 doi:10.20944/preprints202210.0317.v1

Article

Genome-Wide Characterization of Sulfate Transporter Gene Family in Oilseed Crops: *Camelina sativa* and *Brassica napus*

Parviz Heidari [1,*], Soosan Hasanzadeh [1], Sahar Faraji [2], Sezai Ercisli [3] and Freddy Mora-Poblete [4,*]

[1] Faculty of Agriculture, Shahrood University of Technology, Shahrood 3619995161, Iran; sousan.hasanzadeh@gmail.com (S.H.).
[2] Department of Plant Breeding, Faculty of Crop Sciences, Sari Agricultural Sciences and Natural Resources University (SANRU), Sari 4818168984, Iran; sahar.faraji@rocketmail.com.
[3] Department of Horticulture, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey; sezai.ercisli@tubitak.gov.tr
[4] Institute of Biological Sciences, University of Talca, Talca 3460000, Chile;
* Correspondence: heidarip@shahroodut.ac.ir; (P.H.), morapoblete@gmail.com (F.M-P.)

Abstract: Sulfate transporters (SULTRs) are responsible for the uptake of the sulfate (SO_4^{2-}) ions in the rhizosphere by the roots and their distribution in plant organs. In this study, SULTR family members in the genome of the two oilseed crops, *Camelina sativa*, and *Brassica napus*, were identified and characterized based on their sequence structure, duplication events, phylogenetic relationships, phosphorylation sites, and expression levels. Herein, 36 and 45 putative *SULTR* genes were recognized from the genome of *C. sativa*, and *B. napus*, respectively. SULTR proteins were predicted as basophilic proteins with low hydrophilicity in both studied species. According to phylogenetic relationships, we divided SULTRs into five groups, in which SULTRs 3 showed highest variation. Besides, several duplication events were observed between *SULTRs*. The first duplication event was predicted approximately five million years ago between three *SULTRs 3.1* in *C. sativa.* Two subunits were indicated in the 3D structure of SULTRs that the active binding sites differed between *C. sativa* and *B. napus*. According to available RNA-seq data, *SULTRs* showed diverse expression in tissues and response to stimuli. *SULTRs 3* showed an expression in all tissues. *SULTRs 3.1* were more upregulated in response to abiotic stresses in *C. sativa*, while *SULTRs 3.3*, and *SULTRs 2.1* showed an upregulation in *B. napus*. Furthermore, *SULTRs 3* and *SULTRs 4.1* showed an upregulation in response to biotic stresses in *B. napus*. Based on the distribution of cis-regulatory elements in the promoter region, we speculated that *SULTRs* might be controlled by phytohormones such as ABA, and MeJA. Therefore, it seems that *SULTR* genes in *C. sativa* have been more influenced by evolutionary processes and have acquired more diversity.

Keywords: Bioinformatics; Biotic stresses; Regulatory mechanisms; Protein structure; Gene expression; Evolution analysis

1. Introduction

Sulfur (S) is a macronutrient that is required for the biosynthesis of amino acids such as cysteine (Cys) and methionine (Met), vitamins, cofactors, glutathione (GSH), as well as secondary metabolites; thus, (S) is a vital element for plant growth, development and stress responses [1–3]. Root cells uptake sulfate (SO_4^{-2}) form of (S) through a proton co-dependent process. The uptake and assimilation of the sulfate resources available in the environment and sending them to produce essential sulfur (S) metabolites crucial for development and stress responses is reported to be very critical in plants and microbes [4]. The soil sulfate content may be modified through some factors such as activities of dissimilating soil microbes, weathering of S-containing minerals, human activities modifying deposition of S into the ecosystem, and climate changes [1]. Therefore, the available contents of sulfate can be altered in the soil, because of the plants' root systems development absorb the nutrient compounds according to their requirements and material accessibility.

 doi:10.20944/preprints202210.0317.v1

It was reported that in comparison with other micro-nutrients, sulfate seems to contain only a gentle and limited effect on root structures [5]. To meet the required S-containing metabolites synthesis, the membrane transport system and their related metabolic enzymes should optimize the sulfate uptake, S acquisition, storage, and employment [6]. The uptake and distribution of sulfate throughout the plant are facilitated by a network of sulfate transporters (SULTRs) encoded by a multigene family [7]. The H^+/SO_4^{2-} co-transporters SULTRs, are reported to contain the 12 membrane-spanning domains, along with a carboxyl-terminal region, so-called STAS (Sulfate Transporter/AntiSigma-factor) that is suggested to play the important roles in transporters activity and their interactions with other proteins [1,8].

The involvement of SULTRs in S-transportation within plants was first reported by Smith et al. [9]. SULTRs are characterized by 12 transmembrane domains (TMDs) and an AntiSigma factor antagonist (STAS) domain at C-terminus, which is critical for sulfate transporter activity [10]. Genomes of higher plants like *Arabidopsis thaliana*, rice, wheat, sorghum, and apple were reported to have 12, 12, 11, 10, and 9 *SULTR* genes, respectively [11–14]. The SULTR family is well characterized in *Arabidopsis*, and based on their sequence resemblance, function, and location, the sulfate transporters can be divided into four main groups. The first group includes AtSULTR 1.1, AtSULTR 1.2, and AtSULTR 1.3, which all are high-affinity (S) transporters [15]. AtSULTRs 1; 1-2 are co-localized in the root hairs, epidermal, and cortical cells of roots and they both are responsible for sulfate uptake from the soil [16,17]. Nevertheless, despite their common task AtSULTR 1.1 is predominantly function under (S) deficiency, while AtSULTR 1.2 function under either sulfur-sufficient or sulfur-deficient conditions efficiently [18]. AtSULTR 1.3 is localized merely in the phloem and cooperates in the source-sink distribution of sulfate [19]. Group 2 consists of two low-affinity transporters; AtSULTR 2.1 and AtSULTR 2.2, which are responsible for the transportation of sulfate from root to shoot in the vasculature [20]. Group 3 with five members (AtSULTR 3; 1-5) is the largest group. However, their precise function has not been fully established. It has been reported that SULTR 3.1, which transports sulfate into chloroplast, might have a role in helping plants to withstand abiotic stresses [21]. Also, SULTR 3.5 has been reported to co-express with SULTR 2.1 to enhance the uptake and facilitate the root-to-shoot transport of sulfate under (S)-depletion conditions [22,23]. The group 4 transporters, SULTR 4.1 and SULTR 4.2, demonstrated as tonoplast localized transporters that release sulfate from the vacuoles into the cytosol [24,25]. Besides *A. thaliana*, many studies have been conducted to characterize SULTRs in other crops functionally. For instance, 14 putative *SULTR* genes were considered for Rapeseed (*Brassica napus*) which among all of them, only group 1 and group 4 were induced in response to S-deprivation [26]. Twenty eight putative *SULTR* genes were identified in the soybean (*Glycine max*) genome, and *GmSULTR 1.2b* was confirmed to have an important role in sulfate uptake and improving plant tolerance to sulfur deficiency stress [27]. In the potato (*Solanum tuberosum*) genome, 12 *SULTR* genes were identified, and members of group 3 (StSULTR3s) were potentially involved in biotic/abiotic stress responses through MYB TFs, which play crucial role/s in the modulation of StSULTR3s under such circumstances [28]. Maize (*Zea mays L.*) genome includes eight putative *SULTR* genes which all of them except for *ZmSULTR 3.3*, were induced by drought and heat stresses [29]. In addition, various studies have confirmed that *SULTRs* can be responsive to heavy metals exposure [30,31]. Despite the progress made in plant *SULTRs* functional characterization, there are still more important crops that need to be investigated. In this sense, *Camelina sativa* is an oilseed crop of the Brassicaceae family with many qualities such as low input, great adaptation and resistance, short life cycle, and easy genetic transformation have turned *C. sativa* into an ideal model plant [32,33]. Moreover, *C. sativa* has growing importance as a biofuel [34,35], and oilseed plants are typically very (S)-demanding [36], but a study on *C. sativa*'s response to various fertilizers showed that seed yield or oil content of *camelina* seeds did not affect by sulfur fertilization [37]. Altogether, in order to develop S-efficient crops and varieties tolerant to S-deficiency, it is necessary to identify and characterize

 doi:10.20944/preprints202210.0317.v1

SULTRs, especially in low input crops like *C. sativa*. To the best of our knowledge, except for only one study focusing on *C.sativa* under salinity stress that reported an up-regulation of *SULTR 3.4* in *C. sativa* under salinity stress [38], there is no available report on genome-wide analysis of *SULTR* genes in *C. sativa.* In this study, these resources have been employed to distinguish the regulation role of *SULTR* genes in various cellular processes, especially in response to stimuli. We focus on the SULTR sequences in the *C. sativa* and *B. napus* genomes and compare and discuss their adjustment and possible engagement in protection against unfavorable environmental stimuli. We also highlight the potential properties of these genes to ameliorate sulfate uptake.

2. Results

2.1. SULTR properties in Camelina sativa and Brassica napus

In the current study, 36 and 45 putative SULTR genes were recognized from the genome of C. sativa, and B. napus, respectively (Table S1). SULTRs were characterized and compared between two oilseed crops, C. sativa, and B. napus, according to their coding DNA sequence (CDS) and protein length, exon number, the isoelectric point (pI), molecular weight (MW), the grand average of hydropathy (GRAVY) value, and instability index (Table S1 and Table 1). Our results showed that the physicochemical properties of SULTR proteins in the two studied plants are almost close to each other. For instance, MW ranged from 29.07 to 91.99 kDa in C. sativa, and 28.94 to 83.86 kDa in B. napus. Besides, the pI value ranged from 7.41 to 9.93 in C. sativa, and 7.11 to 10.71 in B. napus. Moreover, the GRAVY value varied from 0.271 to 0.624 in C. sativa, and 0.108 to 0.621 in B. napus. Based on the instability index, 83% and 73% of SULTR proteins were predicted as stable proteins in C. sativa, and B. napus, respectively. In addition, the exon number varied from 4 to 20 in C. sativa, and 4 to 19 in B. napus (Figure 1, Table 1). Overall, SULTR proteins were predicted as basophilic proteins with low hydrophilicity.

Table 1. Summary of SULTRs properties in Camelina sativa and Brassica napus. Full details of SULTRs properties are provided in Table S1.

Attributes	*C. sativa*	*B. napus*
CDS length (bp)	801 – 3428	878 – 3428
Protein length (aa)	266 – 829	264 – 758
Exon number	4 – 20	4 – 19
pI	7.41 – 9.93	7.11 – 10.71
MW (KDa)	29.07 – 91.99	28.94 – 83.86
GRAVY	0.271 – 0.624	0.108 – 0.621
Instability index	83% stable	73% stable

2.2. Phylogenetic analysis and classification of the SULTR gene family

In the present study, a phylogenetic tree of SULTR proteins was performed, including 45 SULTRs from *B. napus*, 36 SULTRs from *C. sativa*, 28 SULTRs from *Glycine max*, 12 SULTRs from *Oryza sativa*, and 12 SULTRs from *Arabidopsis thaliana* (Figure 1). The studied SULTRs were classified into five main groups. All SULTRs 4.1 and 4.2 including 16 SULTRs were located in group I, and SULTRs 2.1 and 2.2 were clustered in group II. Besides, 30 SULTRs from class 1.1, 1.2, and 1.3 were found in group III. SULTRs class 3 were separated in group IV and V that SULTRs 3.3 and 3.4 including 28 proteins located in group IV and 34 SULTRs from class 3.1, 3.2, and 3.5 located in group V (Figure 1). SULTRs from monocot model plant, rice, showed a high distance from dicot samples. Moreover, SULTRs from *C. sativa* and *B. napus* were evaluated and compared according to the conserved motifs. Ten conserved motifs were recognized in the protein sequence of SULTRs, in which the motif 6 was not observed in SULTRs 4.1 and 4.2, group I (Figure 2). Ten conserved motifs were identified in SULTRs 2.1 and 2.2, except a SULTR 2.1 from *C. sativa*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

showed eight conserved motifs. Besides, SULTRs from class 1.1, 1.2, and 1.3 as well as class 3.1, 3.2, and 3.5 showed high diversity based on patterns of motifs distribution (Figure 2). Motifs 7 and 2 were frequently observed in SULTR proteins and showed a potential to screen members of this family.

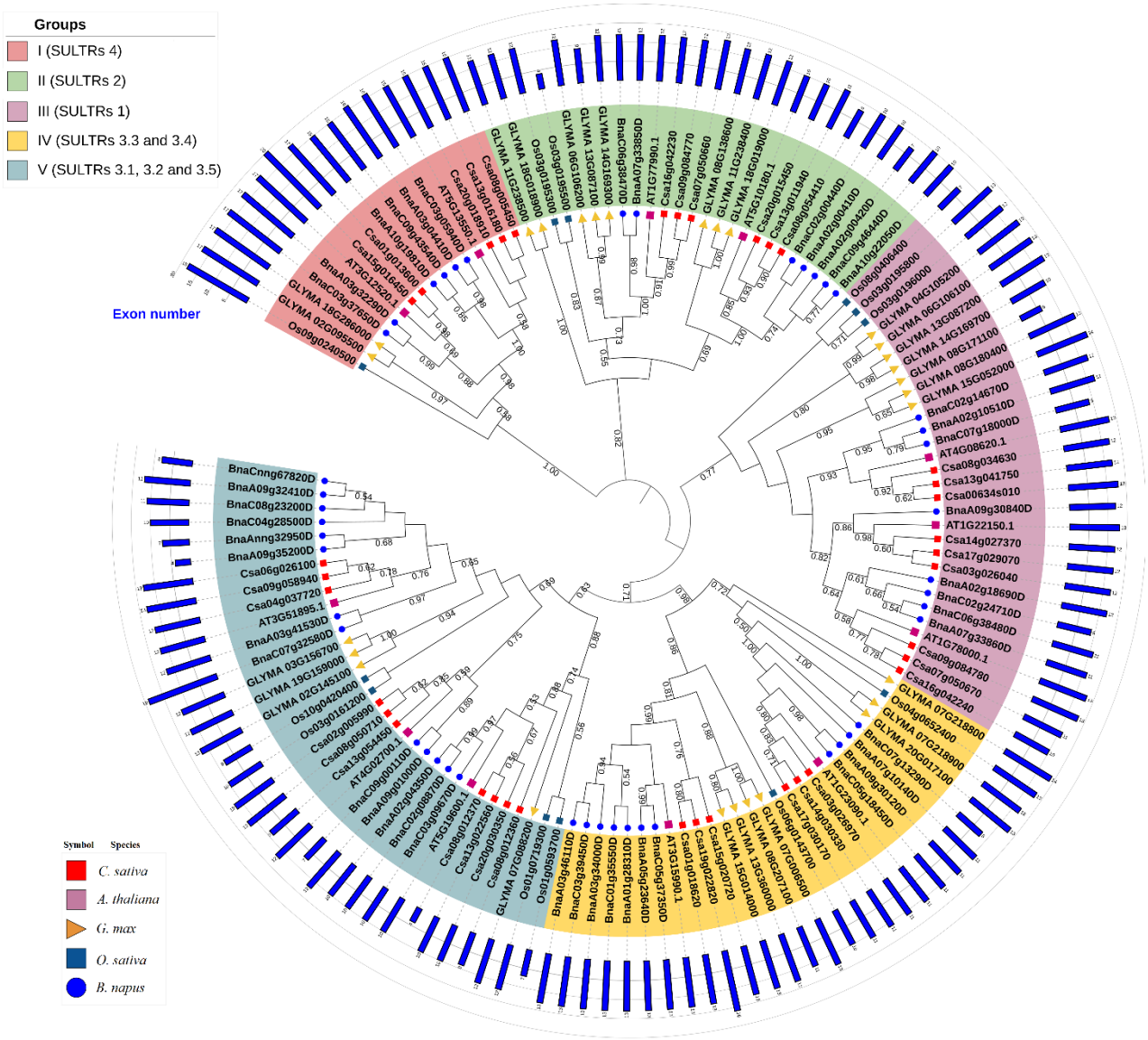


Figure 1. Phylogenetic tree of SULTRs from *Camelina sativa, Brassica napus, Arabidopsis thaliana, Glycine max*, and *Oryza sativa*. The number of exon for each of the SULTR coding genes is shown in blue bar.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

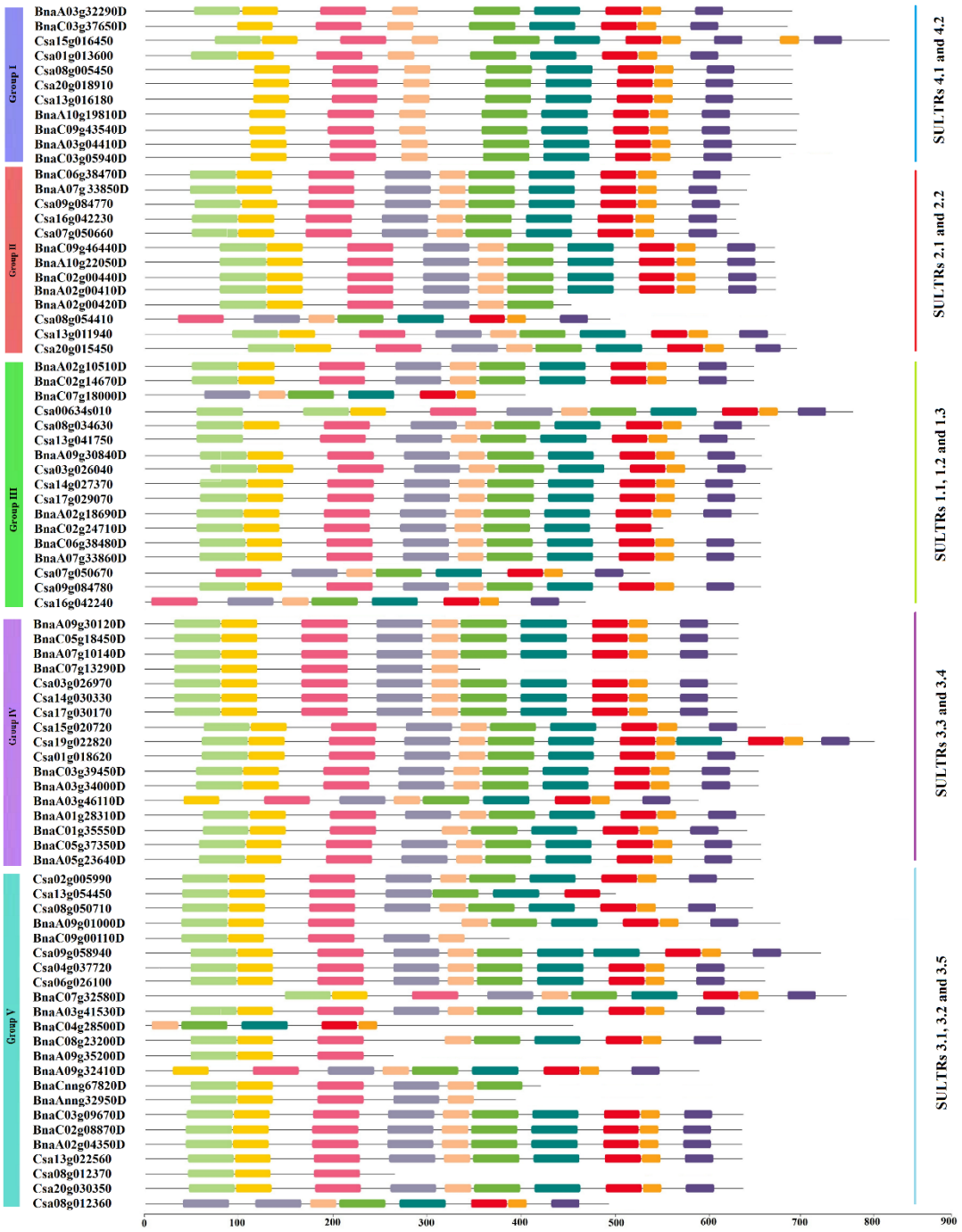


Figure 2. Distribution of conserved motifs in the SULTRs from *Camelina sativa*, and *Brassica napus*. Grouping is based on phylogenetic tree.

2.3. Evolutionary process in MGT genes in Citrullus lanatus, and Cucumis sativus

In this study, to investigate the duplication events in the SULTR gene family in *C. sativa* and *B. napus*, the values of the synonymous (Ks), non-synonymous (Ka), and Ka/Ks of each duplicated gene pair were calculated and presented in Figure 3 and Table S2. The Ks value of *SULTRs* in *C. sativa* was frequently observed between 0.6 and 1.0 (Figure 3a), and Ka/Ks was frequently observed between 0.7 and 0.9 (Figure 3b). In contrast, the frequency of Ks and Ka/Ks of *SULTRs* from the *B. napus* genome differed from *C. sativa*, whereas the Ks value was frequently detected between 1.2 and 1.6 (Figure 3c), and Ka/Ks was frequently observed ranging from 0.3 to 0.5 (Figure 3d). In *C. sativa*, the first duplication event between *SULTRs* was predicted around five million years ago (MYA) between

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

three *SULTRs 3.1*, including *Csa06g026100-Csa04g037720* and *Csa09g058940-Csa04g037720*, while the first duplication in *B. napus* was approximately occurred three MYA between two *SULTRs 3.1*, *BnaA03g41530* and *BnaA09g35200* (Table S2). Several synteny blocks were observed between *SULTRs* from *C. sativa* and *B. napus*. However three *SULTRs* of class 1.3, *Csa17g029070*, *Csa14g027370*, and *Csa03g026040*, four from Class 3, *Csa13g054450*, *Csa08g050710*, *Csa02g005990*, and *Csa08g012360*, and a *SULTR 1.1*, *Csa08g034630*, from *C. sativa* showed less synteny relationships with *SULTRs* from *B. napus* (Figure 4).

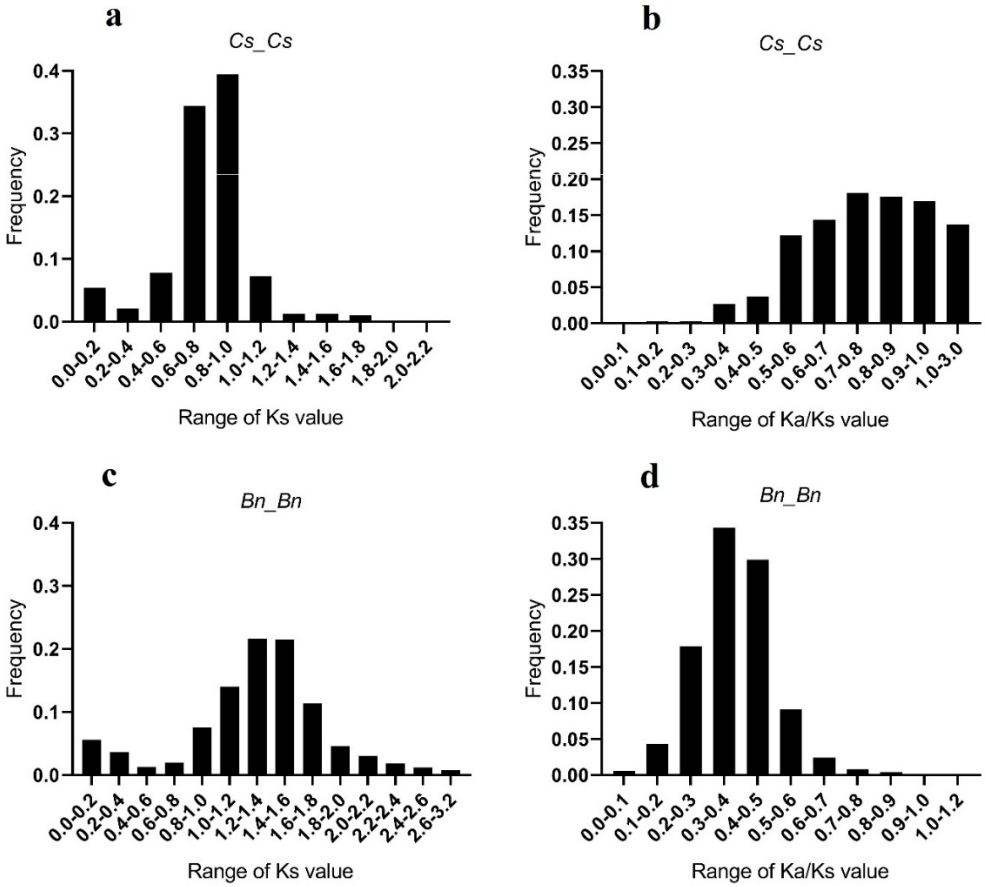


Figure 3. Frequency of Ks value and Ka/Ks in *SULTRs*. Frequency of Ks value (a), and Ka/Ks (b) between *SULTRs* of *C. sativa* (Cs). Frequency of Ks value (c), and Ka/Ks (d) between *SULTRs* of *Brassica napus* (Bn). Full details of duplicated *SULTRs* are provided in Table S2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

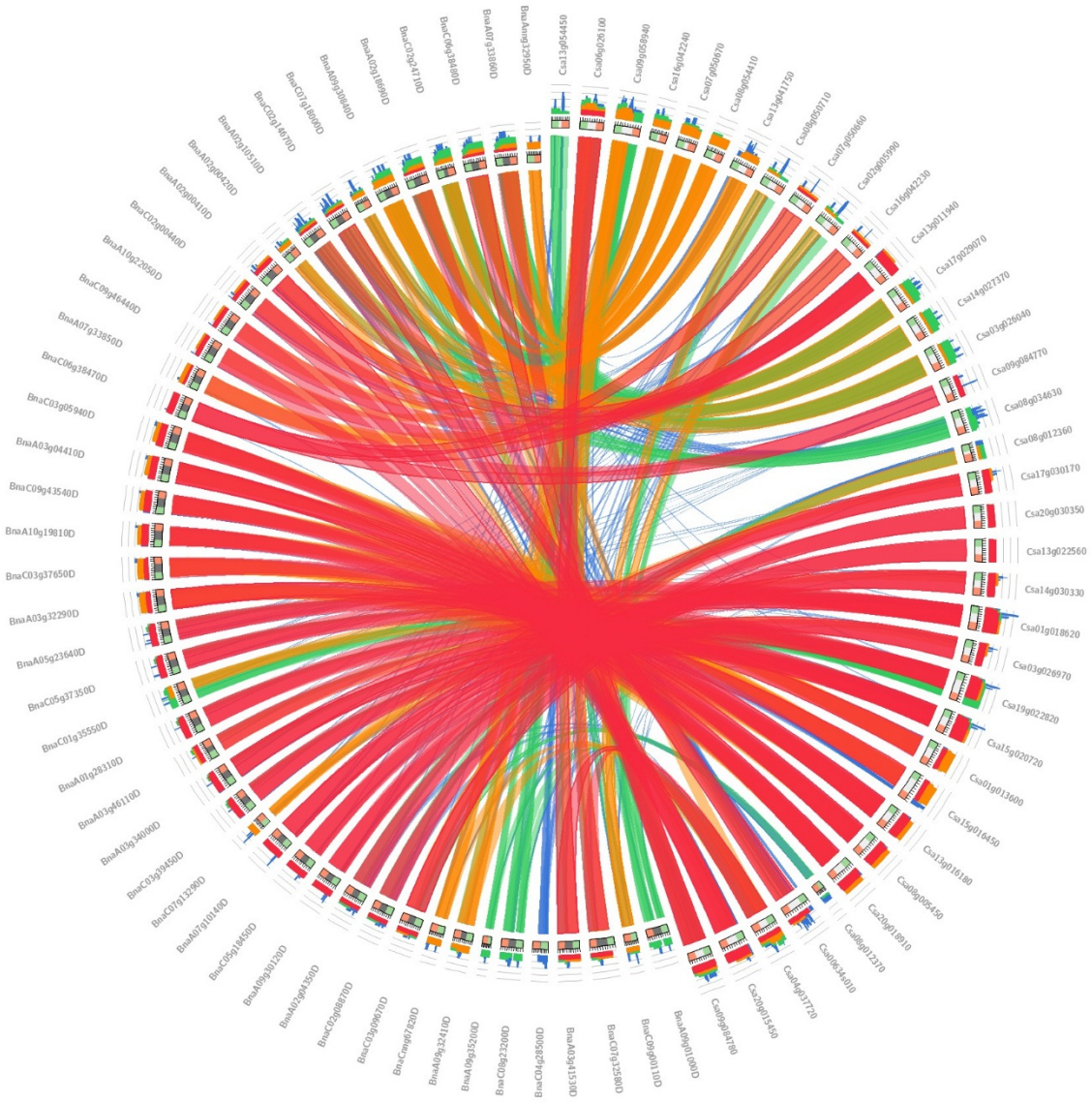


Figure 4. Synteny relationships between *SULTRs* from *Camelina sativa,* and *Brassica napus.*

2.4. Transmembrane structure of SULTRs

SULTR proteins from different groups were compared based on their transmembrane structure *in C. sativa* and *B. napus* (Figure 5). In group I, 12 transmembrane helices and 11 pores were predicted in all SULTRs. However, SULTRs in *B. napus* showed similar structure based on position of transmembrane helices while in *C. sativa,* they were diverse. Besides, the number of transmembrane helices in SULTRs of group II ranged from 10 to 12 in *B. napus* and 8 to 10 in *C. sativa*. Most SULTRs in *B. napus* showed ten transmembrane helices with nine pores, except for BnaC07g18000D with seven transmembrane helices, while the number of transmembrane helices varied between 8 and 11 in *C. sativa*. In group IV, SULTRs of *B. napus* predicted to have ranging 6 to 11 transmembrane helices while in *C. sativa*, the number of transmembrane helices ranged from 9 and 13. SULTRs in group V showed high diversity based on transmembrane structure, and between 4 and 14 transmembrane helices were observed in their structure.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

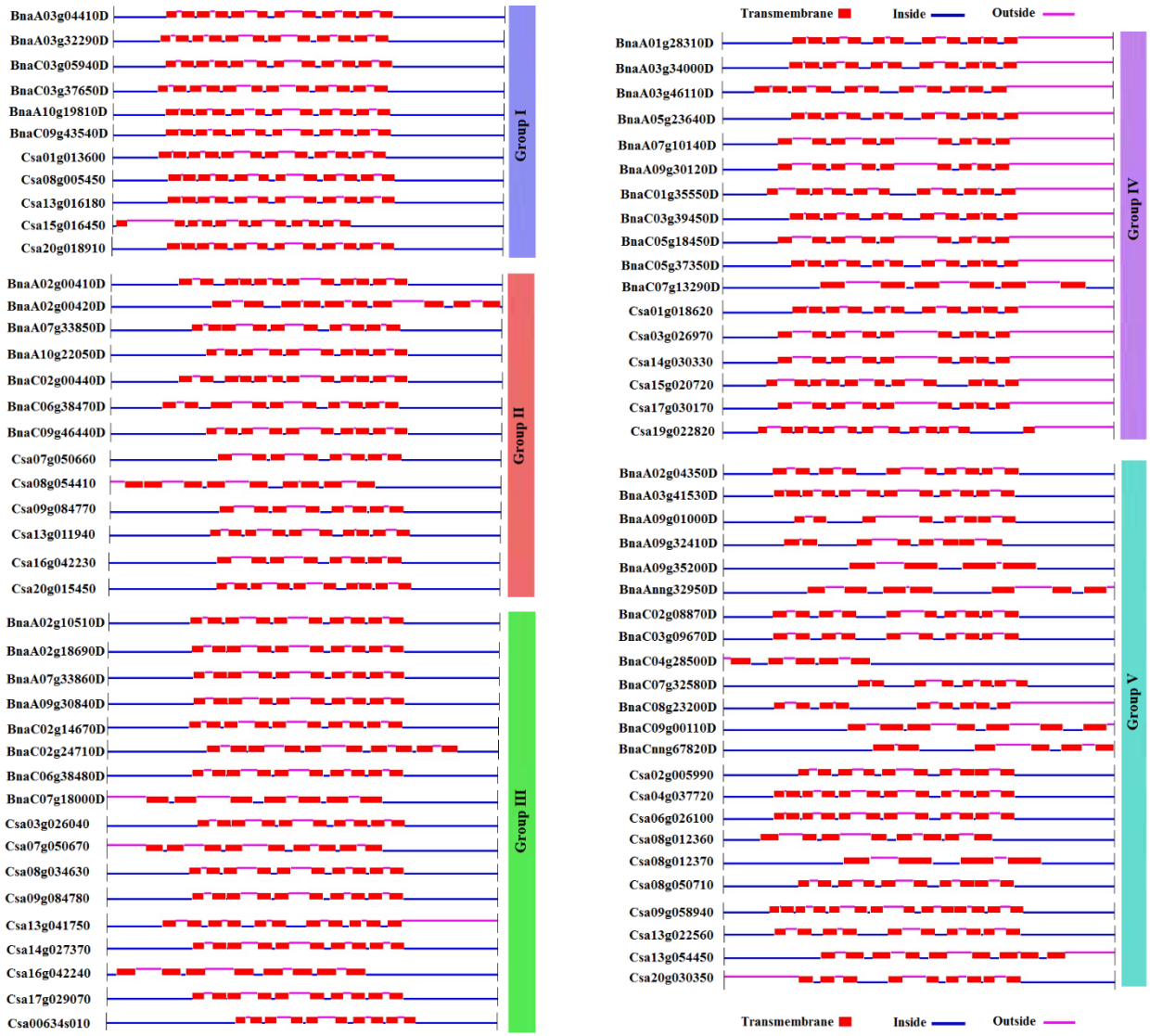


Figure 5. Transmembrane structure of SULTRs in *C. sativa* and *B. napus*. Grouping is based on phylogenetic tree.

2.5.3. D structure analysis of SULTRs

Prediction of the 3D structure revealed that SULTRs in *C. sativa* and *B. napus* have two subunits that the active binding site can be located in small or large subunits (Figure 6). These results showed that SULTRs are diverse between *C. sativa* and *B. napus* (Figure 6). In the candidate SULTRs of group I, valine (VAL), proline (PRO), phenylalanine (PHE), asparagine (ASN), lysine (LYS), glycine (GLY), and serine (SER) amino acids were frequently identified in binding sites of candidate SULTR from *C. sativa*, while PHE, GLY, and leucine (LEU) were frequently observed in pocket sites of candidate SULTR from *B. napus* (Figure 6). In the candidate SULTRs of group II, PHE, GLY, and alanine (ALA) were more recognized as binding sites in *C. sativa*, while PHE, SER, and isoleucine (ILE) were frequently observed in *B. napus*. Besides, six amino acids including SER, aspartic acid (ASP), LYS, ILE, ALA, and tyrosine (TYR) were more recognized in pocket sites of candidate SULTR in *C. sativa* from group III, while in the candidate of *B. napus*, PHE, and threonine (THR) were frequently observed in binding sites. In the candidate SULTRs from group IV, SER, GLY, histidine HIS, and TYR were more identified as binding sites in *C. sativa*, while LEU, ILE, glutamate (GLU), and arginine (ARG) were frequently observed in pocket sites of *B. napus*. In the candidate SULTRs from group V, SER, PHE, ILE, ALA,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

VAL, LEU, and TYR were more recognized as binding sites in *C. sativa*, while ALA, ILE, methionine (MET), VAL, and THR were frequently observed in pocket sites of *B. napus*.

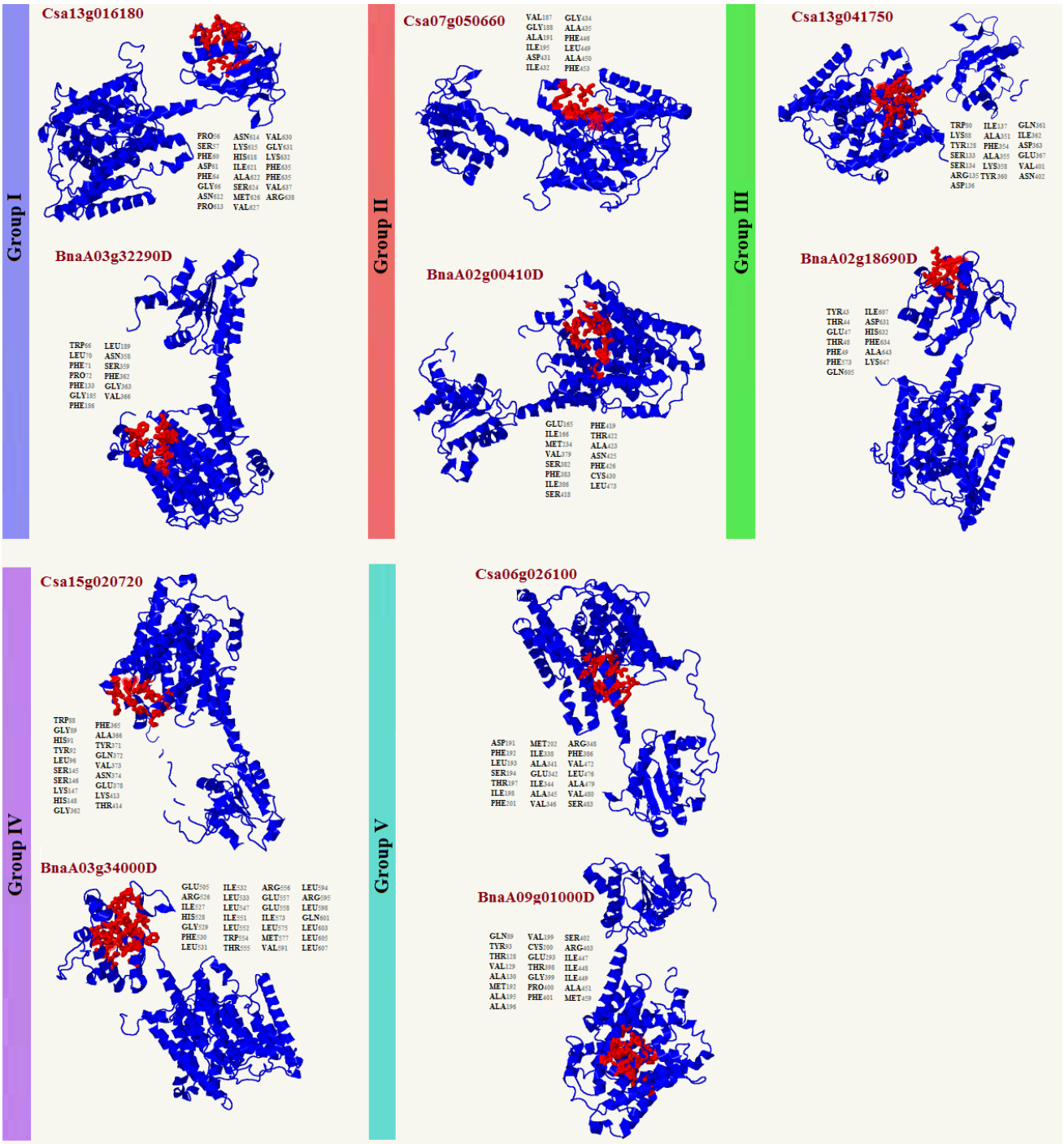


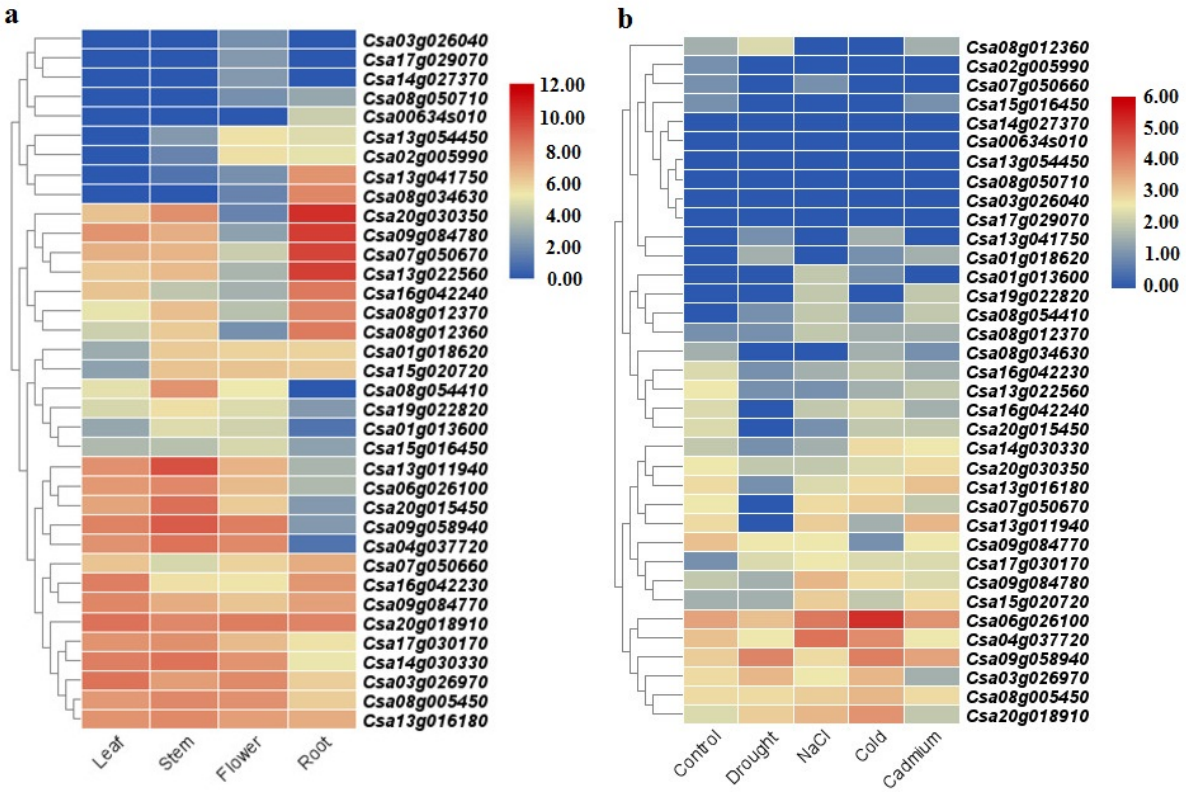
Figure 6. Three-dimensional docking analysis of candidate SULTRs in *C. sativa* and *B. napus*. Ligand-binding sites are highlighted in red, and list of pocket sites are provided beside the protein structure..

2.6. Expression analysis in SULTRs

In this study, the expression patterns of *SULTRs* in *C. sativa* and *B. napus* were illustrated in different tissues and response to stress (Figure 7 and Figure 8). Two *SULTRs* from class 3.5, *Csa20g030350*, and *Csa13g022560*, and two genes of class 1.2 including *Csa09g084780*, and *Csa07g050670* more expressed in roots of *C. sativa*, while three *SULTRs*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

from class 3.1 including *Csa06g026100*, *Csa09g58940*, and *Csa04g0377720* and three *SULTRs 2.1* (*Csa13g011940*, *Csa08g054410*, and *Csa20g015450*) showed a high expression in stem tissues (Figure 7a). In leaf tissues of *C. sativa*, three *SULTRs* from class 3.3 including *Csa17g030170*, *Csa14g030330*, and *Csa03g026970*, two genes from class 2.2, *Csa16g042230*, and *Csa09g084770*, and a *SULTR 4.1*, *Csa20g018910*, showed high expression (Figure 7a). In response to abiotic stresses, *SULTRs 3.1* were induced in *C. sativa* (Figure 7b). For example, *Csa06g026100* and *Csa04g037720* were more expressed in response to cold and salt stress, and *Csa09g058940* was more induced in response to drought, cold, and cadmium stress. In addition, *Csa20g018910* as a chloroplast *SULTR 4.1* was more expressed under cold stress (Figure 7b). Besides, *SULTRs* of *B. napus* showed a diverse expression in tissues and response to abiotic and biotic stresses (Figure 8). Two *SULTRs 2.1*, *BnaA02g00410D*, and *BnaC02g00440D*, a *SULTR 3.4*, *BnaC01g35550D*, and a *SULTR 3.5*, *BnaC02g08870D*, showed high expression in root tissues of *B. napus*, while two *SULTRs 3.2*, *BnaC09g00110D*, and *BnaA09g01000D*, two *SULTRs 3.1*, *BnaA03g41530D*, *BnaC07g32580D*, a *SULTR 3.3*, *BnaC05g18450D*, and a *SULTR 2.2*, *BnaC06g38470D*, expressed in seed (Figure 8a). In stem tissues of *B. napus*, two *SULTRs* from class 3, *BnaA03g41530D*, and *BnaC04g28500D*, showed high expression and three *SULTRs* from class 3, *BnaA09g32410D*, *BnaA07g10140D*, and *BnaC07g13290D*, a *SULTR 2.1*, *BnaC09g46440D*, and a *SULTR 4.1*, *BnaA03g04410D*, expressed in leaf tissues (Figure 8a). Furthermore, two *SULTRs 3.3*, *BnaC05g18450D*, and *BnaA09g30120*, and two *SULTRs 2.1* including *BnaA10g22050D* and *BnaC09g46440D* were more upregulated in response to PEG, NaCl, and ABA treatment (Figure 8b). Interestingly, two *SULTRs 2.1*, *BnaC06g38470D*, and *BnaA07g33850D*, were differentially expressed in response to cold stress in *B. napus*. However, *BnaA07g10140D*, as a *SULTR 3.3*, and *BnaC09g46440D*, as a *SULTR 2.1*, were also upregulated under cold stress. In response to biotic stresses, two *SULTRs 4.1*, *BnaC03g05940D*, and *BnaA03g04410D* showed upregulation in response to a fungal pathogen, *Leptosphaeria maculans*. In addition, a *SULTR 3.4*, *BnaC01g3550D*, and a *SULTR 3.3*, *BnaA07g10140D*, were more induced in response to *Sclerotinia sclerotiorum* and *Bacillus thuringiensis* strain 4f5, respectively (Figure 8b).



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

Figure 7. Expression levels of *SULTRs* based on the available RNA-seq data in different tissues (**a**) and response to abiotic stresses (**b**) in *C. sativa*.

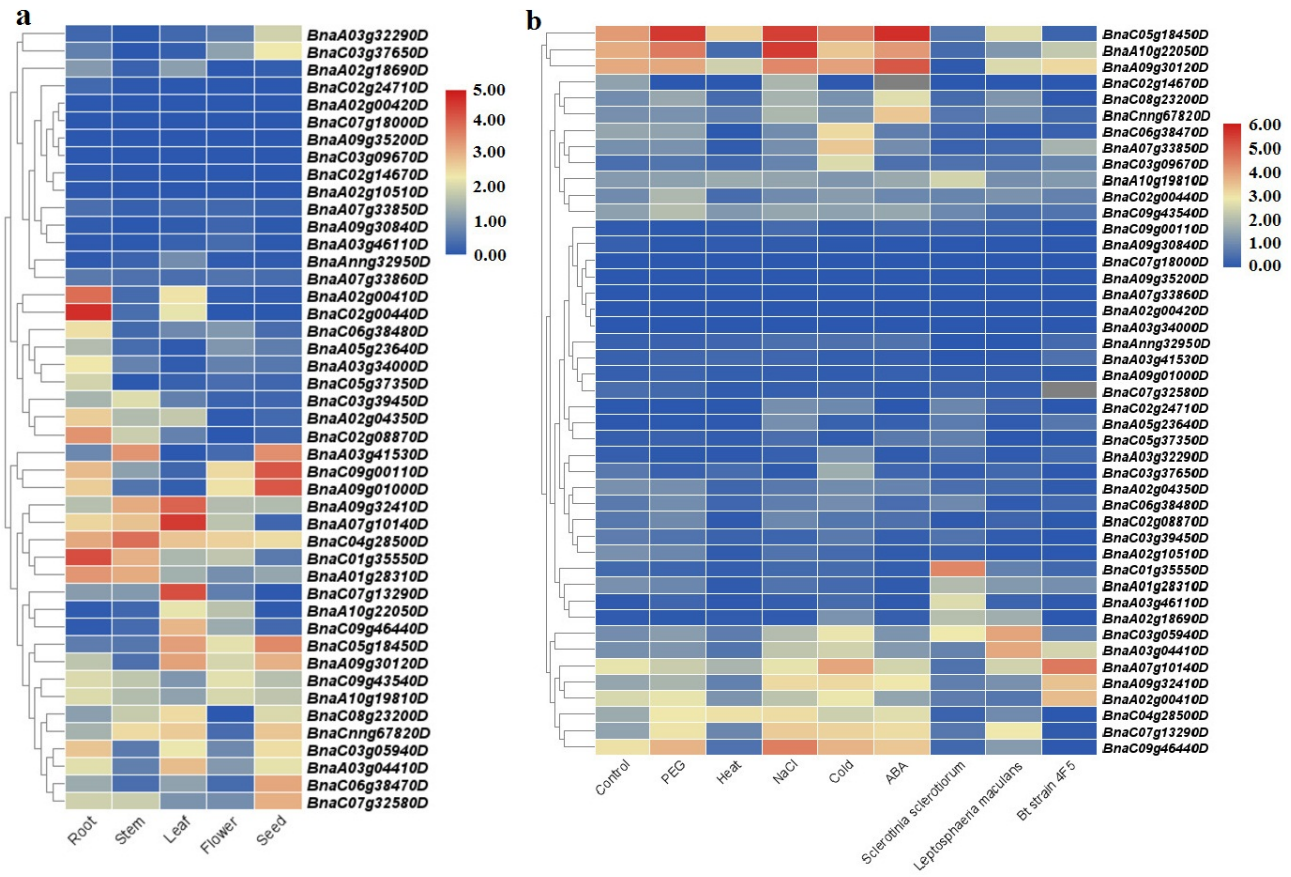


Figure 8. Expression levels of *SULTRs* based on the available RNA-seq data in different tissues (a) and response to abiotic and biotic stresses (b) in *B. napus*.

2.7. Prediction of phosphorylation into SULTRs

The potential phosphorylation sites of SULTRs in *C. sativa* and *B. napus* were predicted based on serine, threonine, and tyrosine amino acids (Figure 9). The potential of phosphorylation sites in SULTRs ranged from 3 (in Csa13g054450 as a SULTR 3.2) to 21 (in Csa08g005450 as a SULTR 4.1) with average of 10.28 sites per protein in *C. sativa* (Figure 9a). Interestingly, SULTRs from class 4.1 showed a high potential for phosphorylation events in *C. sativa*. Besides, SULTRs in *B. napus* showed ranging from a site in BnaC07g18000D, as a SULTR 1.1, to 23 sites in BnaA10g19810D, as a SULTR 4.1 with average of 9.71 sites per protein (Figure 9b). In addition, more phosphorylation sites were predicted in SULTRs 4.1 in *B. napus*.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

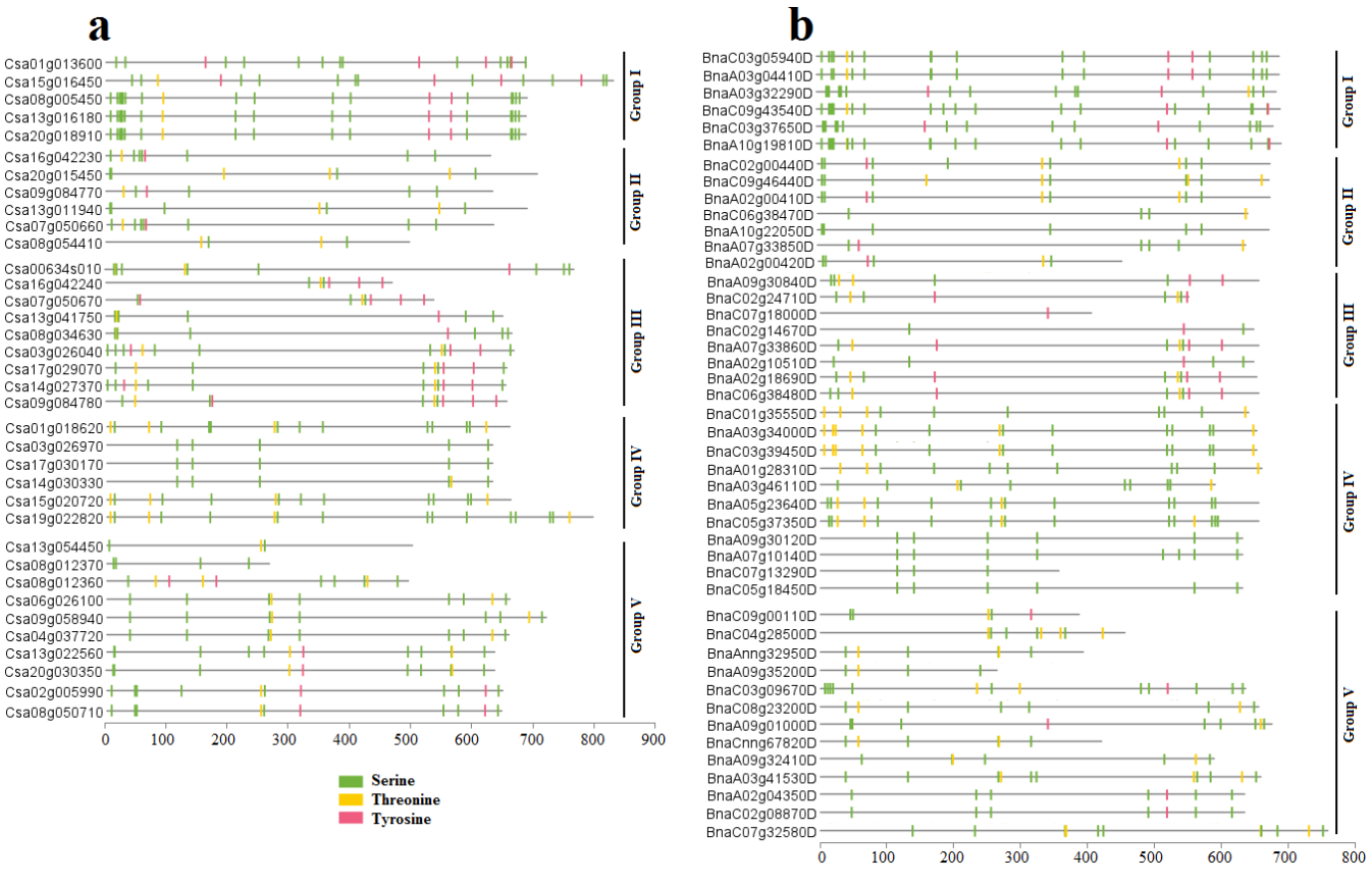


Figure 9. Prediction of phosphorylation site in SULTRs of *C. sativa* (a) and *B. napus* (b) with scores ≥ 0.90 using NetPhos 3.1 Server. Grouping is based on phylogenetic tree.

2.8. Distribution of cis-regulatory elements in promoter site

In this study, the distribution of cis-regulatory elements in the promoter site of *SULTRs* in *C. sativa* and *B. napus* was investigated (Figure 10, Figure S2, and Figure S3). *SULTRs* in *C. sativa* and *B. napus* were compared based on cis elements related to stress and response to hormones (Figure 10). Cis-regulatory elements associated with auxin, ABA, MeJA, GA, and SA responsive were observed in the promoter region of *SULTRs*. Results revealed that cis-regulatory elements of ABA-responsive are frequently distributed in *SULTRs* from *C. sativa*, while MeJA-responsive elements are more observed in *B. napus* (Figure 10). Besides, cis-regulatory elements related to biotic and cold stress were more identified in *SULTRs* from *B. napus* and drought stress-related elements were more observed in the promoter site of *SULTRs* from *C. sativa*.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 October 2022 doi:10.20944/preprints202210.0317.v1

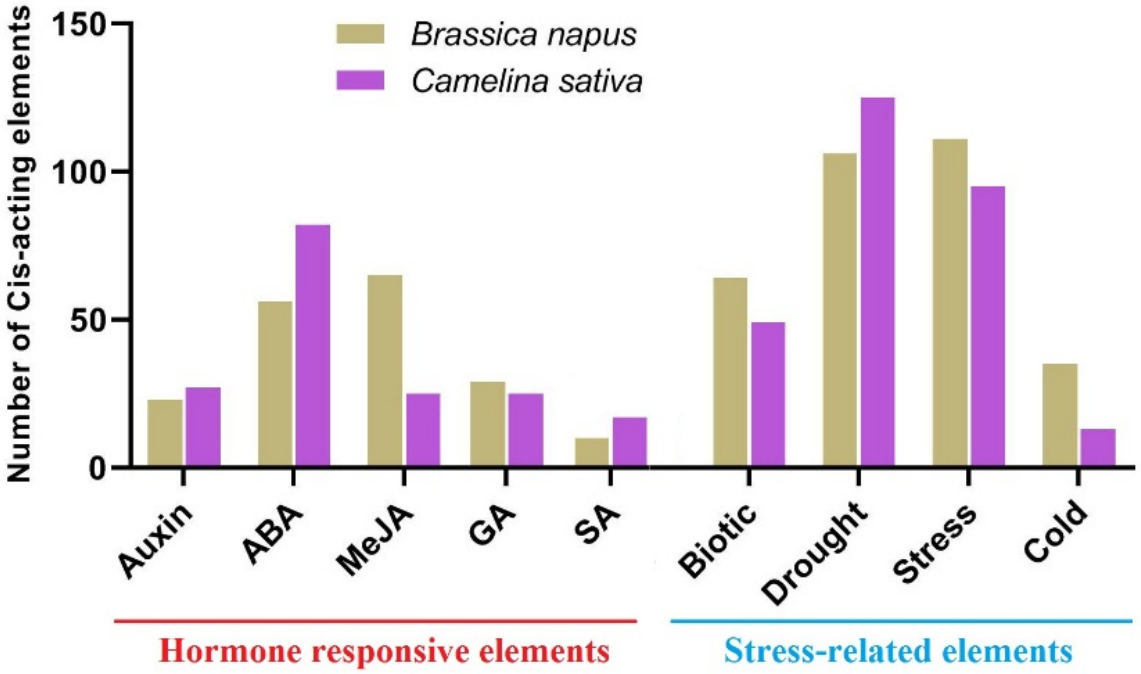


Figure 10. Comparison between *SULTRs* from *C. sativa*, and *B. napus* based on the number of cis-regulatory elements related to hormone, and stress-responsive into promoter sites. More details are provided in figure S2 and figure S3.

3. Discussion

The uptake and distribution of sulfate throughout the plant are facilitated by a network of sulfate transporters encoded by a multigene family (SULTRs) [7]. Due to the important role of sulfate in plants, SULTRs in several plant species have been studied. For instance, genomes of higher plants like *Arabidopsis thaliana*, and rice (12 *SULTRs*), wheat (11 *SULTRs*), sorghum (10 *SULTRs*), and apple (9 *SULTRs*) have been identified [11–14]. In this study, we identified and characterized 36 and 45 putative *SULTR* genes in the genome of *C. sativa*, and *B. napus*, respectively (Table S1). More members of this gene family in *C. sativa*, and *B. napus* may be associated with changes in ploidy levels and genome size as well as duplication events under evolution processes [35,39]. Our investigations revealed that SULTR proteins in the two studied plants, *C. sativa*, and *B. napus*, have the same range of physicochemical properties, i.e., MW, pI, GRAVY, and the instability index. In addition, the exon number ranged from 4 to 20 in *C. sativa*, and 4 to 19 in *B. napus*. Similarities in gene structures may indicate evolutionary events that have occurred significantly in the plant genome [40,41]. Our findings suggest that the exon/intron pattern may provide new insights into evolutionary relationships among members of the gene family and may have originated from a common ancestor. Moreover, it has been reported that exon number affects the expression and genes with less exons can be quickly induced in response to environmental stresses [42,43]. SULTRs have been divided into four main classes based on their location and functions [4]. In this study, different SULTR classes were separated from each other based on phylogenetic analysis and SULTRs class 4 showed more distance from other classes while a high variation was observed between members of SULTRs class 3 (Figure 1). Differences have been observed between model monocot plant, rice, and dicot plants, indicating that diversity in SULTR gene family has occurred after the divergence of monocots and dicots [44,45]. According to the results of conserved motifs in SULTRs, some conserved sites were recognized between SULTR groups, which can use to distinguish a specific group from other groups.

According to phylogenetic results, camellia SULTRs were more similar to SULTRs of *B. napus*, although their evolutionary trends were different. Based on the ka/Ks index, the

 doi:10.20944/preprints202210.0317.v1

first duplication events into SULTR genes occurred in *C. sativa* about 5 million years ago, while in *B. napus*, it occurred 3 million years ago. Furthermore, it seems that other members of SULTR gene family have originated from SULTRs class 3. Besides, Ka/Ks revealed that the duplicated SULTRs in *B. napus* were under purifying (negative) selection while both adaptive (positive) selection and purifying selection were observed in *SULTRs* of *C. sativa* [46]. It suggested that the duplicated genes with conserved functions, pseudogenization, or both were possibly produced by purifying selection [47]. Interestingly, results of comparative synteny revealed that several *SULTRs* from *C. sativa*, including three *SULTRs 1.3*, *Csa17g029070*, *Csa14g027370*, and *Csa03g026040*, four *SULTRs 3*, *Csa13g054450*, *Csa08g050710*, *Csa02g005990*, and *Csa08g012360*, and a *SULTR 1.1*, *Csa08g034630*, showed less synteny relationships with *SULTRs* from *B. napus* (Figure 4). It seems that these genes may have been specifically developed in the evolution of the camellia, and more researches are needed to determine their function.

SULTRs can be classified into four groups based on sequence structure, location, and function [48]. For instance, genes of group 1 and group 2 are more expressed in root cells and vacuolar tissues, respectively [48,49]. Herein, *SULTRs* in *C. sativa* and *B. napus* showed a diverse expression in different tissues and response to stresses. In the roots of *C. sativa*, two *SULTRs 1.2* and two *SULTRs 3.5* were more expressed, while in *B. napus*, two *SULTRs 2.1* (*SULTR 3.4*, and *SULTR 3.5)* showed a high expression in root tissues. In the shoot tissues, *SULTRs 2*, *3*, and class 4 were more expressed. Interestingly, *SULTRs* class 3 showed diverse functions and illustrated an expression in all tissues, indicating that members of this class are not specific to a tissue or an organ. In addition, members of *SULTRs 3* showed a high variation based on the transmembrane structure. Moreover, different expression patterns were observed between members of the SULTR gene family in *B. napus* and camellia in response to stimuli. *SULTRs 3.1* were more induced in response to abiotic stresses in *C. sativa*, while *SULTRs 3.3*, and *SULTRs 2.1* were more upregulated in *B. napus*. Several members of SULTR 3 play multiple roles and have interaction with abscisic acid (ABA) metabolism [21–23]. In the present study, SULTRs 3 and SULTRs 4.1 showed an upregulation in response to biotic stresses, including bacterial and fungal pathogen in *B. napus*. Besides, cis-regulatory elements related to ABA and MeJA responsive frequently observed in promoter sites of SULTRs. We concluded that SULTRs might be controlled by phytohormones, especially hormones related to stress such as ABA, and MeJA. These interactions can effectively induce members of this gene family in response to stress. It can be stated that the expression levels in different *SULTR*s may be correlated with hormone and stress-responsive elements observed in the promoter regions. Prediction of the 3D structure revealed two subunits in SULTRs structure that the active binding site differed between subgroups (Figure 6). PHE, ALA, ILE, and VAL were identified as key amino acids in binding site playing critical roles in the function and the regulation of SULTRs. Post-translational phosphorylation modifications can affect the function and the possible interaction of proteins [50,51]. The prediction of phosphorylation sites in SULTRs revealed that SULTRs 4.1 have a high potential for influencing post-translation modifications such as phosphorylation. Members of this group of transporters, SULTR4.1 and SULTR 4.2 have been found as tonoplast transporters, which allow leaving sulfate from the vacuoles to the cytosol [24,25]. It seems that phosphorylation modifications play key roles on activity of these transporters.

4. Materials and Methods

4.1. Identification of SULTR genes in C. sativa and B. napus

To recognize all sequences related the SULTR family, the amino acid sequence of two conserved domains, including Sulfate_transp (PF00916), and STAS (PF01740) were used as queries in BLASTP of Ensembl Plants (https://plants.ensembl.org/index.html) against protein database of *C. sativa* and *B. napus*. Furthermore, orthologue genes were identified

 doi:10.20944/preprints202210.0317.v1

following the same criteria in *Arabidopsis thaliana*, *Oryza sativa*, and *Glycine max*. All collected sequences were checked using NCBI Conserved Domain Database (CDD) [52], and Pfam database [53] to confirm the presence of domains related to SULTRs. Physiochemical properties, including molecular weight (MW), instability index, isoelectric points (pI), and GRAVY of SULTRs were predicted using the ProtParam tool [54]. The TMHMM Server version 2.0 was used to predict the transmembrane structure of SULTRs in *C. sativa* and *B. napus* [55].

4.2. Phylogenetic and conserved motif analyses

The amino acid sequences of all the recognized *SULTRs* from five plant species, i.e., *C. sativa*, *B. napus*, *A. thaliana*, *O. sativa*, and *G. max,* were aligned using an online tool, Clustal-Omega [56]. The whole phylogenetic relationships were constructed by the Maximum likelihood (ML) method with 1000 bootstrap replicates using the IQ-TREE webserver [57]. Finally, a phylogenetic tree was designed using the interactive tree of life (iTOL version 5) tool [58]. The conserved protein motifs in SULTR members in *C. sativa*, and *B. napus* were identified using the Multiple Expectation Maximization for Motif Elicitation program (MEME version 5.0.5) [59].

4.3. Promoter analysis

In this study, 1500 bp upstream of the start codon of *SULTRs* was selected as promoter site, and these regions in *C. sativa*, and *B. napus* were downloaded from Ensembl Plants. The sequence of each promoter site was screened to identify the conserved cis-acting regulatory elements using the PlantCARE tool [60], and cis-acting elements were classified based on their function.

4.4. Ka/Ks Ratio and duplication Analysis

In the present study, pairs of *SULTR* genes in each species (*C. sativa*, and *B. napus*) with identity of more than 85% were considered as duplicated genes [61]. Besides, the synonymous (Ks) and non-synonymous (Ka) indexes were calculated for all gene pair in each species using the MEGAX software [62]. The time of divergence of duplicated *SULTR* genes was estimated by the following equation, $T = (Ks/2\lambda) \times 10{-}6$. ($\lambda = 6.5 \times 10{-}9$) [63]. In addition, the synteny relationships of SULTRs for each species and between the orthologous of *C. sativa*, and *B. napus* were drawn by Circos software [64].

4.5. Gene expression analysis

Herein, the available RNA-seq data related to *C. sativa* and *B. napus* was screened to extract the expression levels of *SULTR* genes. Four RNA-seq datasets of *C. sativa*, including SRR935368 (root tissue), SRR935362 (leaf tissue), SRR935365 (stem tissue), and SRR935369 (flower) were retrieved from the NCBI gene bank and analyzed. To extract the expression patterns of *SULTRs* in response to stress, RNA-seq datasets related to salt (SRR935382), drought (SRR935380), cadmium (SRR935383), cold stress (SRR935372), and control condition (SRR935385) were used. To analyze raw data, we used the FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and HISAT [65] to check the quality of and map the sequences, respectively. FPKM (fragments per kilobase of exon model per million mapped reads) was used to calculate the transcript levels of each *SULTR* in *C. sativa*. To illustrate the expression levels of *SULTRs* in *B. napus*, we utilized the RNA-Seq data of 18 tissues and in response to biotic and abiotic stresses from rapeseed cultivar ZhongShuang11 (ZS11) via the Brassica Expression Database [66]. The expression patterns of the target genes were extracted based on FPKM values. Finally, heatmaps were constructed based on log2 transformed method using TBtools software [67].

4.6. Prediction of protein 3D structure, modeling, pocket sites, and phosphorylation

 doi:10.20944/preprints202210.0317.v1

Herein, five proteins of each species, *C. sativa*, and *B. napus,* were candidates based on the phylogenetic tree, and finally, the three-dimensional structures of 10 SULTRs were predicted by the Phyre2 server [68]. In the next step, the predicted structures were checked through Ramachandran Plot Analysis [69]. The pocket site of each model was highlighted on the predicted structures. NetPhos 3.1 Server [70]with a potential value of more than 0.90 was applied to predict the phosphorylation site of SULTRs in *C. sativa*, and *B. napus*.

5. Conclusions

In this study, we identified and characterized 36 and 45 putative *SULTR* genes in two important oilseed crops, *Camelina sativa* and *Brassica napus*, as the first report. We found that the first duplication event had occurred in *SULTR* genes of *C. sativa*, and members of this family showed diverse structure and functions. Besides, several *SULTR* genes have uniquely been developed in *C. sativa* under evolution processes. Besides, SULTRs 3 were identified as a class of sulfate transporter family with high diversity. Overall, the results revealed new insights of structure and function of SULTRs in oilseed crops. However, further analyses related to functional studies need to disclose the role of SULTRs in development and growth processes as well as response to stimuli.

Supplementary Materials: Table S1. List of the identified *SULTRs* and their characteristics in *Camelina sativa* and *Brassica napus*. **Table S2** Ka/Ks values predicted in the duplicated gene pairs in the Sulfate transporter family in the *Camelina sativa* and *Brassica napus* genomes. **Figure S1** Synteny analysis of *SULTR* genes in the *Camelina sativa* (a), and *Brassica napus* genomes (b). **Figure S2** Distribution of cis-regulatory elements in promoter site of *SULTRs* in *Camelina sativa*. **Figure S3** Distribution of cis-regulatory elements in promoter site of *SULTRs* in *Brassica napus*

Author Contributions: Conceptualization, P.H., S.H., and S.F.; methodology, P.H., S.F.; software, P.H., S.E., and F.M-P.; writing—original draft preparation, P.H., and F.M-P.; writing—review and editing, P.H., and F.M-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Takahashi, H. Sulfate transport systems in plants: functional diversity and molecular mechanisms underlying regulatory coordination. *J. Exp. Bot.* **2019**, *70*, 4075–4087.
1. Li, Q.; Gao, Y.; Yang, A. Sulfur Homeostasis in Plants. *Int. J. Mol. Sci.* **2020**, *21*, 8926.
3. Faraji, S.; Heidari, P.; Amouei, H.; Filiz, E.; Poczai, P. Investigation and Computational Analysis of the Sulfotransferase (SOT) Gene Family in Potato (Solanum tuberosum): Insights into Sulfur Adjustment for Proper Development and Stimuli Responses. *Plants* **2021**, *10*, 2597.
4. Takahashi, H.; Buchner, P.; Yoshimoto, N.; Hawkesford, M.J.; Shiu, S.-H. Evolutionary relationships and functional diversity of plant sulfate transporters. *Front. Plant Sci.* **2012**, *2*, 119.
5. Gruber, B.D.; Giehl, R.F.H.; Friedel, S.; von Wirén, N. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol.* **2013**, *163*, 161–179.
6. Koprivova, A.; Kopriva, S. Sulfation pathways in plants. *Chem. Biol. Interact.* **2016**, *259*, 23–30.
7. Leustek, T.; Saito, K. Sulfate transport and assimilation in plants. *Plant Physiol.* **1999**, *120*, 637–644.
8. Shibagaki, N.; Grossman, A.R. Binding of cysteine synthase to the STAS domain of sulfate transporter and its regulatory consequences. *J. Biol. Chem.* **2010**, *285*, 25094–25102.
9. Smith, F.W.; Ealing, P.M.; Hawkesford, M.J.; Clarkson, D.T. Plant members of a family of sulfate transporters reveal functional subtypes. *Proc. Natl. Acad. Sci.* **1995**, *92*, 9373–9377.
10. Shibagaki, N.; Rose, A.; McDermott, J.P.; Fujiwara, T.; Hayashi, H.; Yoneyama, T.; Davies, J.P. Selenate-resistant mutants of Arabidopsis thaliana identify Sultr1; 2, a sulfate transporter required for efficient transport of sulfate into roots. *Plant J.* **2002**, *29*, 475–486.
11. Kumar, S.; Asif, M.H.; Chakrabarty, D.; Tripathi, R.D.; Trivedi, P.K. Differential expression and alternative splicing of rice sulphate transporter family members regulate sulphur status during plant growth, development and stress conditions. *Funct. Integr. Genomics* **2011**, *11*, 259–273.

12. Buchner, P.; Parmar, S.; Kriegel, A.; Carpentier, M.; Hawkesford, M.J. The sulfate transporter family in wheat: tissue-specific gene expression in relation to nutrition. *Mol. Plant* **2010**, *3*, 374–389.
13. Akbudak, M.A.; Filiz, E.; Kontbay, K. Genome-wide identification and cadmium induced expression profiling of sulfate transporter (SULTR) genes in sorghum (Sorghum bicolor L.). *Biometals* **2018**, *31*, 91–105.
14. Xun, M.; Song, J.; Shi, J.; Li, J.; Shi, Y.; Yan, J.; Zhang, W.; Yang, H. Genome-Wide Identification of Sultr Genes in Malus domestica and Low Sulfur-Induced MhSultr3; 1a to Increase Cysteine-Improving Growth. *Front. Plant Sci.* **2021**, 2114.
15. Rouached, H.; Secco, D.; Arpat, A.B. Getting the most sulfate from soil: regulation of sulfate uptake transporters in Arabidopsis. *J. Plant Physiol.* **2009**, *166*, 893–902.
16. Zheng, Z.-L.; Zhang, B.; Leustek, T. Transceptors at the boundary of nutrient transporters and receptors: a new role for Arabidopsis SULTR1; 2 in sulfur sensing. *Front. Plant Sci.* **2014**, *5*, 710.
17. Aarabi, F.; Naake, T.; Fernie, A.R.; Hoefgen, R. Coordinating sulfur pools under sulfate deprivation. *Trends Plant Sci.* **2020**.
18. Maruyama-Nakashita, A.; Nakamura, Y.; Yamaya, T.; Takahashi, H. Regulation of high-affinity sulphate transporters in plants: towards systematic analysis of sulphur signalling and regulation. *J. Exp. Bot.* **2004**, *55*, 1843–1849.
19. Takahashi, H.; Kopriva, S.; Giordano, M.; Saito, K.; Hell, R. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. *Annu. Rev. Plant Biol.* **2011**, *62*, 157–184.
20. Takahashi, H.; Watanabe-Takahashi, A.; Smith, F.W.; Blake-Kalff, M.; Hawkesford, M.J.; Saito, K. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in Arabidopsis thaliana. *Plant J.* **2000**, *23*, 171–182.
21. Cao, M.; Wang, Z.; Zhao, Q.; Mao, J.; Speiser, A.; Wirtz, M.; Hell, R.; Zhu, J.; Xiang, C. Sulfate availability affects ABA levels and germination response to ABA and salt stress in A rabidopsis thaliana. *Plant J.* **2014**, *77*, 604–615.
22. Kataoka, T.; Hayashi, N.; Yamaya, T.; Takahashi, H. Root-to-shoot transport of sulfate in Arabidopsis. Evidence for the role of SULTR3; 5 as a component of low-affinity sulfate transport system in the root vasculature. *Plant Physiol.* **2004**, *136*, 4198–4204.
23. Zuber, H.; Davidian, J.-C.; Aubert, G.; Aimé, D.; Belghazi, M.; Lugan, R.; Heintz, D.; Wirtz, M.; Hell, R.; Thompson, R. The seed composition of Arabidopsis mutants for the group 3 sulfate transporters indicates a role in sulfate translocation within developing seeds. *Plant Physiol.* **2010**, *154*, 913–926.
24. Kataoka, T.; Watanabe-Takahashi, A.; Hayashi, N.; Ohnishi, M.; Mimura, T.; Buchner, P.; Hawkesford, M.J.; Yamaya, T.; Takahashi, H. Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in Arabidopsis. *Plant Cell* **2004**, *16*, 2693–2704.
25. Wang, L.; Chen, K.; Zhou, M. Structure and function of an Arabidopsis thaliana sulfate transporter. *Nat. Commun.* **2021**, *12*, 1–8.
26. Parmar, S.; Buchner, P.; Hawkesford, M.J. Leaf developmental stage affects sulfate depletion and specific sulfate transporter expression during sulfur deprivation in Brassica napus L. *Plant Biol.* **2007**, *9*, 647–653.
27. Ding, Y.; Zhou, X.; Zuo, L.; Wang, H.; Yu, D. Identification and functional characterization of the sulfate transporter gene GmSULTR1; 2b in soybean. *BMC Genomics* **2016**, *17*, 1–19.
28. Vatansever, R.; Koc, I.; Ozyigit, I.I.; Sen, U.; Uras, M.E.; Anjum, N.A.; Pereira, E.; Filiz, E. Genome-wide identification and expression analysis of sulfate transporter (SULTR) genes in potato (Solanum tuberosum L.). *Planta* **2016**, *244*, 1167–1183.
29. Huang, Q.; Wang, M.; Xia, Z. The SULTR gene family in maize (Zea mays L.): Gene cloning and expression analyses under sulfate starvation and abiotic stress. *J. Plant Physiol.* **2018**, *220*, 24–33.
30. Huang, S.Q.; Xiang, A.L.; Che, L.L.; Chen, S.; Li, H.; Song, J.B.; Yang, Z.M. A set of miRNAs from Brassica napus in response to sulphate deficiency and cadmium stress. *Plant Biotechnol. J.* **2010**, *8*, 887–899.
31. Kumar, S.; Asif, M.H.; Chakrabarty, D.; Tripathi, R.D.; Dubey, R.S.; Trivedi, P.K. Comprehensive analysis of regulatory elements of the promoters of rice sulfate transporter gene family and functional characterization of OsSul1; 1 promoter under different metal stress. *Plant Signal. Behav.* **2015**, *10*, e990843.
32. Brock, J.R.; Dönmez, A.A.; Beilstein, M.A.; Olsen, K.M. Phylogenetics of Camelina Crantz.(Brassicaceae) and insights on the origin of gold-of-pleasure (Camelina sativa). *Mol. Phylogenet. Evol.* **2018**, *127*, 834–842.
33. Yuan, L.; Li, R. Metabolic engineering a model oilseed Camelina sativa for the sustainable production of high-value designed oils. *Front. Plant Sci.* **2020**, *11*, 11.
34. Ahmadizadeh, M.; Rezaee, S.; Heidari, P. Genome-wide characterization and expression analysis of fatty acid desaturase gene family in Camelina sativa. *Gene Reports* **2020**, *21*, 100894.
35. Faraji, S.; Ahmadizadeh, M.; Heidari, P. Genome-wide comparative analysis of Mg transporter gene family between Triticum turgidum and Camelina sativa. *BioMetals* **2021**, *4*.
36. Lošák, T.; Hlusek, J.; Martinec, J.; Vollmann, J.; Peterka, J.; Filipcik, R.; Varga, L.; Ducsay, L.; Martensson, A. Effect of combined nitrogen and sulphur fertilization on yield and qualitative parameters of Camelina sativa [L.] Crtz.(false flax). *Acta Agric. Scand. Sect. B-Soil Plant Sci.* **2011**, *61*, 313–321.
37. Solis, A.; Vidal, I.; Paulino, L.; Johnson, B.L.; Berti, M.T. Camelina seed yield response to nitrogen, sulfur, and phosphorus fertilizer in South Central Chile. *Ind. Crops Prod.* **2013**, *44*, 132–138.
38. Heydarian, Z.; Yu, M.; Gruber, M.; Coutu, C.; Robinson, S.J.; Hegedus, D.D. Changes in gene expression in Camelina sativa roots and vegetative tissues in response to salinity stress. *Sci. Rep.* **2018**, *8*, 1–22.
39. Abdullah; Faraji, S.; Mehmood, F.; Malik, H.M.T.; Ahmed, I.; Heidari, P.; Poczai, P. The GASA Gene Family in Cacao (Theobroma cacao, Malvaceae): Genome Wide Identification and Expression Analysis. *Agronomy* **2021**, *11*, 1425.

40. Faraji, S.; Filiz, E.; Kazemitabar, S.K.; Vannozzi, A.; Palumbo, F.; Barcaccia, G.; Heidari, P. The AP2/ERF Gene Family in Triticum durum: Genome-Wide Identification and Expression Analysis under Drought and Salinity Stresses. *Genes (Basel).* **2020**, *11*, 1464.
41. Musavizadeh, Z.; Najafi-Zarrini, H.; Kazemitabar, S.K.; Hashemi, S.H.; Faraji, S.; Barcaccia, G.; Heidari, P. Genome-Wide Analysis of Potassium Channel Genes in Rice: Expression of the OsAKT and OsKAT Genes under Salt Stress. *Genes (Basel).* **2021**, *12*, 784.
42. Koralewski, T.E.; Krutovsky, K. V Evolution of exon-intron structure and alternative splicing. *PLoS One* **2011**, *6*, e18055.
43. Heidari, P.; Puresmaeli, F.; Mora-Poblete, F. Genome-Wide Identification and Molecular Evolution of the Magnesium Transporter (MGT) Gene Family in Citrullus lanatus and Cucumis sativus. *Agronomy* **2022**, *12*, 2253.
44. Rezaee, S.; Ahmadizadeh, M.; Heidari, P. Genome-wide characterization, expression profiling, and post- transcriptional study of GASA gene family. *Gene Reports* **2020**, *20*, 100795.
45. Heidari, P.; Faraji, S.; Poczai, P. Magnesium transporter Gene Family: Genome-Wide Identification and Characterization in Theobroma cacao, Corchorus capsularis and Gossypium hirsutum of Family Malvaceae. *Agronomy* **2021**, *11*, 1651.
46. Zhang, Z.; Li, J.; Zhao, X.-Q.; Wang, J.; Wong, G.K.-S.; Yu, J. KaKs_Calculator: calculating Ka and Ks through model selection and model averaging. *Genomics. Proteomics Bioinformatics* **2006**, *4*, 259–263.
47. Visser, R.G.F.; Bachem, C.W.B.; de Boer, J.M.; Bryan, G.J.; Chakrabati, S.K.; Feingold, S.; Gromadka, R.; van Ham, R.C.H.J.; Huang, S.; Jacobs, J.M.E. Sequencing the potato genome: outline and first results to come from the elucidation of the sequence of the world's third most important food crop. *Am. J. Potato Res.* **2009**, *86*, 417–429.
48. Yoshimoto, N.; Takahashi, H.; Smith, F.W.; Yamaya, T.; Saito, K. Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis roots. *Plant J.* **2002**, *29*, 465–473.
49. Gigolashvili, T.; Kopriva, S. Transporters in plant sulfur metabolism. *Front. Plant Sci.* **2014**, *5*, 442.
50. Heidari, P.; Mazloomi, F.; Nussbaumer, T.; Barcaccia, G. Insights into the SAM Synthetase Gene Family and Its Roles in Tomato Seedlings under Abiotic Stresses and Hormone Treatments. *Plants* **2020**, *9*, 586.
51. Heidari, P.; Ahmadizadeh, M.; Izanlo, F.; Nussbaumer, T. In silico study of the CESA and CSL gene family in Arabidopsis thaliana and Oryza sativa: Focus on post-translation modifications. *Plant Gene* **2019**, *19*.
52. Marchler-Bauer, A.; Derbyshire, M.K.; Gonzales, N.R.; Lu, S.; Chitsaz, F.; Geer, L.Y.; Geer, R.C.; He, J.; Gwadz, M.; Hurwitz, D.I. CDD: NCBI's conserved domain database. *Nucleic Acids Res.* **2015**, *43*, D222–D226.
53. Finn, R.D.; Bateman, A.; Clements, J.; Coggill, P.; Eberhardt, R.Y.; Eddy, S.R.; Heger, A.; Hetherington, K.; Holm, L.; Mistry, J. Pfam: the protein families database. *Nucleic Acids Res.* **2014**, *42*, D222–D230.
54. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, M.R.; Appel, R.D.; Bairoch, A. Protein identification and analysis tools on the ExPASy server. In *The Proteomics Protocols Handbook*; Humana Press: Totowa, NJ, 2005; pp. 571–607.
55. Möller, S.; Croning, M.D.R.; Apweiler, R. Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics* **2001**, *17*, 646–653.
56. Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T.J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J.; et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **2011**, *7*, 539.
57. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating Maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274.
58. Letunic, I.; Bork, P. Interactive Tree of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Res.* **2019**, *47*, W256–W259.
59. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37*, W202–W208.
60. Lescot, M.; Déhais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouzé, P.; Rombauts, S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327.
61. Heidari, P.; Faraji, S.; Ahmadizadeh, M.; Ahmar, S.; Mora-Poblete, F. New insights into structure and function of TIFY genes in Zea mays and Solanum lycopersicum: a genome-wide comprehensive analysis. *Front. Genet.* **2021**, *12*, 534.
62. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549.
63. Yang, S.; Zhang, X.; Yue, J.-X.; Tian, D.; Chen, J.-Q. Recent duplications dominate NBS-encoding gene expansion in two woody species. *Mol. Genet. Genomics* **2008**, *280*, 187–198.
64. Krzywinski, M.; Schein, J.; Birol, I.; Connors, J.; Gascoyne, R.; Horsman, D.; Jones, S.J.; Marra, M.A. Circos: an information aesthetic for comparative genomics. *Genome Res.* **2009**, *19*, 1639–1645.
65. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *12*, 357–360.
66. Chao, H.; Li, T.; Luo, C.; Huang, H.; Ruan, Y.; Li, X.; Niu, Y.; Fan, Y.; Sun, W.; Zhang, K. BrassicaEDB: a gene expression database for Brassica crops. *Int. J. Mol. Sci.* **2020**, *21*, 5831.
67. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202.
68. Kelley, L.A.; Mezulis, S.; Yates, C.M.; Wass, M.N.; Sternberg, M.J.E. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* **2015**, *10*, 845–858.

 doi:10.20944/preprints202210.0317.v1

69. Lovell, S.C.; Davis, I.W.; Arendall III, W.B.; De Bakker, P.I.W.; Word, J.M.; Prisant, M.G.; Richardson, J.S.; Richardson, D.C. Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. *Proteins Struct. Funct. Bioinforma.* **2003**, *50*, 437–450.
70. Blom, N.; Sicheritz-Pontén, T.; Gupta, R.; Gammeltoft, S.; Brunak, S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. *Proteomics* **2004**, *4*, 1633–1649.