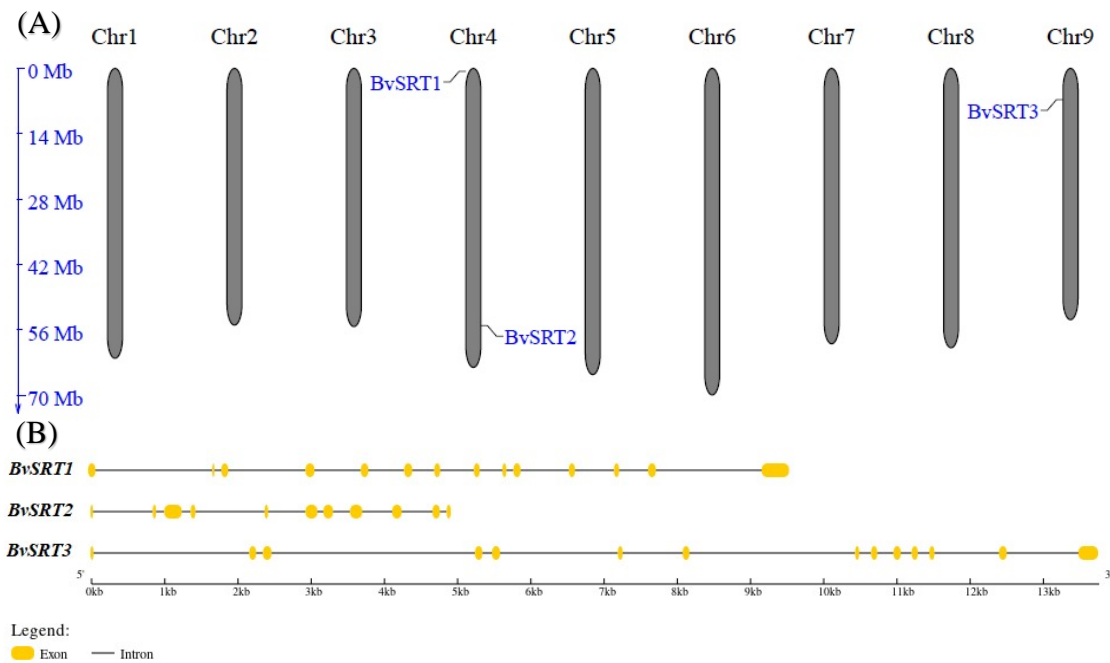
and 2, which are sirtuin\_cat domains (PS50305). Motif 3 exists in BvSRT1 and BvSRT3 is a DHS-like\_NAD/FAD-binding domain (IPR029035), which is catalytic domain of sirtuin family.

### 3.4. Chromosomal distribution, and gene structures

To investigate the chromosomal distribution of the *BvSRT*



**Fig. 2.** Motif analysis of the SIR2-type HDAC proteins in *B. vulgaris*. The MEME online tool and TBtools were used to analyze and construct the domains. Distinct motifs are indicated by different colors and numbers.



**Fig. 3.** Chromosomal distribution and structure of *BvSRT* genes. **(A)** Chromosomal positions of three *SRT* genes in sugar beet genome generated in MG2C tool. The number of the chromosomes is displayed at the top of each chromosome. The genome-scale in megabases (Mb) is given on the left. **(B)** Gene structure analyses of the *BvSRTs* performed in the GSDS 2.0 tool. Exons and introns are indicated by yellow boxes, and black lines, respectively. Kb: kilobases.

genes, they are mapped on the chromosomes by using information from the sugar beet genomic database. The sugar beet *SIR2* gene family is found to be dispersed on chromosomes 4, and 9 (Fig. 3A). Two genes belonging to Class IV and Class II, *BvSRT1* and *BvSRT2* located on chromosome 4. No genes were found on chromosomes 1, 2, 3, 5, 6, 7, and 8.

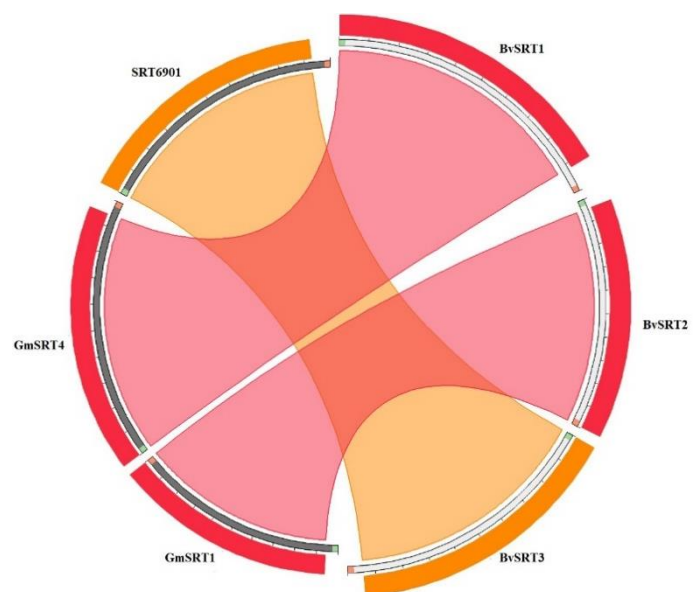
Structures of *BvSRT* genes are indicated in Fig. 3B. The intron-exon numbers ranged from 10 to 14. In *BvSRT2*, there are ten introns, and 11 exons, while *BvSRT1* and *BvSRT3* both have 13 introns and 14 exons. *BvSRT1* and *BvSRT3* exhibited similar exon/intron structures and identical numbers. Taken together, the intron-exon distribution was conserved in Class IV members.

### 3.5. Genomic synteny results

Circoletto results (Darzentas, 2010) used to investigate the evolutionary relationship between the SRTs of sugar beets and other plant species such as *Arabidopsis*, *Oryza sativa*, *Sorghum bicolor*, *Glycine max*, *Vitis vinifera*, *Capsicum annuum*, *Zea mays*, and *Solanum lycopersicum* were shown in Fig. 4. The red and orange colors exhibit the level of evolutionary conservation among *SIR2* proteins. The tool used “score/max” ratio colouring with orange  $\leq 0.75$ , and red  $> 0.75$ . It has been found that *BvSRT1* showed synteny with *GmSRT4*, and the sequence similarity was greater than 75%. *BvSRT2* had syntenic relationships with *GmSRT1*, while the *BvSRT3* possessed synteny with *SRT6901* (*Vitis vinifera* SRT). The lowest similarity was demonstrated in *BvSRT3*-*SRT6901* pairs.

### 3.6. Cis-elements in promoter regions

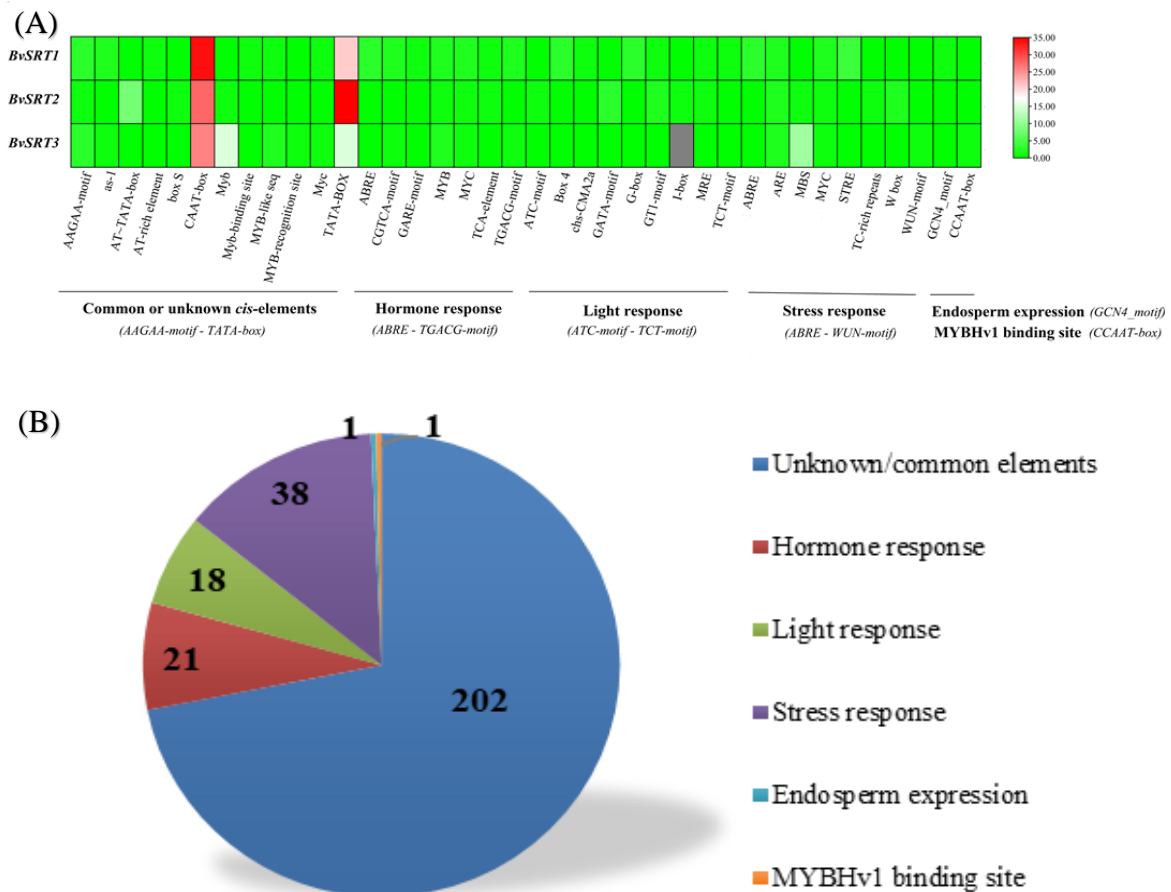
To predict functional characteristics of *BvSRT* genes, *cis elements* in promoters were analyzed by searching the 1500 bp upstream region of the transcriptional activation site.



**Fig. 4.** Representation of genomic synteny in various plant species such as *B. vulgaris*, *A. thaliana*, *S. lycopersicum*, and *O. sativa* identifying the level of conservation at the amino acid sequence level in 2 colors. The red and orange colors exhibit the level and intensity of evolutionary conservation among *SIR2* proteins. The maximum intensity between proteins is shown in orange color.

PlantCARE results showed 36 types of *cis*-acting elements, which were classified into six different groups: common or unknown promoter elements (12), hormone response (7), light response (9), stress response (9), endosperm expression (1) and MYBHv1-binding (1) (Fig. 5A, 5B). There were 276 *cis*-acting elements in total: light (ATC-motif, Box 4, chs-CMA2a, GATA-motif, G-box, GT1-motif, I-box, MRE, TCT-motif), abscisic acid (ABRE, MYC, MYB), gibberellin/methyl jasmonate (MeJA) (GARE-motif, TGACG-motif, CGTCA-motif), salicy-



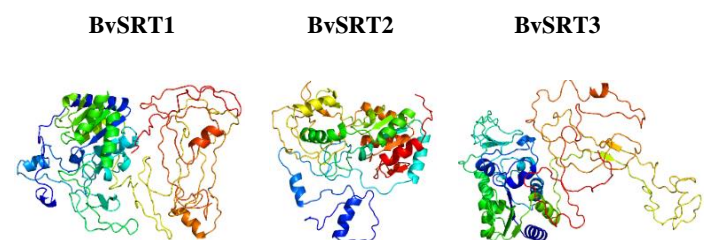


**Fig. 5.** (A) The heatmap demonstrates all *cis*-acting regulatory elements that are found in the promoter regions of *BvSRT1*, *BvSRT2*, and *BvSRT3* genes. (B) Total numbers and functions of *cis*-acting regulatory elements related to promoter regions, hormone response, light-responsiveness, stress-response, endosperm expression, and MYBHv1 binding, which were predicted by PlantCARE software.

lic acid (TCA-element), drought (ABRE, MYB, MYC, MBS) and stress (TC-rich repeats, STRE) response elements, common or unknown promoter elements (AAGAA-motif, as-1, AT~TATA box, AT-rich element, box S, CAAT-box, Myb, Myb-binding site, MYB-like seq, MYB-recognition site, Myc, TATA-box) (Fig. 5A, 5B). The largest group was common or unknown *cis*-elements with 202 members, and the second largest group was stress response-related elements. All *SIR2* genes in *B. vulgaris* contained three stress response-related elements including ARE, MYC, and MYB. MYC is involved in dehydration and ABA response. However, the abscisic acid response element (ABRE) and stress-response element (STRE) were both found only in the *BvSRT1* promoter. TC rich repeats and W box were both present only in the promoter of the *BvSRT2* gene. Two types of *cis*-elements involved in MeJA response (TGACG-motif, CGTCA-motif) existed in *BvSRT1* and *BvSRT3* genes. Wound responsive element, WUN-motif was assumed to be contained in the *BvSRT3*. Hormone-specific *cis*-elements such as GARE-motif (gibberellin), and TCA-element (salicylic acid) existed in the *BvSRT1* promoter. Light response-specific elements, ATC-motif, GATA-motif, and I-box in *BvSRT2* promoter were recorded. The *cis*-regulatory elements that are involved in the light response (Box-4, G-box, TCT-motif) were found in the promoter region of the *SRT1* in *B. vulgaris*. Furthermore, there was only one *cis* element (GCN4\_motif) in *BvSRT1* involved in endosperm expression. MYBHv1 binding site, CCAAT-box was present only in the *BvSRT3* promoter.

### 3.7. Protein 3D structure

A protein bioinformatics tool, Phyre2 has been widely used to predict and analyze protein structures, functions, and mutations (Kelley et al., 2015). 3D models of *BvSRT1*, *BvSRT2*, *BvSRT3* proteins obtained from Phyre2 server were formed with >90% confidence at 54%, 73%, 52% coverage and 53%, 43%, 48% identity, respectively. The 3D protein structure models are shown in Fig. 6. Images are colored by rainbow N → C terminus. The identity and coverage were found 43%, and 75% for *BvSRT2*, respectively. Alpha-helix is the secondary structure element for *BvSRT1* (15%), *BvSRT2* (26%), and *BvSRT3* (14%). The  $\beta$ -strands are distributed by 22% (*BvSRT1*), 10% (*BvSRT2*), and 20% (*BvSRT3*). Templates



**Fig. 6.** Predicted 3D structures of *BvSRT1*, *BvSRT2*, and *BvSRT3* proteins. The protein models were built in the Phyre2 web portal by using amino acid sequences.

used to form 3D structural homology of BvSRT1, 2, 3 include NAD<sup>+</sup>-dependent protein deacetylase sirtuin 2 (c3zg6A, c3pkiF, c3k35D), DHS-like NAD/FAD binding domain (d1y5a1, d1j8fa, d2b4ya1), NAD<sup>+</sup>-dependent deacetylase sirtuin 3 (c3glsC), transcriptional regulatory protein sir2 homolog (c3jwpA), NAD-dependent protein deacetylase sirtuin 4 orthologue, etc.

#### 4. Discussion

Gene activity is controlled by epigenetic mechanisms including DNA methylation, histone modifications and RNA interference (RNAi) in eukaryotic cells and these mechanisms are used by plants to survive under different environmental conditions (Yuan et al., 2013; Salgotra and Gupta, 2019). Histones are highly conserved globular proteins whose N-terminal tails are located at the surface of the nucleosome for post-translational modifications (PTMs) that regulate transcription through controlling the accessibility of the transcriptional machinery to certain genomic regions (Kouzarides, 2007). In eukaryotes, histone deacetylase enzymes (HDACs) remove the acetyl group from the tails of the core histones, and lead to transcriptional repression. Epigenetic regulation of biological processes such as development and stress response in sugar beet is unknown. Furthermore, there are few research articles regarding the roles of histone modifications or histone modifiers in sugar beet cultivars (Yolcu et al., 2016; Yu et al., 2023; Yolcu et al., 2024). Among histone modifier proteins, RPD3/HDA1-type HDACs and HATs have been recently identified and characterized in *B. vulgaris* (Yu et al., 2023; Yolcu et al., 2024). Other histone modifiers associated with histone methylation, and phosphorylation have not yet been identified and characterized in sugar beet. This work performed *in silico* analyses of *B. vulgaris* SIR2 genes by using bioinformatics tools or databases and examined their physiochemical properties, subcellular localization, phylogenetic relationships, gene structure, motif distribution, chromosomal distribution, genomic synteny, promoter *cis*-elements, and protein 3D structure models. Plants have fewer SIR2 family proteins than fungi and animals. Here, 3 SIR2 members were identified in *B. vulgaris*, and they were phylogenetically classified into two classes (Class II and IV). The number of sugar beet SIR2 subfamily is not similar to other plant species. For example, *Arabidopsis*, rice, tomato, maize, pepper, grape, *P. mume*, *Sorghum*, Tartary buckwheat and litchi include only two SIR2 proteins (Pandey et al., 2002; Huang et al., 2007; Aquea et al., 2010; Zhao et al., 2014; Peng et al., 2017; Zhang et al., 2020; Cai et al., 2022; Du et al., 2022; Meng et al., 2022; Yan et al., 2023), while the wheat, tea plant, and soybean contain a total of 6, 4 and 4 SIR2 proteins, respectively (Yang et al., 2018; Yuan et al., 2020; Shu et al., 2021). Like SIR2 proteins from *F. tataricum* (Yan et al., 2023), *S. bicolor* (Du et al., 2022), class IV proteins (BvSRT1 and BvSRT3) have more than 400 amino acids in length, respectively. All SIR2 family proteins in sugar beet contained a SIR2 domain, consistent with SIR2 proteins in distinct plant species, such as *Arabidopsis* (Pandey et al., 2002), *O. sativa* (Zhang et al., 2016), *S. lycopersicum* (Zhao et al., 2014), *Z. mays* (Zhang et al., 2020), *F. tataricum* (Yan et al., 2023), *V. vinifera* (Aquea et al., 2010), *L. chinensis* (Peng et al., 2017), *T. aestivum* (Shu et al., 2021), *C. annuum* (Cai et al., 2022) and *C. sinensis* (Yuan et al., 2020). This suggests that sugar beet SIR2-type HDACs may have similar functions to their homologous genes identified in other plant species. The

conserved motifs of the BvSRT proteins were similar, especially for BvSRT1 and BvSRT3, which may be due to the functional similarities. Additionally, SIR2 proteins in *B. vulgaris* showed amino acid similarities with *G. max* and *V. vinifera* SIR2 proteins. No SIR2 proteins from rice indicated synteny with beet proteins. Prediction of protein-3D structures is important to find out the biological functions of proteins. In the present work the homology modeling was used (Kelley et al., 2015), which depends on SIR2s from different organisms. Protein models presented above contained NAD<sup>+</sup>-dependent protein deacetylase sirtuin 2 and DHS-like NAD/FAD-binding domains.

This work predicted nuclear, cytoplasmic, and mitochondrial localizations of BvSRT proteins. Previous studies demonstrated that SIR2-type HDACs were localized in different subcellular compartments, such as the nucleus, cytoplasm, chloroplast, and mitochondria. For instance, AtSRT1 was localized in the cytoplasm/nucleus (Pandey et al., 2002), but the OsSRT1 and SiSRT1 were present in the nucleus (Huang et al., 2007; Zhao et al., 2014). WOLF PSORT prediction tool and experimental results both indicated the nuclear localization of SiSRT1, which may demonstrate the reliability of prediction tools. Consistently with *B. vulgaris* SRT2 protein, OsSRT2, and AtSRT2 were localized in the mitochondrion (König et al., 2014), but SiSRT2 was found in both the nucleus and cytoplasm (Zhao et al., 2014). Interestingly, SbSRT1, SbSRT2, CsSRT2, and CsSRT3 were assumed to be localized in the chloroplast, suggesting that they may have different functions in *Sorghum* and *C. sinensis* (Yuan et al., 2020; Du et al., 2022). In contrast, WOLF PSORT predicted peroxisomal localization of the BvSRT1 protein. RPD3/HDA1-type HDAC proteins in sugar beet showed nuclear and cytoplasmic localization, while the localization of BvHDAC4 near nuclei implied its location in the chloroplast or mitochondrion (Yu et al., 2023). Taken together, it is difficult to interpret SIR2 protein localizations in *B. vulgaris* without wet lab studies. Further experimental studies are needed to verify the results from the prediction data mentioned above.

Transcription profiles are mediated by *cis*-acting regulatory elements that play key roles in the expression of genes involved in environmental stress response, and plant development (Biłas et al., 2016; Marand et al., 2023). Recently identified RPD3/HDA1-type HDACs in *B. vulgaris* contained *cis*-elements related to light, stress, and hormones (Yu et al., 2023), consistent with PlantCARE data of the present study. Three SIR2 genes in *B. vulgaris* have 15 stress-responsive elements including ARE, MYB, and MYC. Furthermore, the most abundant stress-related *cis*-element was recorded for MBS in the BvSRT3 gene. Interestingly, even though the BvSRT1 and BvSRT3 belong to the same class, and have similar motifs and exon/intron structures, no MBS and WUN-motifs were found in the BvSRT1 promoter. Many abiotic stress-inducible genes have *cis*-regulatory elements such as ABRE (Narusaka et al., 2003), which was found only in the promoter of BvSRT1. Wheat TaSRT1D and TaSRT2U promoter regions were also found to have ABRE elements (Shu et al., 2021). In a recent study, ABA treatment remarkably inhibited the transcription of *O. sativa* SRT and *S. bicolor* SRT2 genes, suggesting that ABA could regulate the expression of HDAC genes (Fu et al., 2007; Du et al., 2022). MYB transcription factors along with MBS are required for transcription of drought-induced genes. Parallel with BvSRT results, MBS and WUN-motif also existed in the promoters of RPD3/HDA1-type HDACs in *B. vulgaris* (Yu et al., 2023). Similarly, MYB and MBS elements were present in promoter regions of the *S. bicolor* SRTs (Du et al., 2022). MeJA-

specific *cis*-elements, CGTCA, and TGACG motifs were found in sugar beet *SRT1* and *SRT3* genes. CGTCA-motif also induces defense mechanisms under environmental constraints such as salt, drought, and low temperature stresses (Kaur et al., 2017). Similarly, promoter regions of *P. mume SRT1* and *SRT2* genes contained hormone and stress response-related *cis*-elements (Meng et al., 2022). In *F. tataricum*, *FtSRT1* and *FtSRT2* genes also contained *cis*-elements correlated with light response and MeJA response. It has been reported that a SIR2 homolog in rice, OsSRT1 negatively regulates leaf senescence by inactivating the transcription of JA biosynthesis genes and *PECTIN METHYLESTERASE1 (OsPME1)*. ChIP assays revealed direct binding of OsSRT1 to the promoter of *OsPME1*, and *OsPME1* transcript levels were enhanced in the *SRT1* RNAi plants (Fang et al., 2016). Thus, the effects of hormone treatments on SIR2 proteins and their gene expression patterns should be investigated in *B. vulgaris*. Gene expression data showed the differential expression patterns of *FtSRT* and *V. vinifera SRT6901* genes in distinct tissues such as stems, leaves, roots, fruits, and flowers (Aquea et al., 2010; Yan et al., 2023). *SRT6902* in *V. vinifera* was expressed in an organ-specific manner. Transcript levels of *P. mume SRT2* were higher in flowering buds than in leaves and stems, whereas *PmSRT1* was remarkably expressed in stems (Meng et al., 2022). *RPD3/HDA1-type HDAC* genes were expressed in response to salt, drought and low-temperature stresses (Yu et al., 2023). In *Sorghum* plants, elevated histone acetylation levels were recorded under cold, heat, osmotic, and salt stresses (Du et al., 2022). Moreover, transcript levels of *FtSRT1* and *FtSRT2* genes fluctuated under different light wavelengths, suggesting their involvement in the light response (Yan et al., 2023). In the present study, 9 types of light response-related *cis*-elements were assumed to be present in the *BvSRT1* and *BvSRT2* promoter regions. There were no light response-specific *cis*-elements found in the *BvSRT3* promoter. At least eight types of light-responsive *cis*-elements were determined in each *RPD3/HDA1* gene of sugar beet (Yu et al., 2023). However, the effect of different light conditions on the transcriptional activity of *SRT* genes is unknown in sugar beet. In addition to hormone-, light- and stress-correlated *cis*-elements, the core promoter elements such as TATA box (71), and CAAT box (88) were included in the *BvSIR2* genes that regulate the appropriate initiation of the transcription process by RNA polymerase II (Biłas et al., 2016). A higher number of stress-related *cis*-regulatory elements may correlate with the stress-induced upregulation of *BvSRT* genes. Taken together, monocotyledonous and dicotyledonous plant species both share similar *cis elements*. However, up to date, no

experimental findings have been reported on how and whether the *SIR2* genes of *B. vulgaris* respond to environmental stresses, hormone treatments, and light conditions. Thus, wet-lab studies along with *in silico* analysis should be performed to further characterize the functions of *BvSIR2* genes.

Overall, the findings presented above provide insight into the potential roles of sugar beet *SIR2-type HDAC* genes. *In-silico* analyses could help plant biologists select genes encoding histone deacetylation proteins for further functional characterization.

## 5. Conclusion

The SILENT INFORMATION REGULATOR2 (SIR2) proteins are NAD<sup>+</sup>-dependent protein deacetylases, found in different organisms such as bacteria, mammals, fungi, and plants. They participate in development, stress response, and energy metabolism in plants. In the present study, an *in silico* analysis of SIR2 family members was done in sugar beet. Three SIR2 family HDAC-encoding genes (*BvSRT1*, *BvSRT2*, and *BvSRT3*) were identified from the sugar beet genome, and they were located on chromosomes 4 and 9. The phylogenetic analysis exhibited that 3 sugar beet SRTs were divided into two classes: Class II (*BvSRT2*) and IV (*BvSRT1* and *BvSRT3*). SIR2 family proteins were confirmed to have an SIR2 domain (PF02146) using by SMART and NCBI CDD databases. Through the MEME tool, the conserved motifs were found to range from 6 to 50 amino acids in length, while the GSDS showed that the intron-exon numbers ranged from 10 to 14. The *BvSRT1* and *BvSRT3* proteins were assumed to be localized in both the nucleus and cytoplasm, while the *BvSRT2* protein was located in the mitochondrion. Promoter analysis showed the potential involvement of *BvSRT* genes in hormone regulation, light response, abiotic stress response, and meristem expression. This study might provide preliminary information for further research on SIR2-type histone deacetylases in sugar beet. However, experimental findings are needed to confirm the data obtained from bioinformatics tools/databases and examine the transcript abundance of SIR2-encoding genes under different stresses, light conditions, and hormone treatments.

**Conflict of interest:** The author declares that she has no conflict of interests.

**Informed consent:** The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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