Genome-wide identification of 2-oxoglutarate and Fe (II)-dependent dioxygenase family genes and their expression profiling under drought and salt stress in potato (#84244)

First revision

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Genome-wide identification of 2-oxoglutarate and Fe (II)-dependent dioxygenase family genes and their expression profiling under drought and salt stress in potato

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2-Oxoglutatrate-dependent dioxygenases (2OGDs) comprises of 2-Oxoglutatrate and Fe(II)-dependent dioxygenases (2ODD) enzyme family. 2OGDs are involved in the biosynthesis of various compounds like gibberellin, ethylene etc, and in various catabolism pathways such as auxin catabolism, salicylic acid catabolism. Despite their important roles *2ODDs* have not been studied in potato, which is the third most important crop globally. In this study, comprehensive genome wide analysis was done to identify all *2ODDs* in potato and the putative genes were analysed for the presence of signature 2OG-FeII_Oxy (PF03171) domain and the conserved DIOX_N (PF14226) domain. A total of 205 *St2ODDs* were identified and classified into eight groups based on their function. The physiochemical properties, gene structures and motifs were analysed and gene duplication events were also searched for *St2ODDs.* The active amino acid residues responsible for binding with 2-oxoglutarate and Fe (II) were conserved throughout the *St2ODDs.* The three dimensional (3D) structures of the representative members of Flavanol synthase (FNS), 1-aminocyclopropane-1-carboxylic acid oxidases (ACOs) and Gibberellin oxidases (GAOXs) were made and docked with their representative substrates and the potential interactions are visualised. The *St2ODDs* were also analysed for their expression patterns in abiotic stresses like heat, salt, and drought. We found altered expression levels of *St2ODDs* under abiotic stress conditions which was further confirmed for drought and salt stress using qRT-PCR. The expression levels of *St2ODD115, St2ODD34,* and *St2ODD99* were found to be upregulated in drought stress with 2.2, 1.8, and 2.6 fold change respectively. While after rewatering the expression levels were normal. In salt stress, the expression levels of *St2ODD151, St2ODD76, St2ODD91,* and *St2ODD34* were found to be upregulated after 24 hour (h), 48 hour (h), 72 hour (h), and 96 hour (h). Altogether, the elevated expression levels suggests the importance of *St2ODDs* under abiotic stresses i.e. drought and salt. Overall our study provided a knowledgebase for *2ODDs* gene family in potato which can be used further to study the important roles of *2ODDs* in potato plant.

Genome-wide identification of 2-oxoglutarate and Fe (II)-dependent dioxygenase family genes and their expression profiling under drought and salt stress in potato

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Abstract

2-Oxoglutatrate-dependent dioxygenases (2OGDs) comprises of 2-Oxoglutatrate and Fe(II)-dependent dioxygenases (2ODD) enzyme family. 2OGDs are involved in the biosynthesis of various compounds like gibberellin, ethylene etc, and in various catabolism pathways such as auxin catabolism, salicylic acid catabolism. Despite their important roles *2ODDs* have not been studied in potato, which is the third most important crop globally. In this study, comprehensive genome wide analysis was done to identify all *2ODDs* in potato and the putative genes were analysed for the presence of signature 2OG-FeII_Oxy (PF03171) domain and the conserved DIOX_N (PF14226) domain. A total of 205 *St2ODDs* were identified and classified into eight groups based on their function. The physiochemical properties, gene structures and motifs were analysed and gene duplication events were also searched for *St2ODDs.* The active amino acid residues responsible for binding with 2-oxoglutarate and Fe (II) were conserved throughout the *St2ODDs.* The three dimensional (3D) structures of the representative members of Flavanol synthase (FNS), 1-aminocyclopropane-1-carboxylic acid oxidases (ACOs) and Gibberellin oxidases (GAOXs) were made and docked with their representative substrates and the potential interactions are visualised. The *St2ODDs* were also analysed for their expression patterns in abiotic stresses like heat, salt, and drought. We found altered expression levels of *St2ODDs* under abiotic stress conditions which was further confirmed for drought and salt stress using qRT-PCR. The expression levels of *St2ODD115, St2ODD34,* and *St2ODD99* were found to be upregulated in drought stress with 2.2, 1.8, and 2.6 fold change respectively. While after rewatering the expression levels were normal. In salt stress, the expression levels of *St2ODD151, St2ODD76,*

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1.Introduction

The 2-Oxoglutarate-dependent dioxygenases (2OGDs) are iron containing, non-heme enzymes, that localize in cytosol and converts 2-Oxoglutarate to succinate and carbon dioxide. 2OGDs are ubiquitously distributed in nature ranging from bacteria, fungi, plants, to vertebrates and parts in various oxygenation and hydroxylation reactions ;(Carolis & Luca 1994; Cheng et al., 2014; Jiang et al., 2021). 2OGDs superfamily comprise largest enzyme family in plant genome after cytochrome P450 monooxygenases (CYPs). 2OGDs are very divergent in amino acid sequences and are involved in various metabolic pathways as well. Proline hydroxylase was the first identified 2OGD which requires ferrous ion as cofactor and α-ketoglutarate, ascorbate (Hutton & Tappel, 1966; Ge et al., 2021). Earlier genome exploration of six model plant species classified 2OGDs in three broad classes: DOXA, DOXB, and DOXC based on their amino acid sequence similarity (Kawai, Ono & Mizutani., 2014a; Wang et al., 2022). Among the three classes of 2OGDs proteins, DOXA is composed of *Escherichia coli* AlkB plant homologs which undergoes oxidative demethylation of alkylated nucleic acids and histones (Falnes et al., 2002)(Trewick et al., 2002). DOXB class undergoes hydroxylation of proline in cell wall protein synthesis (Keskiaho et al.; Hieta & Myllyharju, 2002). DOXC class is the most important class of 2OGDs as it is involved in plant metabolism and are involved in various pathways including steroidal glycoalkaloids (SGA), and flavonoid biosynthesis (Hagel & Facchini, 2018) (Sonawane et al., 2022). In DOXC class, 2-Oxoglutatrate and Fe(II)-dependent dioxygenases (2ODD) gene family lies. 2ODDs have the signature 2OG-FeII_Oxy (PF03171) domain and also have the conserved DIOX_N (PF14226) domain (Kawai, Ono & Mizutani, 2014b). 2ODDs are involved in plant secondary metabolism. They also serves important roles in various biosynthesis and catabolism pathways including, Gibberellin biosynthesis, Ethylene biosynthesis, Auxin catabolism, Salicylic acid catabolism, and SGA metabolism, (Farrow et al., 2014; Sonawane et al., 2022). The versatility of 2ODD enzymes

in various biosynthetic pathways for important metabolite synthesis and normal plant functioning makes the study of 2ODD gene family important (Pan et al., 2017).

Potato (*Solanum tuberosum)* is an important cash crop, and is the world's third most important crop. It is globally consumed and is a rich source of carbohydrates and vitamins. It is also used to make commercial food products as well (You et al., 2019; Alok et al., 2022; Zaki & Radwan, 2022). However, various anti-nutritional compounds are also associated with potato like α-solanine and α-chaconine which belongs to *St2ODDs*. A-solanine and α-chaconine are plant secondary metabolites and concentrations ranging 200-400 mg are toxic to humans (Machado, Toledo & Garcia, 2007) (Liu et al., 2021). Prior studies have targeted these secondary metabolites and reported to reduce the concentration of these antinutritional compounds through metabolic profiling (Nakayasu et al., 2018). So it becomes important to study the *2ODD* gene family.

Besides this potato yield is affected by various abiotic stresses like heat, drought and salt, and therefore, it becomes important to identify stress responsive *St2ODDs* affecting the secondary metabolites. Tuber yield is of much interest in order to develop new crop varieties resistant to elevated abiotic stresses in nature .

In potato, the *2ODD* gene family has not been characterised. In this study, *St2ODD*s belonging to the DOXC class were studied and identified 205 *2ODD* genes. Further these identified genes were systematically analysed for their gene structure, conserved motifs, physiological properties, evolutionary relationship, chromosomal distribution, duplication events i.e. tandem duplication, and segmental duplication. Active sites were also predicted for substrate binding and co-factor (Fe-II) binding. 3D structures of Flavanol synthase (FNS), 1-aminocyclopropane-1-carboxylic acid oxidases (ACOs) and Gibberellin oxidases (GAOXs) were predicted and docking interations were studied with specific substrates. In addition, the expression levels of *St2ODD* genes were measured under abiotic stresses: salt, drought and heat, to explore their roles with respect to each condition which was further validated for salt, and drought stress by qRT-PCR. Our results identified *St2ODD* genes in potato showing changes in expression under drought, salt stress and established knowledge domain and theoretical basis for further improvement of potato and potato breeding.

2.Materials and Methods

2.1.Retrieval and identification of potential *2ODDs* genes from potato

Firstly, the proteome of *S. tuberosum* was retrieved from Spud DB (http://spuddb.uga.edu) DM1-3 v6.1 Click or tap here to enter text.(Pham et al., 2017). Reference sequence of *2ODDs* of *S. lycopersicum, S. chacoense, S. melongena, Manihot esculents, Capsicum annuum, Arabidopsis thaliana, Nicotiana tabacum* were downloaded from National Centre for Biotechnology Institute (NCBI) protein database. The reference genes were Blastp (e values of <0.001) searched against potato proteome (Verma & Singh, 2021). Secondly, Hidden Markov Model (HMM) profiles of *St2ODDs* were fetched from the pfam database and the retrieved sequences were searched (e value cut-off 1e-05) against HMM profiles. The candidate sequences obtained from both the methods were considered as putative *2ODDs* genes and were further analysed for *2ODDs* domains (2OG-FeII_Oxy, pfam03171 and DIOX_ N, pfam14226) using various servers like SMART (http://smart.embl-heidelberg.de/) (Letunic, Khedkar & Bork, 2021), and NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler-Bauer et al., 2015).

2.2.Gene structure, motif analysis and chromosomal mapping

The diversity of *St2ODDs* genes were further analysed by studying the gene structure and conserved protein domains. To visualize the intron/exon structure, the online tool Gene Structure Display Server (GSDS) 2.0 (http://gsds.gao-lab.org/index.php) (Hu et al., 2015) was used. Conserved motifs were identified using MEME online website (https://meme-suite.org/meme) with maximum number of motifs, 10; optimum width of each motif, between 50 and 100 residues; other parameters were set to default values (Bailey et al., 2015). Chromosomal locations of *2ODDs* were collected from Click or tap here to enter text. Spud DB (http://spuddb.uga.edu) DM1-3 v6.1 (Pham et al., 2017), and were distributed across the 12 chromosomes of potato using TBtools (https://github.com/CJ-Chen/TBtools/releases) (Chen et al., 2020). Nomenclature was given based on the order of chromosomal location of the genes.

2.3.Phylogenetic analysis and gene duplication

Multiple protein sequence of 205 identified genes were aligned using ClustalW in Molecular Evolutionary Genetics Analysis MEGA 7.0 (Kumar et al., 2016) (https://www.megasoftware.net/) and the phylogenetic tree was constructed with neighbour-joining (NJ) method with 1000

bootstrap replicates and complete deletion. The phylogenetic tree constructed was further analysed with ITOL tool (https://itol.embl.de/upload.cgi) (Letunic & Bork, 2007). Gene duplication events were considered with 80% or more identity with e value 1e-10. The synonymous substitution (Ks) rates and nonsynonymous substitution (Ka) rates of duplicated *2ODD* genes were calculated using Pal2nal http://www.bork.embl.de/pal2nal (Suyama, Torrents & Bork) and selection pressure was evaluated by calculating Ka/Ks ratio (Shumayla et al., 2019).

2.4.Protein Structure Analysis and 3D modelling

The physio-chemical properties and subcellular localization of *2ODDs* proteins were calculated using ProtParam ExPasy server (https://web.expasy.org/protparam/) (Walker et al., 2005) and ProtComp version 9.0 server (http://www.softberry.com) (Emanuelsson et al., 2000). SWISS-Model was used for 3D structure prediction of *St2ODDs* (Waterhouse et al., 2018) and visual representation of 3D structures of *St2ODDs* were done using UCSF Chimera (Pettersen et al., 2004). Ligand 3D models with PubChem CID 51 (2-Oxoglutaric acid), specific to FNS and GaOX and PubChem CID 769 (Bicarbonate) specific to ACCs were retrieved from PubChem database in SDF format (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2016) and converted in PDB format using PyMOL (Schrödinger, LLC).

2.5.Protein-ligand docking evaluation

The Molecular docking of *St2ODDs* and their various ligands were performed using AutoDock 4. The 3D conformation of various ligands were retrieved from PubChem in SDF format PubChem CID 51 (2-Oxoglutaric acid) and PubChem CID 769 (Bicarbonate). PyMOL Version 2.0 (Schrödinger, LLC) was utilized for converting it in PDB format. The PDB files were then converted to PDBQT format using AutoDock 4 (Morris et al., 2009). Various parameters were assigned including addition of non-polar hydrogens and gasteiger charges. Grid boxes were generated with different dimensions in X,Y, and Z directions which is shown in the supplemental information (Table S2). The proteins and ligands were docked with energy range of 4 and exhaustiveness set to 8 and the best conformation was selected having lowest free energy (Anand et al., 2022). The protein-ligand hydrophobic and hydrogen bond interactions were represented with BIOVIA Discovery Studio Visualizer (https://www.3ds.com/products-

services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/)Click or tap here to enter text..

2.6.Identification of abiotic stress responsive *2ODDs* genes

Sequence Read Archive (SRA) data corresponding to project number SRP056128, SRP229183, and SRP237987 for drought, heat and salt were downloaded from NCBI SRA. Differentially expressed genes were identified using Trinity-V 2.03 package, Transcript abundance was calculated in FPKM (Fragments per kilo-base of transcript per million mapped reads) and the heat map of expressed 2*ODD* genes under various stress conditions were visualized usingClick or tap here to enter text. TBtools (https://github.com/CJ-Chen/TBtools/releases) (Chen et al., 2020). Putative 2*ODD* genes responsive to stress were validated using RT-qPCR (Verma et al., 2022).

2.7.Plant materials and Treatments

Solanum tuberosum cv Kufri jyoti plantlets were received from ICAR-Central Potato Research Institute (CPRI, Shimla) and were maintained in growth chamber in soil under 16 hour (h) light/8 hour (h) dark photoperiod, 24^0 C, under 60 % humidity. The maintained seedlings were watered regularly. For drought stress, watering was with-held to mimic severe drought conditions for three days followed by rewatering for three days. The leaves of drought treated plants, after rewatering and control plants were harvested immediately in liquid nitrogen and then stored in -80° C. For salt stress, 4 weeks old plants grown in soil were subjected to 500 mmol/L NaCl. The salt treated plants were collected after 0 h, 24 h, 48 h, 72 h, and 96 h time courses. Collected leaf samples were stored immediately in liquid nitrogen followed by -80° C for further experimentation.

2.8.RNA isolation, cDNA preparation and qPCR analysis

Total RNA from the drought and salt stressed along with control leaf samples were extracted from the samples (Ghawana et al., 2011). cDNA was synthesized using the Superscript III first strand cDNA synthesis kit (Invitrogen USA) according to the manufacturer's instructions. Elongation factor 1 alfa (*ef1α*) was the reference gene for expression normalization. The expression levels of *St2ODD* genes in potatoes subjected to drought and salt stress were measured using qRT-PCR. Primers were designed using Primer 3 software (http://primer3.ut.ee) (Untergasser et al., 2012)

and the primer sequences are shown in the supplemental information (Table S3). The correlative expression data were calculated using Livak's method (Livak & Schmittgen, 2001). Three technical replicates of each biological replicates were performed in the qRT-PCR experiment.

3.Results

3.1.Identification of *2ODDs* in potato

The *2ODDs* belongs to the *2OGDs* gene superfamily and are involved in various plant metabolic pathways which are explored in previous studies (You et al., 2019; Farrow et al., 2014; Hagel & Facchini, 2018). Here, to identify the *2ODDs* in potato whole proteome was BLASTP searched against the reference *2ODDs* of related plants and all the putative genes were retrieved and examined for the presence of domains associated with *2ODDs* i.e. 2OG-FeII_Oxy (PF03171) and DIOX_N (PF14226) using various bioinformatics tools like SMART, and NCBI Conserved Domain Search. Eventually, a total of 205 *St2ODDs* were confirmed having the *2ODD* domains: 2OG-FeII_Oxy (PF03171) signature domain and DIOX_N (PF14226) domain. The physiochemical properties of these identified *St2ODDs* were studied and the coding sequence (CDS) lengths, genomic sequence lengths, molecular weights (MWs), isoelectric points (PIs), and grand average of hydropathicity index (GRAVY) of these genes are shown in Table S1. The MWs of *St2ODDs* ranged from 22.3 kDa to 108.5 kDa. *St2ODD30* had the smallest amino acid sequence of 198 aa and *St2ODD54* had the largest amino acid sequence of 910 aa. The exonic numbers ranged between 2 to 14 exons and the predicted PIs values ranged from 4.68 to 8.94, suggesting that *St2ODDs* consists of both basic and acidic proteins. The Subcellular localization analysis suggested that *St2ODDs* in potato were expressed either in the cytoplasm or secreted extracellularly. *St2ODDs* consists of many plant secondary metabolites which are localized in cytosol (Xu et al., 2008). This study predicted the same and gave insight to the basic aspects of *St2ODDs*.

3.2.Chromosomal distribution and gene duplication of *St2ODD* genes

The chromosomal location of 205 *St2ODDs* were identified using database and a chromosomal distribution map of *St2ODDs* were constructed (Fig. 1). The chromosomal distribution showed

that *St2ODDs* are widely distributed across the 12 chromosomes of potato. Chromosome 2 and 9 has the maximum of *St2ODD* genes (35/205) each. Chromosome 5 has the minimum number of *St2ODDs* (3/205). Our results suggests that most of the *St2ODDs* were distributed across the proximal ends of the chromosomes. For the expansion and generation of gene families, gene duplication plays a vital role which needed to be studied for *St2ODDs* in *S. tuberosum*. Thus, we analysed gene duplication events for *2ODDs* in *S. tuberosum* (Hofberger et al., 2015). Gene duplication events occurs due to uneven crossing over, chromosome duplication. The genes with 80% or more identity located on the same chromosome, were considered as duplication events. Tandem duplication is defined earlier as the duplication event within 5 Mb region of a chromosome while other duplication events are characterized as segmental duplication (Agarwal et al., 2016). Gene duplication events were analysed for *St2ODDs* genes and the identified events were confirmed for tandem duplication and segmental duplication for their genome wide expansion. On the basis of chromosomal location, gene clusters were observed on chromosome 1, 2, 6, 7, 9, and 11 (Fig. 1). A total of 139 duplicated genes were found on *S. tuberosum* genome which showed sixty seven percent of *St2ODDs* were originated by duplication events i.e. tandem and segmental (Fig. 1). Further, fifty-six percent of duplication events were tandem duplications and forty-four percent of duplication events were segmental duplications. This suggests that most of the expansion of *St2ODDs* in *S. tuberosum* is due to tandem duplication. The Synonymous (Ks) and Non-synonymous (Ka) values were calculated and their ratios (Ka/Ks) depicted the nature of selection. The Ka/Ks ratio > 1 depicts positive selection, Ka/Ks = 1 depicts neutral selection, and Ka/Ks < 1 depicts purifying selection or negative selection. For *St2ODDs* the Ka/Ks ratios were calculated and then evaluated whether the selection pressure was the driving force for evolution or not (Liu et al., 2014). In this study, 134/139 of duplicated *St2ODDs* experienced negative or purifying selection (Table 1) among them thirty three pairs of duplication events of *St2ODDs* had Ka/Ks less than 0.3 suggesting their lower functional divergence during evolution However 5 pairs of duplicated *St2ODDs* experienced positive selection..

3.3.Phylogenetic analysis of *2ODDs* in potato

To understand the phylogenetic relationship between all the identified *St2ODDs*, phylogenetic tree was constructed. Based on the phylogenetic tree *St2ODDs* could be divided into eight groups (1-8) on their functional aspects (Fig. 2) which were based on gene ontology studies and homology

with characterized *A. thaliana* proteins. Amongst them the largest group was containing 2-Oxoglutarate dioxygenases (2OGs). While 38 genes were having gibberellin oxidases (GAOXs) function. Flavanone Synthase (FNS) function was observed in 22 genes and 10 genes have 1-aminocyclopropane-1-carboxylic acid oxidases (ACOs) function. Other functional groups were observed including downy mildew resistance 6 (DMR6), senescence related genes (SRGs), Dioxygenase for auxin oxidation, and jasmonate induced oxygenase genes.

3.4.Gene structure and motif analysis of *St2ODDs*

St2ODDs have diversified gene structure as exonic number of the identified *St2ODDs* varied from 2 to 14 in number (Fig. 3A). *St2ODD54* contained maximum number of exons (14), according to Fig. 3. Next the protein sequence of all *St2ODDs* were examined for the presence of different motifs using Multiple Expectation Maximization for Motif Elicitation (MEME). We identified ten different conserved motifs named motif 1 to motif 10 (Fig. 4) dispersed throughout the protein sequences. Four motifs (motif 1, 2, 3, and 4) were distributed throughout on *St2ODDs* and motif 5-10 were present specifically in different clusters. Similar clusters showed similar distribution of motifs based on their functions. The conserved motifs and their sequences are shown in the supplemental information (Table S4).

Further the identified motifs were analysed and motif 6 contained active site amino acid residues Asparagine (N), Tyrosine (Y), Histidine (H) and Aspartic acid (D). Motif 1 contained active site amino acid residues Histidine (H), Arginine (R) and Serine (S) which are conserved throughout the eight groups showing the importance of motif 6 and motif 1 in *St2ODDs* enzyme family for functioning. The amino acids N, Y, R, and S are responsible for interacting with 2 OG and the amino acids H, H and D are responsible for interacting with Fe (II), which is a cofactor (Takehara et al., 2020). The conserved active amino acid residues suggests their potential role in interactions with their substrate and the functioning of these identified *St2ODDs.*

3.5.Protein-Ligand interactions of *St2ODDs*

The 3D structures of representative members of FNS, GaOx, and ACOs each group based on their function were modelled using SWISS-MODEL on the basis of homology. All the selected groups have vital roles in flavonoid biosynthesis, gibberellin biosynthesis and ethylene biosynthesis which

needed to be studied for further understanding the substrate binding and functioning of these proteins and are shown in Fig. 5. The active amino acid residues for 2OG binding and iron co-factor binding with FNS were previously described (Sun et al., 2015). Similarly, the active amino acid residues responsible for 2OG and iron co-factor binding with GaOx were previously described (Takehara et al., 2020). For ACOs the active residues were previously described and used as reference for finding the active amino acid residues in *St2ODDs*. (Zhang et al., 2004)The multiple sequence alignment of the 3 groups having FNS, GaOx, and ACO function were made and visualized in which the active amino acids N, Y, R, and S responsible for interacting with 2 OG were conserved for binding with FNS and GaOx. For ACO the binding site for bicarbonate were also conserved i.e. R and R. However the co-factor binding i.e. Fe-II, the amino acids (H, H, and D) were conserved in the 3 groups and showed in Fig. 6. These conserved residues suggests the functional importance of these residues for the possible interactions with substrates and co-factors. In this study few *St2ODDs*, have altered active amino acid residues inferring their possible functional divergence. For further validating the the involvement of these active amino acid residues, docking was performed. Hydrogen and hydrophobic interactions were studied between *St2ODD29, St2ODD124* of GaOXs and 2-Oxoglutaric acid (CID 51). *St2ODD85, St2ODD87* of FNS and 2-Oxoglutaric acid (CID 51). *St2ODD118, St2ODD120* of ACOs and Bicarbonate (CID 769) (Fig.7A-B). The docking results of *St2ODD29* and 2-Oxoglutaric acid showed hydrogen bond interaction with R292 and hydrophobic interactions with S294, Q234, E94. The docking results of *St2ODD124* and 2-Oxoglutaric acid showed hydrogen bond interactions with R263, S265 and hydrophobic interactions with L205, S207. Interactions of *St2ODD118* and bicarbonate showed hydrogen bonds with R244, Q239 and hydrophobic interactions with A238, Y162. Interactions of *St2ODD120* and bicarbonate showed hydrogen bonds with R235 and hydrophobic interactions with R175, H234, H177 (Fig.7C-D). The docking results of *St2ODD85* and 2-Oxoglutaric acid showed hydrogen bond interaction with S317 and hydrophobic interactions with R315, Y232. The docking results of *St2ODD87* and 2-Oxoglutaric acid showed hydrogen bond interactions with S317, L264, I316 and hydrophobic interactions with R315 (Fig. 7E-F). These results validated the role of the conserved amino acids in stabilizing and interacting with the substrates responsible for leading to the plant pathways.

3.6.Expression pattern of *St2ODDs* under drought, salt, and heat stresses

Abiotic stresses plays a crucial role in the growth and yield of the potato and to explore the potential roles of *St2ODDs* against these abiotic stresses, expression levels of *St2ODDs* under drought, salt, and heat treatments were measured using available RNA-seq data. On the basis of log 2 fragments per kilobase of transcript per million fragments (FPKM) values, expression patterns were identified (Fig. 8). Under salt stress, 71 genes were differentially expressed, 39% (15/38) of gibberellin oxidases (GAOXs) which part in GA biosynthesis and catabolism pathway are expressed in salt stress (Hu et al 2021) and 30 % (3/10) of 1-aminocyclopropane-1-carboxylic acid oxidases (ACOs) which part in ethylene biosynthesis are expressed in salt stress (Chang et al., 2019) (Fig7). Under heat stress, seven genes were upregulated among them *St2ODD113* belongs to GAOXs and fifteen genes were downregulated *St2ODD125* and *St2ODD119* encodes GAOXs and ACOs respectively (Fig. 7). Under drought stress, twenty nine genes were differentially expressed *St2ODD130, St2ODD54,* and *St2ODD25* were upregulated. Thirteen genes were downregulated under drought stress. *St2ODD73*, *St2ODD10, St2ODD34, St2ODD99, St2ODD127, St2ODD17, St2ODD125,* and *St2ODD30* belongs to the GaOx group. *St2ODD115* and *St2ODD56* belongs to the ACOs group. *St2ODD195, St2ODD189,* and *St2ODD193* belongs to the FNS group. The expression patterns of *St2ODDs* gene family suggested their role in various stress conditions and some of them are downregulating or upregulating in all three stresses or in groups suggesting their role in accordance to abiotic stress.

3.7.Effect of *St2ODDs* expression under drought and salt stress

To evaluate the role of *St2ODDs* under drought stress and salt stress the gene expression levels of nine *St2ODDs* were done. The gene expression levels of *St2ODD54, St2ODD25, St2ODD130, St2ODD22,* and *St2ODD112* were measured under drought treatment after 3 days and in rewatering after 3 days by qRT-PCR. For salt treatment, the gene expression levels of *St2ODD138, St2ODD76, St2ODD91,* and *St2ODD34* were measured after 24 h, 48 h, 72 h, and 96 h. The genes were selected based on the FPKM values, the higher value change genes were selected and validated via qRT-PCR.

The expression levels of *St2ODDs* under drought stress were analysed in which *St2ODD130*, *St2ODD54* and *St2ODD25* showed increased relative expression under drought stress with 2.26 Fold change (FC), 1.71 Fold change (FC) and 2.5 Fold change (FC) respectively (Fig. 9A). However, the relative expression of *St2ODD22,* and *St2ODD112* downregulated in drought

condition with 0.47 FC, and 0.45 FC respectively but showed significant change in FC after rewatering.

Under salt stress, the expression levels of the *St2ODDs* were measured in one month old seedlings at different time points, i.e. 24 h, 48 h, 72 h, and 96 h. Four genes showed increased expression levels i.e. *St2ODD138, St2ODD76, St2ODD91* and *St2ODD34* throughout the salt stress which is in accordance with the expression patterns of the available RNASeq data (Fig. 9B). *St2ODD138* was upregulated with FC of 1.77 after 24 h, 3.04 FC after 48 h, 4.1 FC after 72 h, and 5.71 FC after 96 h. *St2ODD76* was upregulated with FC of 1.42 after 24 h, 3.44 FC after 48 h, 5.58 FC after 72 h, and 8.66 FC after 96 h. *St2ODD91* was upregulated consistently throughout with FC of 3.61 after 24 h, 5.18 FC after 48 h, 7.6 FC after 72 h, and 16.7 FC after 96 h (Fig. 8B) *St2ODD34* was upregulated with FC of 1.35 after 24 h, 1.99 FC after 48 h, 3.3 FC after 72 h, and 3.71 FC after 96 h.

The elevated relative expression levels suggests the potential involvement of *St2ODDs* with respect to the abiotic stresses which poses huge agricultural loss globally to the potato.

4.Discussion

Potato is the third most important crop consumed globally and is of great significance to humans. Its growth, tuber size and tuber number is affected by various environmental stresses (Schafleitner et al., 2007; Eiasu, Soundy & Hammes, 2007). Various abiotic factors affecting potato yield are salinity, drought, and heat (Deblonde & Ledent, 2001; Levy & Veilleux, 2007). The *2ODDs* belongs to the *2OGDs* superfamily which is the second largest family in plants. They plays a crucial role in an array of biological processes involved in plant metabolism including in gibberellin biosynthesis, ethylene biosynthesis, auxin catabolism, etc and are involved in secondary metabolism and are of great research interest (Farrow et al., 2014). However, *2ODDs* are also elevated under various abiotic stresses including cold, salt, drought stress (Mahajan, Sudesh & Yadav, 2014; Meng et al., 2015; Wang et al., 2020a). Genome wide analysis is an important approach for identifying the biological roles of *St2ODDs* gene family in potato. The *2ODDs* have been characterized previously in *S. lycopersicum* and *C. sativa*, interestingly, the *St2ODDs* have not been characterised in *S. tuberosum* (Potato) (Wei et al.; Zhu et al., 2022).

The approach for identifying *St2ODDs* using BlastP search the known proteins of related families against potato proteome. HMM profiles of *St2ODDs* from Pfam database were searched against HMM profiles were followed as reported earlier (Shumayla et al., 2019; Verma & Singh, 2021). Our study promotes the evolutionary, functional, structural aspects, interaction aspects of *St2ODDs* family genes with different substrates in potato. Here we identified a total of 205 *2ODDs* in potato which is comparable to the 131 *Sl2ODDs* identified by Wei et al., 2021 in tomato having various functions like ACOs, GAOxs, and F3H etc. The identified *St2ODDs* were thoroughly distributed across the 12 chromosomes of potato and the gene duplication events i.e tandem and segmental duplication revealed that majority of duplication events (56%) are due to tandem duplications. Moreover, sixty seven percent of *St2ODDs* family expansion is due to the duplication events. The non-synonymous (Ka) and synonymous (Ks) values and their ratios were calculated for determining the nature of selection for the expansion of *St2ODDs* in potato. The results infer that most of the *St2ODDs* were expanded as a result of negative selection or purifying selection and have lower functional divergence.

The identified *St2ODDs* were analysed for evolutionary relationship and based on their functions classified in eight groups having ACOs, involving in ethylene biosynthesis (Chang et al., 2019), GAOxs, involving in gibberellin biosynthesis (Huang et al., 2015), FNS, involving in flavonoid biosynthesis pathway (Tohge, Perez De Souza & Fernie, 2017) and the classification is consistent with the previous studies (Wei et al., 2021). All the identified *St2ODDs* have conserved 2OG-FeII_Oxy (PF03171) signature domain and also have the conserved DIOX_N (PF14226) domain which is a characteristic of the *2ODD* superfamily (Kawai, Ono & Mizutani, 2014b). Motif analysis showed that similar clusters have similar motif structure and Motif 1, 2, 3, and 6 were present throughout the *St2ODDs* inferring to have important roles in the activity of *St2ODDs.* Motif 6 contained active site amino acid residues Y, H & D and motif 1 contained active site amino acid residues H, R and S inferring their roles in binding with specific substrate and cofactor i.e. Fe(II). Few motifs were specific to certain genes like motif 8 and 10 was specific to St2ODD203, St2ODD187, St2ODD93 etc. The roles of these specific motifs further needs to be studied for their precise relationship and they may have functional specificity in different class.

The protein sequences of the identified *St2ODDs* were aligned and the active amino sites were predicted, the alignment showed that *St2ODDs* possessed ($HxDx_nH$ and YxnRxS) motifs specific to Fe(II) and 2ODD binding (Takehara et al., 2020). Similarly, sequence alignment of FLS1 (flavonol synthase), belonging to the 2ODD superfamily in various plants including *T. aestivum, A .thaliana, Dendrobium officinale* etc. showed similar active amino acid residues (Yu et al., 2021). The active amino acid residues were also conserved in ANS (anthocyanidin synthase) belonging to the 2ODD (Xu et al., 2008) and inferring their functional importance for the functioning of these enzymes. However, few *St2ODDs* didn't have the conserved active amino acid residues suggesting plausible alterations in their functionality during evolution. The protein 3D structures of the *St2ODDs*representative members of GaOx, FNS, and ACC were made and visualised for active residues Y, R, and S and RxR(Fig5) responsible for the binding with 2OG and bicarbonate respectively. All the represented members had the residues in close vicinity in the protein 3D structures as shown in earlier studies (Takehara et al., 2020.) which confirms the presence of binding sites for the substrate. After docking with specific substrates, results showed potential hydrogen and hydrophobic interactions between *St2ODD29, St2ODD124* of GaOXs and 2-Oxoglutaric acid (CID 51). *St2ODD85, St2ODD87* of FNS and 2-Oxoglutaric acid (CID 51). *St2ODD118, St2ODD120* of ACOs and Bicarbonate (CID 769). Various hydrophobic interactions were involved which may facilitates the stabilisation of the complex. Our results suggests the presence of conserved residues in *St2ODDs* and their potential interactions with their respective substrates. The physiochemical properties of these identified *St2ODDs* were analysed and various properties like amino acid residues, molecular weight and exonic number i.e. 2 to 14 in number were in compliance with the previous studies (Wei et al., 2021) which infers the similarity in physiochemical properties of the 2ODDs across plants.

Abiotic stresses plays an important role in the growth, and, yield of the plant. Potato is influenced by various abiotic stresses including heat, salt, and drought stress. Heat stress depends upon the degree and duration of heat which triggers the production of reactive oxygen species (ROS) and oxidative stress which alters the metabolism, growth and productivity of plants (Hasanuzzaman et al., 2013). Salt stress is responsible for hindering plant growth, photosynthesis, and germination as well (Wang et al., 2021). Drought stress also hinders plant growth and affects photosynthesis due to the stomatal closure. Plant growth hormones like auxins, gibberellins etc. also modulates

response towards drought (Aroca, 2013) and 2ODDs are responsible for the biosynthesis of these hormones ultimately affecting plant response to these stresses.

In this study, the expression patterns of *St2ODDs* were observed under heat, salt, and drought stress. The results showed an upregulated response of 34 genes when administered with salt stress. Out of 71 differentially expressed genes, 15 genes belongs to the GAOXs and 3 genes belongs to the ACOs, which are involved in the biosynthesis of gibberellins and ethylene subsequently. Various 2ODDs have been expressed in plant systems to check their role with administered abiotic stresses. Overexpressed F3H from *Camellia sinensis* in tobacco showed increased salt tolerance (Mahajan, Sudesh & Yadav, 2014) similarly FNS1 (*T. aestivum*), belonging to the 2ODD superfamily was overexpressed in *A. thaliana* and showed an increased root length under salt stress.

Under heat stress, seven genes were upregulated after 6 hours but downregulated after 3 days while two genes were upregulated throughout under heat stress. Fifteen genes were downregulated under heat stress. Previous studies have reported the role of 2ODDs under heat stress protection. Overexpression F3HL protein of *S. lycopersicum* in tobacco reported increased expression levels of *Sl*F3HL under chilling stress and faster seed germination, growth levels when compared to control (Meng et al., 2015). Likewise, the identified genes associated with heat stress may be associated with heat stress protection and can be studied.

Under drought stress, twenty nine genes were differentially expressed *St2ODD130, St2ODD54,* and *St2ODD25* were upregulated. Thirteen genes were downregulated under drought stress. Overexpression studies in *Arabidopsis* showed FNS1 of *P. nutans* and LDOX2 of *Reaumuria trigyna* conferred increased resistant to drought by improving antioxidant capacity and increased tolerance to drought, UV-B subsequently in *Arabidopsis* (Wang et al., 2020b), (Li et al., 2021). The expression patterns may suggest the role of *St2ODDs* with the elevated abiotic stresses.

The expression levels of *St2ODDs* were checked using qRT-PCR under drought stress after 3 days and in rewatering after 3 days in which *St2ODD130, St2ODD54,* and *St2ODD25* showed increased relative expression under drought stress and normal expression after rewatering. The expression

levels of *St2ODDs* were also checked using qRT-PCR under salt stress after 24h, 48h, 72h, and 96h. Four *St2ODDs* showed increased expression levels which is in compliance with the expression patterns of RNAseq data . All these results infer the potential roles of the identified *St2ODDs* in potato under different abiotic stresses which further needs to be studied using overexpression systems and could be used for genome editing studies for improving desired traits like tolerance to abiotic stresses in potato. Overall our study forms a knowledgebase and provides functional insights in the *2ODD* gene family in potato which can be further explored to identify new aspects of *2ODDs*. The roles of the identified *St2ODDs* could further be validated by *in-vitro* studies. Overexpression and gene knock-down studies using designer nucleases like CRISPR/Cas could be used to validate the potential roles of *St2ODDs* in different abiotic stresses (Alok et al., 2021).

Conclusion

In conclusion, our results gave insight in the *St2ODDs* which was not studied earlier. Various aspects of *St2ODDs* including chromosomal locations, gene structures, motif analysis, gene duplications, and evolutionary relationships were studied which were in accordance with previous studies. The structural aspects of *St2ODDs* were explored and confirmed for the presence of conserved active sites and their potential interactions with their substrates. The docking and structural domains of identified *St2ODDs* may contribute in future studies. Some candidate genes having roles in various abiotic stresses like heat, salt, and drought have been identified in accordance to the expression pattern which was further validated for drought stress and salt stress by qRT-PCR. *St2ODD130, St2ODD25* and *St2ODD54* was found to be upregulated under drought stress and *St2ODD76, St2ODD91, St2ODD138* and *St2ODD34* showed significant FC under salt stress. The identified genes having roles in association with abiotic stresses i.e. drought stress and salt stress can be further explored. Overexpression studies, CRISPR/Cas could further validate the role of *St2ODDs*.

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Credit authorship contribution statement

KS; conceived the idea, designed the experiments, analysed the results and finalized the manuscript. HC and A; collected and analysed the data, compiled the results. HC; performed wet lab experiments and wrote the manuscript.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Figures Legends:

Figure 1: Schematic representation of the chromosomal distribution of *St2ODD* genes across the twelve chromosomes of potato and the black lines represent the duplicated gene pairs.

Figure 2: Phylogenetic analysis of *St2ODDs* in potato. The *St2ODD* amino acid sequences were aligned using ClustalW, the phylogenetic tree was constructed using neighbour-joining (NJ) method with 1000 bootstrap and complete deletion in MEGA 7.

Figure 3: Gene Structures of *St2ODDs*. Gene structure of *St2ODDs* representing exons, introns, and upstream/downstream are represented by blue, black lines, and orange respectively.

Figure 4: Motif composition *in St2ODDs*. *St*2ODDs conserved motifs were represented in different colored boxes: motif 1 (red), motif 2 (cyan), motif 3 (light green), motif 4 (purple), motif 5 (mustard), motif 6 (dark green), motif 7 navy), motif 8 (pink), motif 9 (orange), and motif 10 (yellow).

Figure 5: A schematic representation of the 3D structure of representative members of **A-B** GaOx (*St2ODD29* and *St2ODD124*). 2-OG binding sites Y, R, and S are represented in red color, while

Fe(II) binding sites H, D, and H are represented in blue color. **C-D** ACC (*St2ODD118* and *St2ODD120*). Bicarbonate binding sites R, and R are represented in pink color, while Fe(II) binding sites H, D, and H are represented in blue color. **E-F** FNS (*St2ODD85* and St2ODD87). 2-OG binding sites N, Y, R, and S are represented in red color, while Fe(II) binding sites H, H, and D are represented in blue color.

Figure 6: Multiple alignment of **A)** GaOx function containing *St2ODDs*. Active amino acids Tyrosine (Y), Arginine (R), and Serine (S) responsible for interacting with 2 OG are represented with blue triangles. The active residues Histidine (H), Histidine (H) and Aspartate (D) responsible for interacting with Fe (II) are represented with red triangles. **B)** FNS function containing *St2ODDs.* Active amino acids Asparagine (N), Tyrosine (Y), Arginine (R), and Serine (S) responsible for interacting with 2 OG are represented with blue triangles. The active residues Histidine (H), Histidine (H) and Aspartate (D) responsible for interacting with Fe (II) are represented with red triangles. **C)** ACC function containing *St2ODDs.* V Active amino acids Arginine (R), and Arginine (R) responsible for interacting with bicarbonate are represented with yellow triangles. The active residues Histidine (H), Histidine (H) and Aspartate (D) responsible for interacting with Fe (II) are represented with red triangles.

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Tables Legends:

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Supplementary Tables

804 **Table S1:** Predicted sequence features of *St2ODDs*.

805 **Table S2**: Grid box dimension co-ordinates (X, Y, and Z) and binding affinity in Kcal/mol of the

806 ten groups (1-10).

807 **Table S3:** Primers used for qRT-PCR.

808 **Table S4:** Sequences of conserved motifs (1-10) in *St2ODDs*.

809 **Table S5:** Raw Data for qRT-PCR result for drought stress and salt stress.

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812

Figure 1

Figure 1

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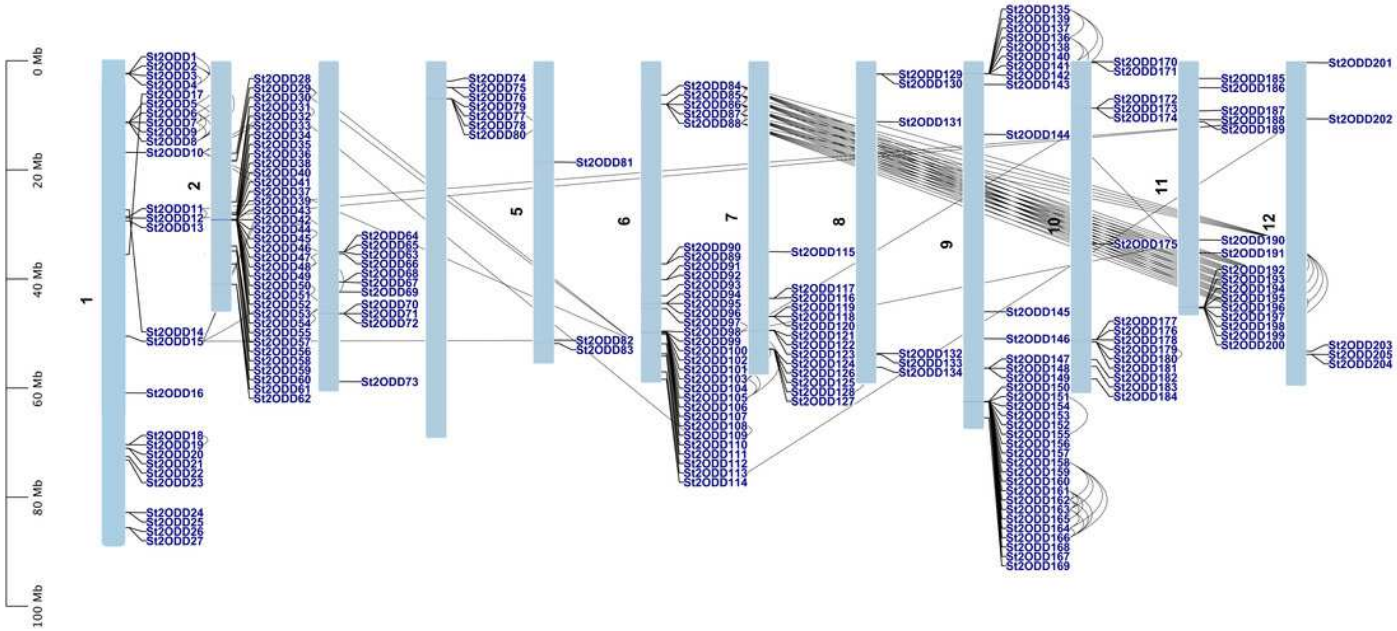

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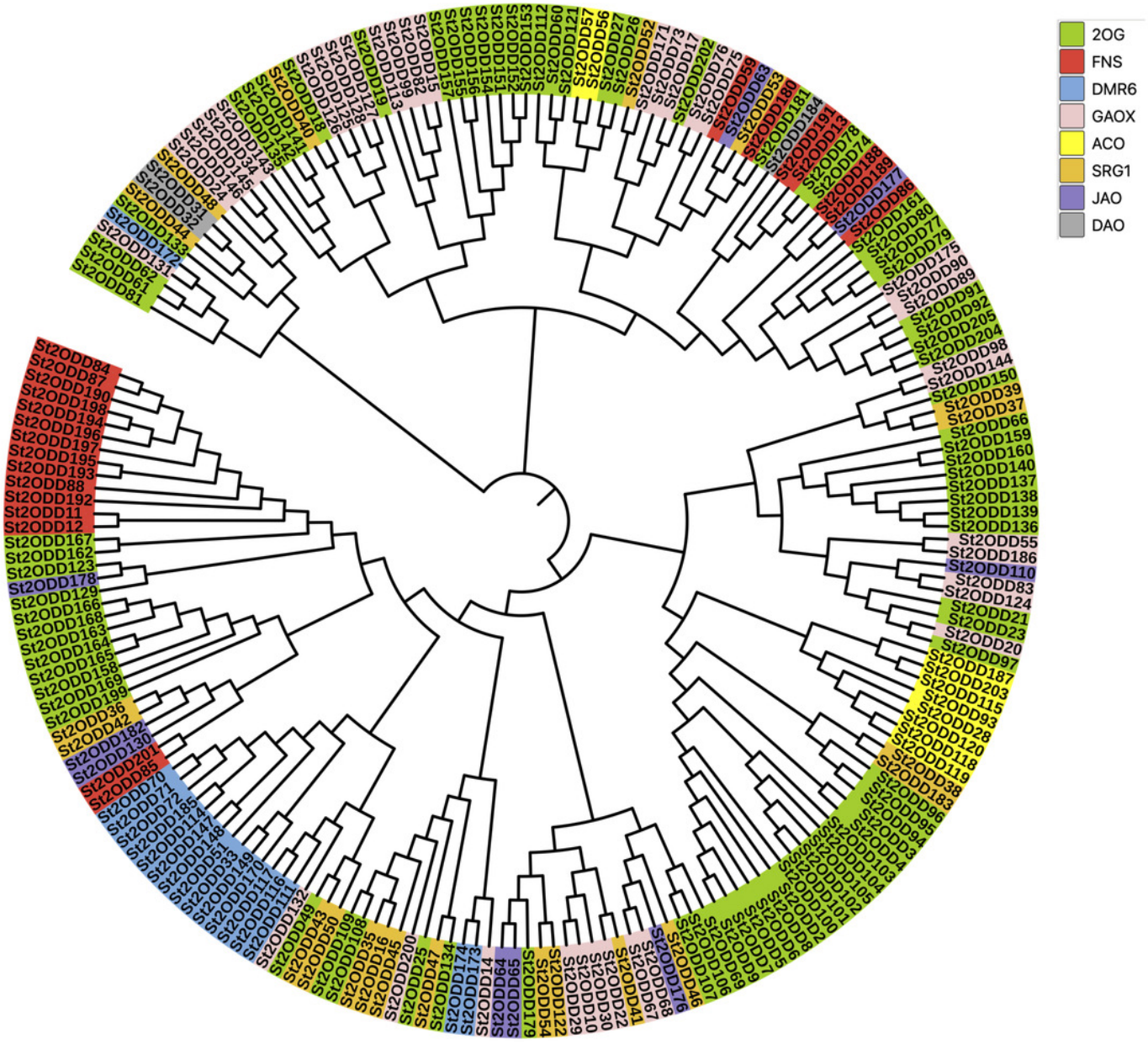

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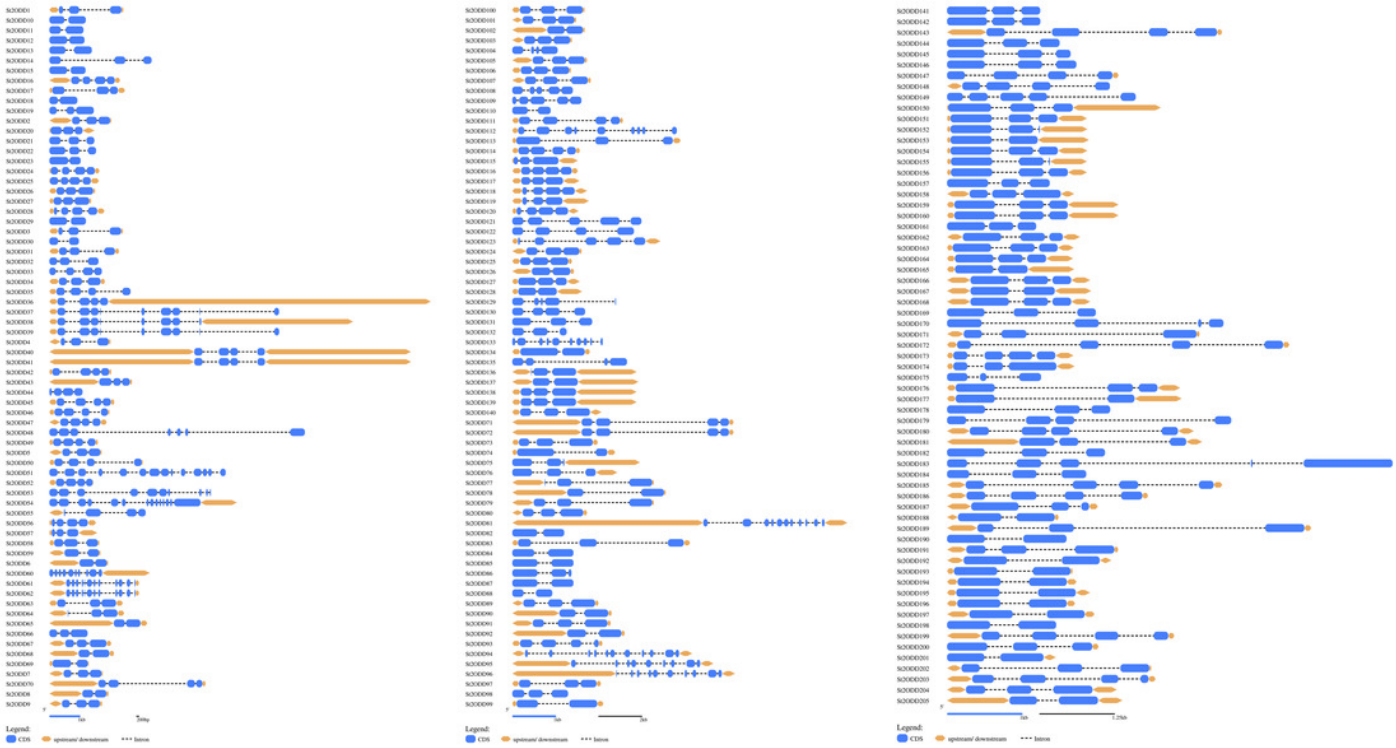

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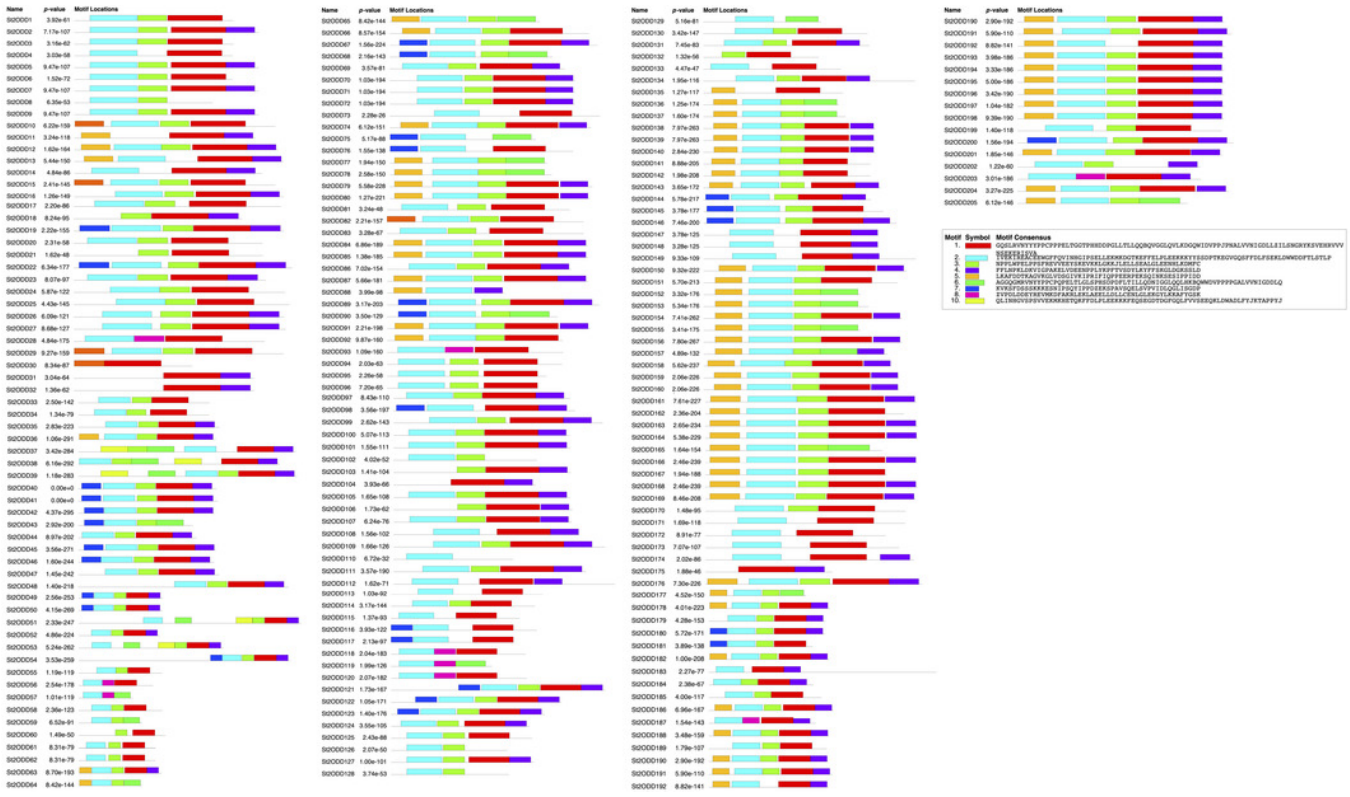

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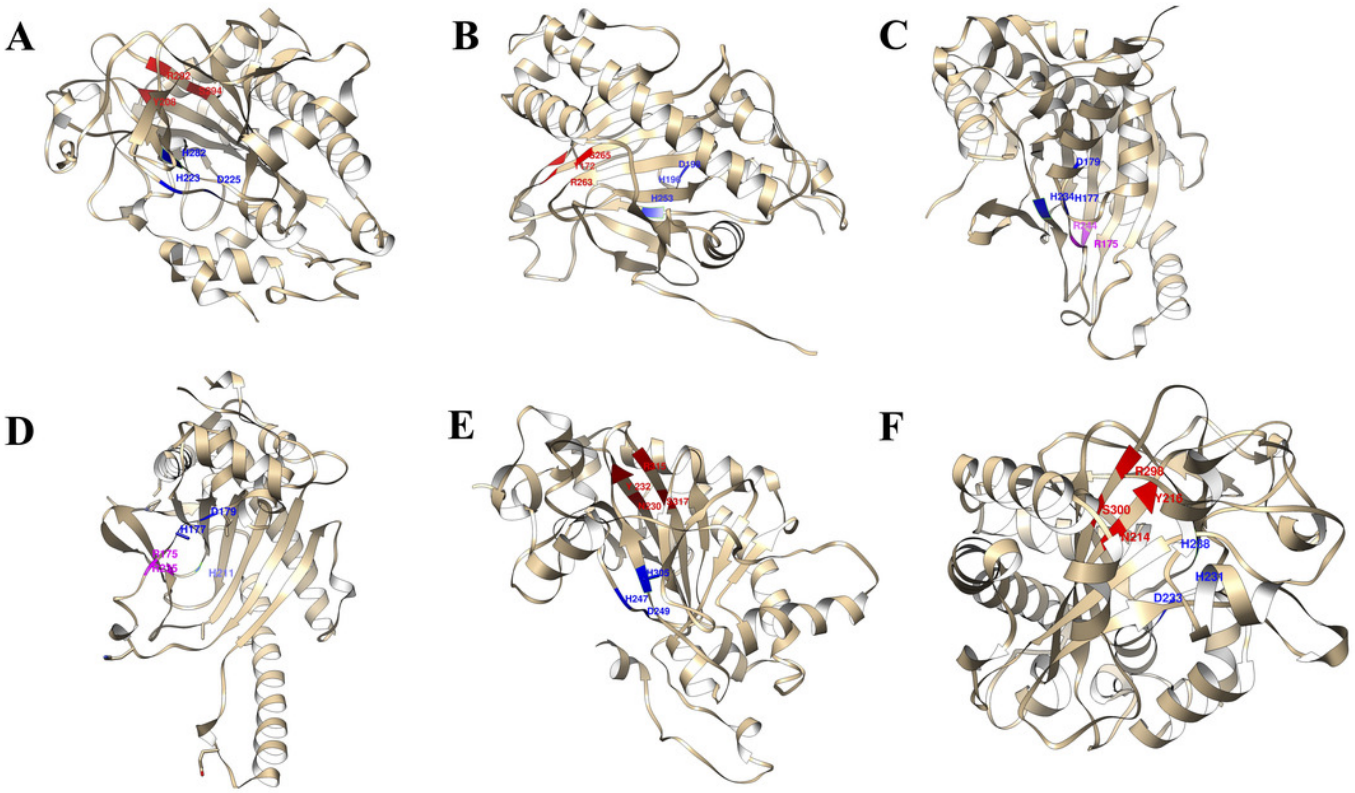

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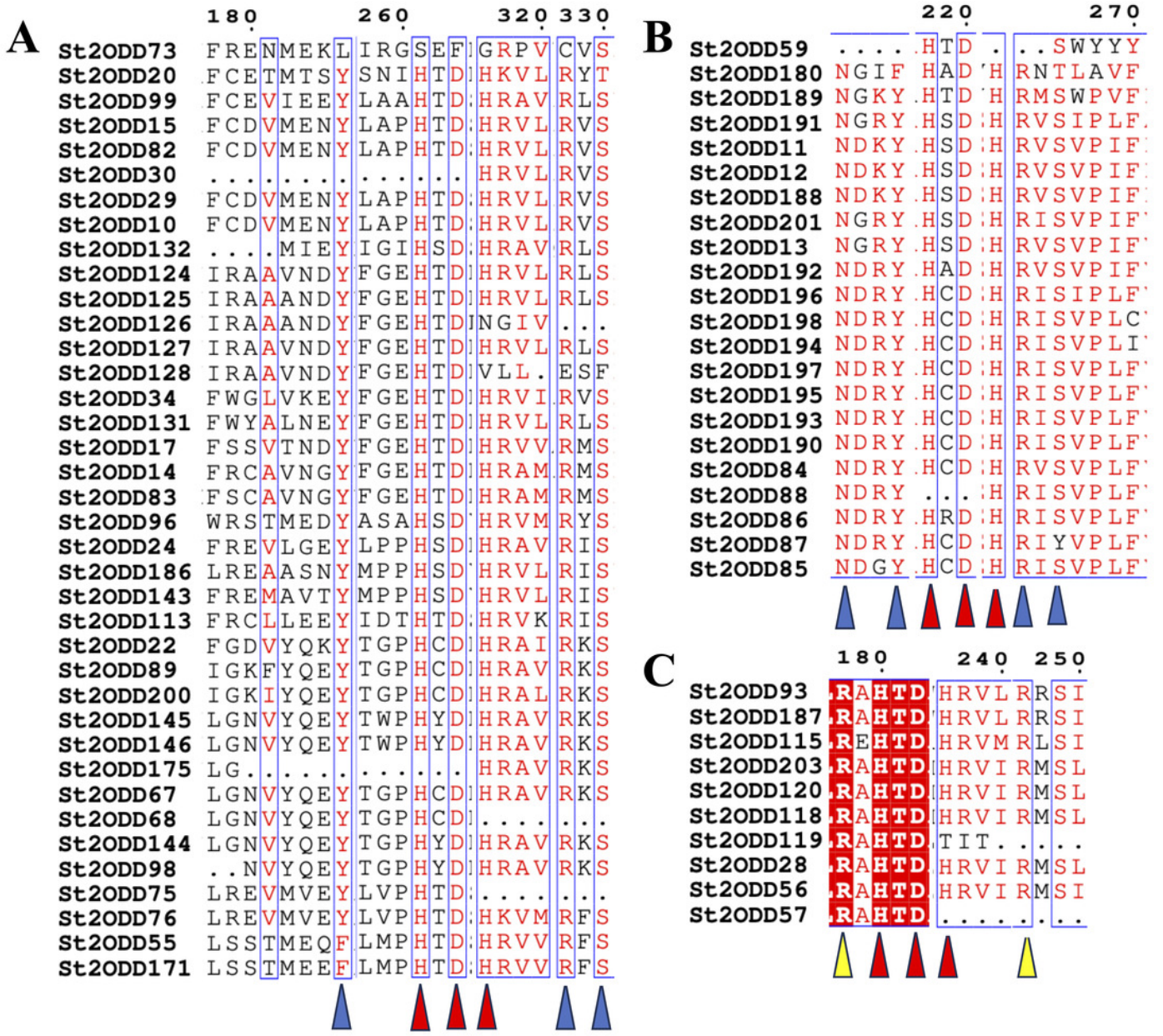
A
180
260
320 330
St2ODD73 FRENMEKLIRGSEFIGRPVCVS
St2ODD20 FCETMTSYSNIHTDIHKVLRYT
St2ODD99 FCEVIEEYLAAHTDIHRAVRLS
St2ODD15 FCDVMENYLAPHTDIHRVLRVS
St2ODD82 FCDVMENYLAPHTDIHRVLRVS
St2ODD30 .....HRVLRVS
St2ODD29 FCDVMENYLAPHTDIHRVLRVS
St2ODD10 FCDVMENYLAPHTDIHRVLRVS
St2ODD132 ....MIEYIGIHSDIHRAVRLS
St2ODD124 IRAAVNDYFGEHTDIHRVLRLS
St2ODD125 IRAAANDYFGEHTDIHRVLRLS
St2ODD126 IRAAANDYFGEHTDINGIV...
St2ODD127 IRAAVNDYFGEHTDIHRVLRLS
St2ODD128 IRAAVNDYFGEHTDIVLL.ESF
St2ODD34 FWGLVKEYFGEHTDIHRVIRVS
St2ODD131 FWYALNEYFGEHTDIHRVLRLS
St2ODD17 FSSVTNDYFGEHTDIHRVVRMS
St2ODD14 FRCAVNGYFGEHTDIHRAMRMS
St2ODD83 FSCAVNGYFGEHTDIHRAMRMS
St2ODD96 WRSTMEDYASAHSDIHRVMRYS
St2ODD24 FREVLGEYLPPHSDIHRAVRIS
St2ODD186 LREAASNYMPPHSDIHRVLRIS
St2ODD143 FREMAVTYMPPHSDIHRVLRIS
St2ODD113 FRCLLEEYIDTHTDIHRVKRIS
St2ODD22 FGDVYQKYTGPHCDIHRAIRKS
St2ODD89 IGKFYQEYTGPHCDIHRAVRKS
St2ODD200 IGKIYQEYTGPHCDIHRALRKS
St2ODD145 LGNVYQEYTWPHYDIHRAVRKS
St2ODD146 LGNVYQEYTWPHYDIHRAVRKS
St2ODD175 LG.....HRAVRKS
St2ODD67 LGNVYQEYTGPHCDIHRAVRKS
St2ODD68 LGNVYQEYTGPHCDI.....
St2ODD144 LGNVYQEYTGPHYDIHRAVRKS
St2ODD98 ..NVYQEYTGPHYDIHRAVRKS
St2ODD75 LREVMVEYLVPHTDI....
St2ODD76 LREVMVEYLVPHTDIHKVMRFS
St2ODD55 LSSTMEQFLMPHTDIHRVVRFS
St2ODD171 LSSTMEEFLMPHTDIHRVVRFS
B
220
270
St2ODD59 ....HTD..SWYYY
St2ODD180 NGIF HADHRNTLAVF
St2ODD189 NGKY.HTDHRMSWPVF
St2ODD191 NGRY.HSDIHRVSIPLF
St2ODD11 NDKY.HSDIHRVSVPIF
St2ODD12 NDKY.HSDIHRVSVPIF
St2ODD188 NDKY.HSDIHRVSVPIF
St2ODD201 NGRY.HSDIHRISVPIF
St2ODD13 NGRY.HSDIHRVSVPIF
St2ODD192 NDRY.HADIHRVSVPIF
St2ODD196 NDRY.HCDIHRISIPLF
St2ODD198 NDRY.HCDIHRISVPLC
St2ODD194 NDRY.HCDIHRISVPLI
St2ODD197 NDRY.HCDIHRISVPLF
St2ODD195 NDRY.HCDIHRISVPLF
St2ODD193 NDRY.HCDIHRISVPLF
St2ODD190 NDRY.HCDIHRISVPLF
St2ODD84 NDRY.HCDIHRVSVPLF
St2ODD88 NDRY...IHRISVPLF
St2ODD86 NDRY.HRDIHRISVPLF
St2ODD87 NDRY.HCDIHRIYVPLF
St2ODD85 NDGY.HCDIHRISVPLF
C
180
240
250
St2ODD93 RAHTDHRVLRRSI
St2ODD187 RAHTDHRVLRRSI
St2ODD115 REHTDHRVMRLSI
St2ODD203 RAHTDHRVIRMSL
St2ODD120 RAHTDHRVIRMSL
St2ODD118 RAHTDHRVIRMSL
St2ODD119 RAHTDTIT.....
St2ODD28 RAHTDHRVIRMSL
St2ODD56 RAHTDHRVIRMSI
St2ODD57 RAHTD.....

Figure 7

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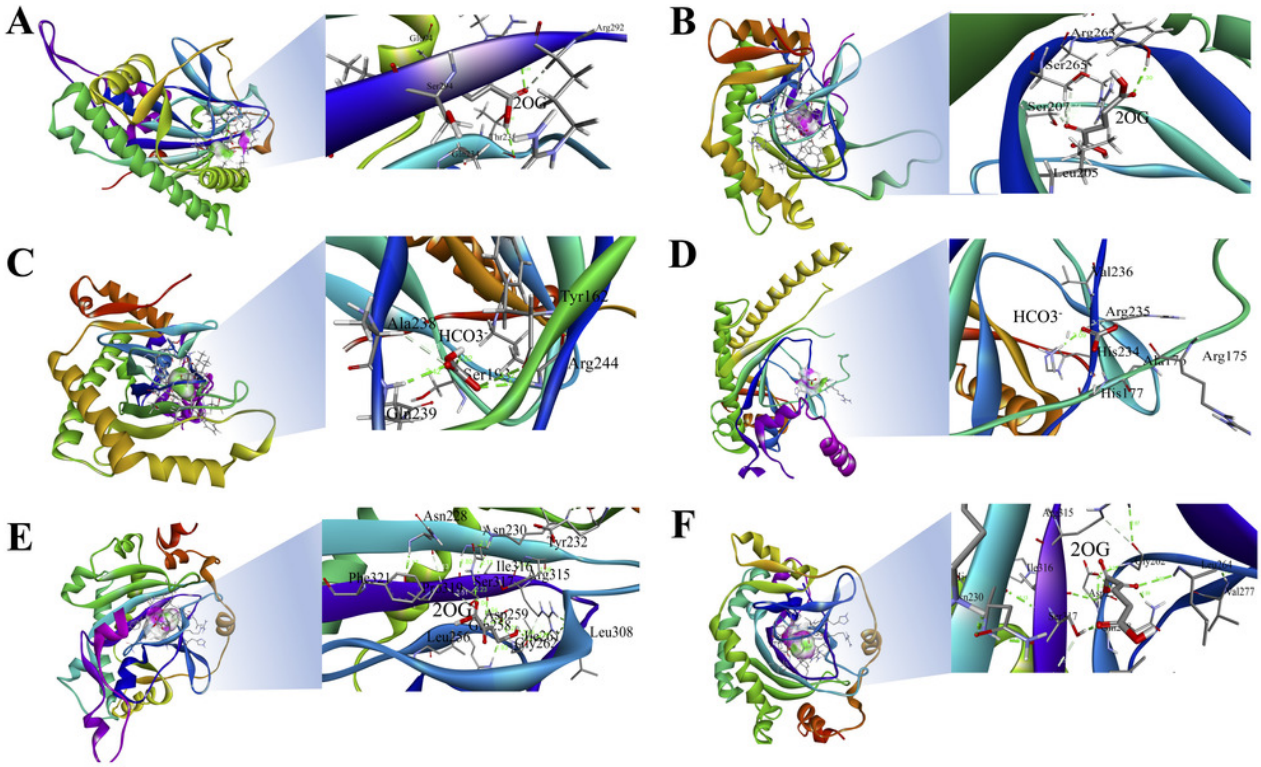

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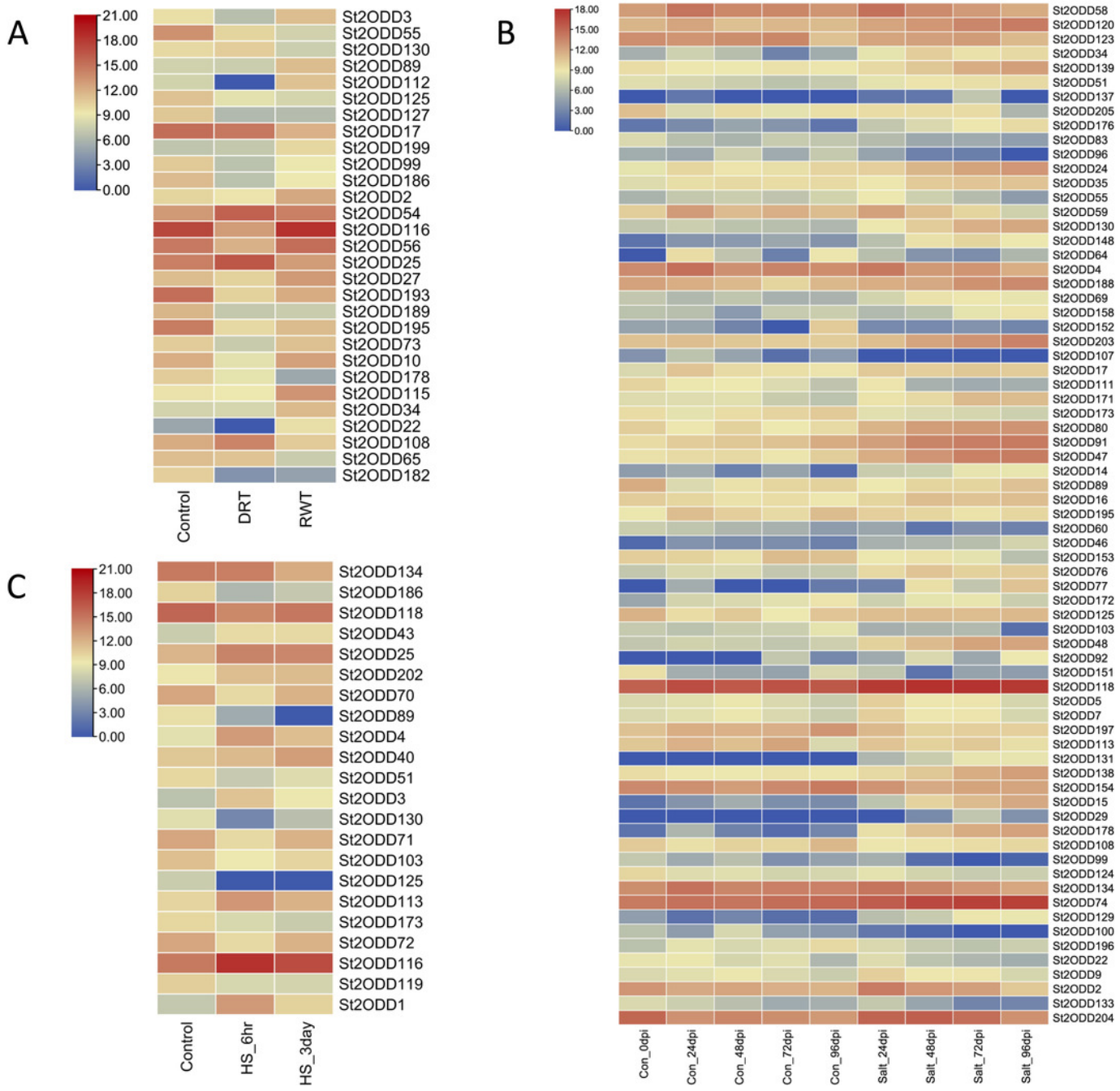

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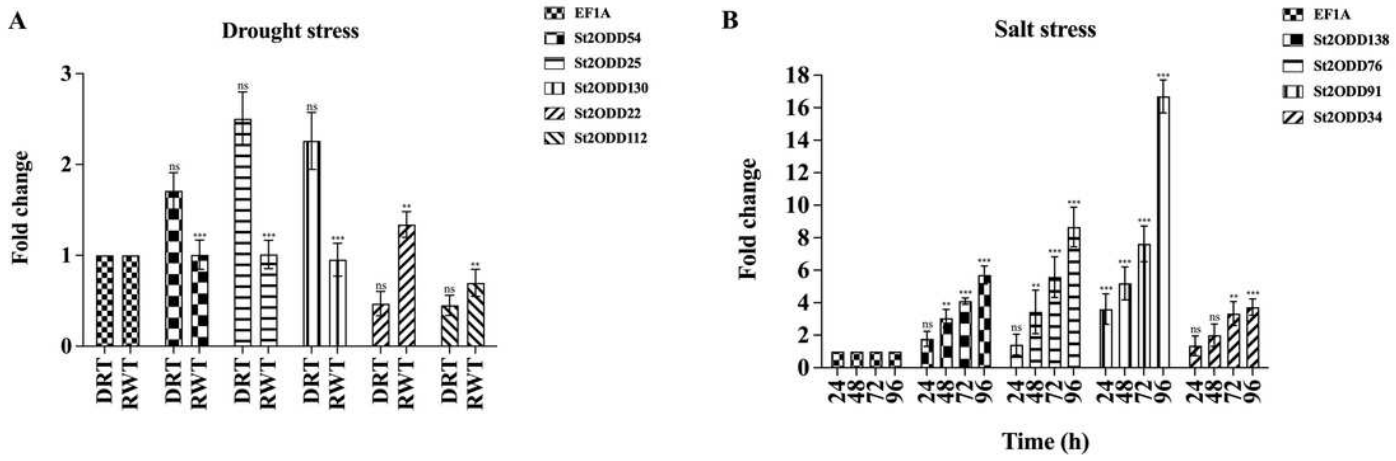

Table 1(on next page)

Table 1

: Duplicated *St2ODD* gene pairs with nonsynonymous substitution (Ka) rates and synonymous substitution (Ks), Ka/Ks, selection type, and duplication type.

1

Dup Gene 1	Dup Gene 2	Ka	Ks	Ka/Ks	Selection	Duplication type
St2ODD144	St2ODD4	0.06569628	0.04190762	1.56764532	Positive	TD
St2ODD11	St2ODD7	0.00663721	0.01490115	0.44541598	Negative	SD
St2ODD2	St2ODD6	0.09652673	0.1050776	0.9186233	Negative	SD
St2ODD2	St2ODD5	0.00663721	0.01490115	0.44541598	Negative	SD
St2ODD2	St2ODD8	0.0186345	0.02378455	0.78347065	Negative	SD
St2ODD3	St2ODD4	0.06572274	0.04184643	1.57056977	Positive	TD
St2ODD5	St2ODD8	0.00970602	0.01008984	0.9619597	Negative	TD
St2ODD6	St2ODD7	0.08704825	0.08833728	0.98540787	Negative	TD
St2ODD6	St2ODD8	0.00439755	0.01007854	0.43632812	Negative	TD
St2ODD6	St2ODD9	0.08704825	0.08833728	0.98540787	Negative	TD
St2ODD7	St2ODD8	0.00970602	0.01008984	0.9619597	Negative	TD
St2ODD10	St2ODD15	0.05577109	0.19061954	0.29257806	Negative	SD
St2ODD10	St2ODD29	0.0237322	0.08128247	0.29197198	Negative	SD
St2ODD10	St2ODD30	0.04992497	0.1109401	0.45001735	Negative	SD
St2ODD10	St2ODD82	0.0528703	0.18635514	0.28370723	Negative	SD
St2ODD11	St2ODD12	0.06128759	0.15116171	0.40544385	Negative	TD
St2ODD11	St2ODD188	0.07898557	0.23428441	0.33713543	Negative	SD
St2ODD12	St2ODD188	0.036845	0.1478565	0.24919433	Negative	SD
St2ODD15	St2ODD29	0.06293806	0.20941455	0.30054294	Negative	SD
St2ODD15	St2ODD30	0.10259873	0.20577881	0.49858742	Negative	SD
St2ODD15	St2ODD82	0.06273337	0.21128955	0.29690712	Negative	SD
St2ODD18	St2ODD19	0.10511845	0.30308326	0.34683028	Negative	TD
St2ODD29	St2ODD30	0.03556222	0.06253621	0.56866604	Negative	TD
St2ODD29	St2ODD82	0.04824609	0.19460705	0.24791543	Negative	SD
St2ODD30	St2ODD82	0.09859509	0.21095941	0.46736519	Negative	SD

St2ODD31	St2ODD32	0.02620672	0.05593442	0.46852574	Negative	TD
St2ODD33	St2ODD114	0.06716191	0.13402513	0.50111431	Negative	SD
St2ODD42	St2ODD45	0.06402212	0.19993341	0.32021722	Negative	TD
St2ODD44	St2ODD47	0.07057711	0.29750572	0.23722942	Negative	TD
St2ODD44	St2ODD48	0.09400143	0.19247944	0.48837128	Negative	TD
St2ODD46	St2ODD53	0.16970163	0.40972685	0.41418235	Negative	TD
St2ODD47	St2ODD48	0.14488402	0.38971926	0.37176511	Negative	TD
St2ODD50	St2ODD51	0.12069077	0.34853361	0.34628157	Negative	TD
St2ODD51	St2ODD53	0.17161054	0.46632126	0.36800925	Negative	TD
St2ODD53	St2ODD54	0.35046972	0.81523819	0.42989856	Negative	TD
St2ODD77	St2ODD80	0.08738575	0.2360608	0.37018323	Negative	TD
St2ODD84	St2ODD85	0.04315687	0.186145	0.23184547	Negative	TD
St2ODD84	St2ODD86	0.04954394	0.20793712	0.23826406	Negative	TD
St2ODD84	St2ODD87	0.04834956	0.19757062	0.2447204	Negative	TD
St2ODD84	St2ODD190	0.04339296	0.12568765	0.34524439	Negative	SD
St2ODD84	St2ODD193	0.05668885	0.14432865	0.39277613	Negative	SD
St2ODD84	St2ODD194	0.07353332	0.256353	0.28684399	Negative	SD
St2ODD84	St2ODD195	0.0561702	0.15104611	0.37187453	Negative	SD
St2ODD84	St2ODD196	0.06644118	0.26447813	0.25121618	Negative	SD
St2ODD84	St2ODD197	0.07020124	0.25027297	0.28049867	Negative	SD
St2ODD84	St2ODD198	0.06255558	0.270784	0.23101655	Negative	SD
St2ODD85	St2ODD87	0.01434164	0.01789127	0.8015998	Negative	TD
St2ODD85	St2ODD86	0.0223309	0.01945004	1.14811592	Positive	TD
St2ODD85	St2ODD190	0.05515156	0.15103153	0.36516583	Negative	SD
St2ODD85	St2ODD88	0.02882001	0.04695144	0.61382592	Negative	TD
St2ODD85	St2ODD195	0.07291675	0.19592791	0.3721611	Negative	SD
St2ODD85	St2ODD193	0.06928318	0.19005848	0.3645361	Negative	SD

St2ODD85	St2ODD198	0.0776672	0.26823273	0.28955153	Negative	SD
St2ODD85	St2ODD194	0.08839285	0.27488677	0.32156095	Negative	SD
St2ODD85	St2ODD196	0.08118562	0.27035025	0.30029793	Negative	SD
St2ODD85	St2ODD197	0.09097925	0.26529128	0.34294097	Negative	SD
St2ODD86	St2ODD87	0.02496672	0.01955304	1.27687138	Positive	TD
St2ODD86	St2ODD88	0.04117256	0.04574588	0.90002773	Negative	TD
St2ODD86	St2ODD190	0.06137565	0.16833057	0.36461381	Negative	SD
St2ODD86	St2ODD193	0.07485809	0.17729158	0.42223152	Negative	SD
St2ODD86	St2ODD194	0.09216987	0.265857	0.34668965	Negative	SD
St2ODD86	St2ODD195	0.07931784	0.21289575	0.37256657	Negative	SD
St2ODD86	St2ODD196	0.0857194	0.27485218	0.31187456	Negative	SD
St2ODD86	St2ODD197	0.09645194	0.3121203	0.30902169	Negative	SD
St2ODD86	St2ODD198	0.0833017	0.26559101	0.31364654	Negative	SD
St2ODD87	St2ODD88	0.0361952	0.04295253	0.84267917	Negative	TD
St2ODD87	St2ODD190	0.06040845	0.16179674	0.37336008	Negative	SD
St2ODD87	St2ODD193	0.07461881	0.2016231	0.37009057	Negative	SD
St2ODD87	St2ODD194	0.09053363	0.27815609	0.32547781	Negative	SD
St2ODD87	St2ODD195	0.07806541	0.20848701	0.37443779	Negative	SD
St2ODD87	St2ODD196	0.08464087	0.27357136	0.30939228	Negative	SD
St2ODD87	St2ODD197	0.09643984	0.27850651	0.34627499	Negative	SD
St2ODD87	St2ODD198	0.08239731	0.27192378	0.3030162	Negative	SD
St2ODD88	St2ODD190	0.06748491	0.15156264	0.44526081	Negative	SD
St2ODD88	St2ODD193	0.0784197	0.20164162	0.38890631	Negative	SD
St2ODD88	St2ODD194	0.10073583	0.23401431	0.43046866	Negative	SD
St2ODD88	St2ODD195	0.08762846	0.18971131	0.46190424	Negative	SD
St2ODD88	St2ODD196	0.09043122	0.25011537	0.36155802	Negative	SD
St2ODD88	St2ODD197	0.11408173	0.24645762	0.4628858	Negative	SD

St2ODD88	St2ODD198	0.09807243	0.22515042	0.43558626	Negative	SD
St2ODD97	St2ODD101	0.15082731	0.28041998	0.5378622	Negative	TD
St2ODD97	St2ODD103	0.1491623	0.29948343	0.49806528	Negative	TD
St2ODD97	St2ODD105	0.14054039	0.27248961	0.51576422	Negative	TD
St2ODD98	St2ODD144	0.02450309	0.07493749	0.32698035	Negative	SD
St2ODD98	St2ODD175	0.19572533	0.36390979	0.53784025	Negative	SD
St2ODD101	St2ODD103	0.150478	0.30131704	0.4994009	Negative	TD
St2ODD101	St2ODD105	0.04306739	0.0408264	1.05489048	Positive	TD
St2ODD103	St2ODD105	0.15353419	0.31519188	0.48711341	Negative	TD
St2ODD108	St2ODD109	0.06700429	0.10432615	0.64225783	Negative	TD
St2ODD113	St2ODD189	0.66649649	1.77888311	0.37467133	Negative	SD
St2ODD118	St2ODD120	0.0261915	0.2951278	0.08874629	Negative	TD
St2ODD124	St2ODD126	0.10690827	0.4964094	0.2153631	Negative	TD
St2ODD126	St2ODD128	0.05344949	0.20377605	0.26229523	Negative	TD
St2ODD129	St2ODD130	0.05101614	0.11788919	0.4327466	Negative	TD
St2ODD135	St2ODD136	0.21553725	0.34973463	0.61628799	Negative	TD
St2ODD135	St2ODD141	0.10181782	0.19313922	0.52717322	Negative	TD
St2ODD135	St2ODD142	0.09506626	0.18714606	0.50797894	Negative	TD
St2ODD136	St2ODD141	0.11602076	0.28011867	0.4141843	Negative	TD
St2ODD136	St2ODD142	0.1067933	0.28093034	0.38014157	Negative	TD
St2ODD141	St2ODD142	0.01818822	0.02946714	0.61723748	Negative	TD
St2ODD144	St2ODD175	0.07633702	0.1946334	0.39220926	Negative	SD
St2ODD145	St2ODD146	0.00756468	0.03783308	0.19994879	Negative	TD
St2ODD151	St2ODD155	0.08795754	0.26721516	0.32916373	Negative	TD
St2ODD158	St2ODD162	0.14084357	0.25515971	0.55198202	Negative	TD
St2ODD158	St2ODD163	0.10637851	0.19789956	0.53753791	Negative	TD
St2ODD158	St2ODD164	0.12623217	0.15711323	0.80344711	Negative	TD

St2ODD158	St2ODD166	0.09902427	0.20454306	0.4841243	Negative	TD
St2ODD161	St2ODD162	0.1484228	0.35333191	0.42006623	Negative	TD
St2ODD161	St2ODD163	0.09515891	0.29623739	0.32122519	Negative	TD
St2ODD161	St2ODD165	0.15729244	0.33400836	0.47092367	Negative	TD
St2ODD161	St2ODD166	0.08157109	0.3142541	0.25957048	Negative	TD
St2ODD162	St2ODD163	0.09001811	0.17228699	0.52248933	Negative	TD
St2ODD162	St2ODD166	0.08374729	0.15777214	0.53081162	Negative	TD
St2ODD162	St2ODD165	0.10199099	0.18093047	0.56370268	Negative	TD
St2ODD163	St2ODD166	0.04675867	0.12542874	0.37279076	Negative	TD
St2ODD163	St2ODD164	0.0975672	0.18354877	0.53156007	Negative	TD
St2ODD164	St2ODD166	0.09209668	0.20413856	0.4511479	Negative	TD
St2ODD179	St2ODD180	0.0794928	0.16823277	0.47251674	Negative	TD
St2ODD190	St2ODD193	0.04521834	0.15013735	0.30117979	Negative	SD
St2ODD190	St2ODD194	0.06695894	0.24483643	0.27348438	Negative	SD
St2ODD190	St2ODD195	0.04405385	0.16497941	0.26702635	Negative	SD
St2ODD190	St2ODD196	0.05864168	0.22252378	0.26352996	Negative	SD
St2ODD190	St2ODD197	0.06108833	0.24501942	0.24932036	Negative	SD
St2ODD190	St2ODD198	0.05607785	0.22850289	0.24541419	Negative	SD
St2ODD193	St2ODD194	0.06368396	0.20051302	0.31760512	Negative	TD
St2ODD193	St2ODD195	0.02291405	0.09777482	0.23435536	Negative	TD
St2ODD193	St2ODD196	0.05668303	0.22011799	0.25751201	Negative	TD
St2ODD193	St2ODD197	0.06233667	0.23956419	0.26020865	Negative	TD
St2ODD193	St2ODD198	0.05412739	0.19669002	0.27519131	Negative	TD
St2ODD194	St2ODD195	0.06576495	0.24323853	0.27037227	Negative	TD
St2ODD194	St2ODD196	0.03951081	0.08266321	0.47797329	Negative	TD
St2ODD194	St2ODD197	0.07011406	0.18379532	0.38147899	Negative	TD
St2ODD194	St2ODD198	0.02650612	0.03671861	0.72187153	Negative	TD

St2ODD195	St2ODD196	0.05616442	0.23301649	0.24103195	Negative	TD
St2ODD195	St2ODD197	0.05797008	0.24041471	0.24112535	Negative	TD
St2ODD195	St2ODD198	0.05105475	0.23904667	0.21357651	Negative	TD
St2ODD196	St2ODD197	0.05664229	0.20893578	0.27109905	Negative	TD
St2ODD196	St2ODD198	0.02408395	0.0849296	0.28357542	Negative	TD
St2ODD197	St2ODD198	0.05920475	0.18579026	0.31866447	Negative	TD

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