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# Analysis of *SOS1* Promoter in Various Plant Species for WRKY Transcription Activation



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#### Abstract

WRKY transcription factors play a critical role in plant stress response to salinity tolerance. SOS1 promoter alignment was analyzed for WRKY binding domain for Arabidopsis, Cochlearia, Tomato, Rice, Salicornia and Wheat using multialin. The analysis using Plant CARE showed Arabidopsis and tomato have one, Wheat and two Salicornia species have two, Rice has four and Cochlearia has exceptional six WRKY binding domains present in their SOS1 promoter. We hypothesize that WRKY transcription factor play an important role in response to abiotic stress and needs further investigation for their possible function in salt stress tolerance.

Keywords: SOS1, WRKY, Transcription, Salt stress

### **Mini Review**

Salinity is a major constraint to food production as the percentage of agricultural land that is affected by high salinity is continuously increasing throughout the world [1]. The extent of the problem is illustrated by the fact that one-third of the global arable land or half of the irrigated arable land is significantly affected by salinization [2]. Seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set are adversely affected by salinity in many crops [3]. The tolerance to soil salinity varies greatly among plant species [4]. Among cereals, rice is highly salt-sensitive whereas barley is relatively salt-tolerant. Variation in salt tolerance is more pronounced in the dicotyledonous crops, wild plant species such as salt bush (Atriplex halimus, A. vesicaria) and several members of the Chenopodeaceae (Suaeda sp., Salicornia sp.) can grow at salinity levels far higher than that of seawater [2,4].

The SOS-signaling pathway mainly involved in salt tolerance in plants, consists of three main components including SOS1, SOS2 and SOS3. At SOS3 encodes a Ca2+-binding protein, which is sensitive to cytosolic Ca2+ level [5]. One of the consequences of salt stress is an increase in the cytoplasmic Ca2+ concentration. This increase in Ca2+ is sensed by SOS3, and it activates SOS2, which is a Ser/Thr protein kinase [6]. This SOS2-SOS3 complex ultimately phosphorylates and activates NHX1 and other transporters involved in vacuolar Na+ transport along with SOS1 [7]. SOS1 is an electro-neutral Na+/H+ exchanger that is specific for Na+. GUS expression under AtSOS1 promoter exhibited a high promoter activity in root epidermal cells (particularly at root tip), and in stellar cells throughout the plant [8]. Atsos1 mutants are extremely salt-sensitive and have combined defects in Na+ extrusion and long-distance transport of Na+ from root to shoot [5,7]. Moreover, AtSOS1 mRNA is more abundant in roots than in shoots [8]. Thus, the suggested roles of SOS1 are:

a. to pump Na+ back into the soil solution

b. to decrease Na+ delivery to the shoot under salt exposure by its retrieval from xylem [9]. Similar functions for SOS1 has been proposed for Popular, Thellungiella salsuginea, wheat and rice [10-13].

The interaction between transcription factors and cisacting regulatory sequences in plant promoters is the key step involved in the altered regulation of gene expression under stress conditions [14]. It has been shown that differential gene expression contributes to the salt tolerance that are strongly and rapidly induced in response to abiotic stress [15]. WRKY transcription factors (TFs) have shown to bind to conserved DNA motifs in the promoter region involved in abiotic salt stress. In Arabidopsis, 72 members of WRKY family have been described [16]. Characteristic for these factors is their conserved WRKY DNAbinding domain (W-box) in the promoter region of targeted genes. WRKY transcription factors regulate transcript levels of these targeted genes upon binding to the W-box (TTGAC/CT) promoter element [15]. These TFs are defined by a conserved DNA binding domain of  $\sim$ 60 amino acids containing the nearly invariant stretch

WRKYGQK followed by a unique zinc-finger pattern of Cys and His residues. WRKYs are sub-divided into three groups in Arabidopsis model plant based on WRKY domains [17].

C. hollandica T. acativum	-CTATTTGTAGAGAAAATGG-AGGGATTACCAAAGAGAAAT-CGAAGACGACGACGACGGAGAGAGAGAGACGACGGCGGAGCCGAGGAG
0. sativa	CETTER TRACEARTER TO TRACEARTE CONTROLATE TO TRACEARTE CONTROLATION TO TRACE AND TRACEARTER TO TRACEARTER TO TRACE AND TRACEARTER TRACEARTER TO TRACEARTER TO TRACEARTER TRACEARTER TRACEARTER TO TRACEARTER
S. brachiata	
<ol> <li>S. dolichostachy, S. lycopersicum</li> </ol>	
A thaliana	ATAAATTTAACGTGAAGAATTGAAAGGTATCAGATTAATGTTTTGGATCAGCCGAATAGAGTCAGGCTAATCCAAATTCTCAAATATTTTCGAAACCTTA
C. hollandica	ggcgagttgaggaagagagaga <mark>tgac</mark> ggcacccgagaaaagaaaagacgacgaggagaaaaaagacgac
T. acetivum O. sativa	GACGCCATTCGACAAAGGAGATGCCGAATTCCGCTCCTCACCGGATCCGACTGGCTTATC-GGCGTAACGGGGATCGGCGAACTCGACCCCGAC CTTAATTTTACACACAACACTCCAAGGCATTATGGCAATACCTAGGTTTGTTTGACACCACAGAATGAGTCTATACACAGGG
S. brachiata	CTTAKTTTAKALAKA
S. dolichostachy S. lycopersicum	
A. thaliana	AACACATCAAACTCCACGAACTAAACACACTCAACACATATCAAAATCCAC-ATTATAAATGTATTTTTGGTAGGCTAGTCGTCTAATTCTAAAA
C. hollandica	GALGALGAGGTAGGATGACGATGACGATGGC-CACCCACGAAGAGATGACGACGACGAAGGAAGAGACGACGAGCGAG
T. acetivum	GGCTAAGCTTCCGATGCAGCATATGAAGCTTTGCTCTCCCCTACGACGCCTTCACGGTAACGATGAAGCCAGAGTCGTCGGGGTCATGAGTG
O. sativa	TTCATAGCCAAAAAAAAAGCCCAAAAGTAAAGTCATGGATGCTAAGT-GCAGTTTACTTGTGAACCTGAAGA-GGAAAATC-TATTCTCCGGTGTTA
S. brachiata	САСАЛААСАСТАСАЛОГСТСАССТАЛАЛАТСАЛТАСТОСАТАТСАЛАТАСТСАТАЛОТАТАЛСАТТАТТСАТТСАТТССАТТССАТТАСТАТТ 
S. lycoperaicum	
A. thaliana	TAGCCAA-TTTACATTTGCAATTGTTTATTCAAAAAGTACAAC-AGTAAGAGGTCTTATCAATTAAATCATAAGAAAAATATGTCCCAAAATGT-CAAA
C. hollandica	CGCCGGA-AGTTAAATCGTCGGGAGAAAAGAGAGAGAAAGTGGGGCTAGGGTTTGTTT
T. acativum	CRATCIAGAATCA/CATCATCACCCCCGGTCCCGGGTCACGAGCACGACATAGCTACCGACCAAGCAATCGCCGACAAACTTCAACCACGATTACCA-CAAG
0. sativa S. brachiata	салалсалтстилаттстисосислосиссатстилаласитстилиттслооститстоосситосалтсилстоттсто <del>тстсис</del> -с ттстисисистилилитилситисисисилиттстилилитититилититтстилотстсилстиссилстиссистсилитисстст
	TTGTAGACACTTAANAATAACATTAACAATACAAATTCTGATAAAATATATATATATATATATATATATATATA
S. lycoperaicum	алсталалатсалалталтсатасталталалтаСатассаталсасатттссассталастататалалалтстстттттттаса
A. thaliana	ARACCARCTACGARTATTTCTTTTCCTTTTARGGCCAARCCAGGTTTGGARTACTTATTACAARTAARCTCARTTCAARTAARTTCTTGTACACAAR
C. hollandica T. agetivum	CCACAATTGGGGAAAATTTCAAATTATCAATTTCATGAATAACTTC-TTTGAAAAAAATCATGGTGGCATTTTCAAAATAGTAACTCACAAATTGGGTTA AAGCCAGATGGCGATCCAAAGCGCCAATTGTCACGTGACGGGGACACACACATAGTACTCCCTCC
0. sativa	AND CLAUR TO CLAUPT TO TRADUCTURATION TO TRADUCTURATION AND AND AND AND AND AND AND AND AND AN
S. brachiata	алалалалалаласаттасасассасалсастоссассталалатастатссататсалат-астсалалтталаттталатттталасса
S. dolichostach	<mark>уд</mark> балалалаласыстабысыскаласыстасбабатетсысыталалаты табталаты балат-абтелеликтаталаттталактттталасса
	ANTOTCTATOTCCTCATAAACAAAATCTAATTAATATTATTTTATTTTATTTTATTTATTA
A. thaliana	TTARGELETARTTECHTOTGRAGGT-ACCATTGREATERCACATCGACATCTATATGACATCAACCATCAALAATCCAAAACCAACCATATATTATTATTTAT
C. hollandica	AAAGGATTTTTCCTTTAAATGTTGGTCACTTTAGTAAGCTCTTCATTCTTGGGCTTATGTTAGTCAATA <mark>TGACCTGATTAT</mark> GGGTCACTTTAGTGAATTT ACACGCTTCAACTTTAACGTAAATTTAACTATCAAGACCGATTGCGGCGGGCG
T. aestivum O. sativa	ALAUGTTUARUTTUARUTTUARUTTUARUTAANATTUARUTUUKUKUKUKUKUKUKUKUKUKUKUKUKUKUKUKUKUK
<ol> <li>brachiata</li> </ol>	AAAAAGTCCCACTGTCAAAAAAAAAAAAAAAA
S. dolichostach S. lycopersicum	ХА ХАХАХАХАКТСТ-АХХАХАХАТТААХАХАХАХАХАХАХАХАХАХАХА
A. thaliana	ATTTTTTTGTCATCATTATATATCCATTAATTTATCTAATTCTGGT-TCTAATAATACTCTTGGTCAGAAAAAATATAAACATTGAAGAATTGGTCGGCTG
C. hollandica	ATTETTALARTALARTATICAL TALARTAL AND THE CONTROL AND THE AND TH
T. acativum	GTAACTTTTTTCTCCCGCCGCAGTCGGTCTCGTTAAATTTATGGTCAAAGTTCGACCTCGGGAAACGTGGACGCACTATATTTTGGAATGGAGG
O. sativa	GACTTATTGAGTAGTAGTAATTTGAAAAATAAGTCTAGGATATTTTAAAGAAATTTAGCATG-TTGAAAAGCATGCTCATTATAAAATGAGAGGATGGTTG
S. brachiata	CTAATGAGAAAAAATGTCAAATCCTTGCCATTAATATGTTACGGACGG
<ol> <li>Golichostach</li> <li>S. lycopersicut</li> </ol>	
A. thaliana	алалттотсалалатататассасалалататсаталтсаталасалалтталтасталаттталттталттталттталсастассастастатас
C. hollandica	ATGATCATAATCATCATCATCACA-ACGATTGGGATCCAATGATGATAATT-ATAATAAATACATATCTTTT-TGTTACATAATTATTATAATTATAATAT GAGTACAGACTAAACTGGT-GCCGTCAGATGAGATGAGAT
T. acetivum O. sativa	GAGTACAGACTAAACTGOT-GOCCOTCAGATGAGATGAGATGOCATCATAAATAATCTAGCGATTACTCTOCTCTCCGOCCCCGTCCCATCT TAGCTGCTGTCACCCCAATCGAGATCCAATCAAAGAAAAGAGAAAAAGGGTGAGCAAACAATCAATCAATCAATCAATCAATCA
S. brachiata	TAATAGTTGTACACCTATCATCTGGC-TCTCTCATCCCTTGAAAACAAACAAACAAACAACTAATGATGATACATTGATGAT-GATAAGCATCTAT
S. dolichostach	XX TAATAGTTGTACAACCTATCATCTGCC-TCTCTCATCCCTTGAAAACAAACAAAACA
A. thaliana	ACOTOTOTATOTATAGCTCTATAAGT-AFTTACTCTCTTTCAGCTATTTATTTTTCAGTGAACGAGCATTCTTCTTCTTCCTCTGTGTTGTT
C. hollandica	ACTIONATIONAL CLAIMAGE AT THE CONTRACT AND A CONTRACT A
T. acetivum	GATCTGATGTGCCACCTCGTCTTGTCTGATCCCACAGCGAAGAAAAGAA
0. sativa	AGTGAGGAGGCTGCTGCATCTTCTTCTTCTTCCCCCCTTTGCCTTGGATTTTCCACTTGCACTTGCACTTCCTCCTCCTCATCAGTAATCC ATTACTCCCCTAAAATCTTGTTGACTCCTCTTTAATTTTGCAACTTCATCTTTTT7-CCT-CACTTTCTCTTTACTCAAAATAATC
S. brachiata S. dolichostachya	ATTACTCCCTAAAATCTTGTTGACTCCCTTTAATTTTGCAACTTCATCTTTTTT-CCT-CACTTTCTCTTTCATCAAAAAAATC ATCTATATTACTCCCTAAA-TCTTGTTGACTCCTCTTTTAATTTTAATTTTGCAACTTCATCTTTTTTTCCT-CACTTTCTCTTTCATCTAAAAAATC
	CTTATTTTACATCARACTCTTCARACTCTTCARACTCACTC
A. thaliana	ССТТСТТХСАТАТАТТСАААТААА
C. hollandica	TCTTCTTGTTANGTANTGCGATTCGAACG-CGAGGGGGGGGGGGGGGGGGGACAGGATCATGGAGGA
T. acetivum O. sativa	GTCTCTTCTCTTCTCCCTCGGCGACGGCGGCGGCGCC
S. brachiata	ANTTATTCTCTTCTTCCGCCATTGTTG-CAGCCAGGCAGCC
10000000000000000000000000000000000000	ANTTATTCTCTCTTCCCCCCTTCTTC-CACCCACCCCACCC
<ol> <li>S. dolichostachya</li> </ol>	

**Figure 1:** Arabidopsis thaliana (*A. thaliana*), Cochlearia x hollandica (*C. hollandica*), Oryza sativa (*O. sativa*), Salicornia brachiate (*S. brachiate*), Salicornia dolichostachya (*S. dolichostachya*), Solanum lycopersicum (*S. lycopersicum*) and Triticum aestivum (*T. aestivum*) showing WRKY binding domain (TGAC) in SOS1 through their promoter alignment (multalin.toulouse.inra.fr/multalin/). Alignment shows six WRKY binding site in C. hollandica, four in O. sativa, two in T. aestivum, S. brachiate, S. dolichostachya and one in A. thaliana and S.

lycopersicum respectively.

Three WRKY genes namely AtWRKY18, AtWRKY40 and AtWRKY60 showed to form a complex networking during salt and osmotic stress where AtWRKY40 antagonized the putative functions of AtWRKY18 and AtWRKY60 [18]. Thus, these WRKYTFs form a highly interacting regulatory network that modulates gene expression by acting as either transcription activator or repressor during stress response. Similarly ZmWRKY33 and TaWRKY71 over expression in Arabidopsis were shown to be induced by salt stress [19-20]. Tomato plants containing 35S::SIWRKY3 showed

reduced oxidative stress and proline contents with the expressions of SOS1 and SOS2 significantly induced in the transgenic plants [21]. Overexpression of GhWRKY34 also promoted expression of SOS1and SOS2 in transgenic plants through activating the SOS pathway with enhanced activity of Na+/H+ antiporter SOS1 [22]. As WKRYs are clearly involved in the abiotic stress tolerance, WRKY specific DNA binding domains in SOS1 promotor of various plant species that could be involved in salt tolerance were analyzed. The promoter region of the SOS1 from different

plants species for WKRYs binding domain; W-box were align using for Arabidopsis thaliana (A. thaliana), Cochlearia x hollandica (C. hollandica), Oryza sativa (O. sativa), Salicornia brachiate (S. brachiate), Salicornia dolichostachya (S. dolichostachya), Solanum lycopersicum (S. lycopersicum) and Triticum aestivum (T. aestivum) (Figure 1). WRKY cis-acting elements were found presents in the promoter regions of SOS1 using PlantCARE database. We found that O. sativa contains four W-box cis-elements and T. aestivum has two W-box cis-elements in their promoter region. A. thaliana and S. lycopersicum has one each W-box whereas S. brachiate and S. dolichostachya have two W-box cis elements in their promoter region. We speculate that as compared to wheat, rice is a more salt sensitive crop and therefore it has more W-box cis elements in the promoter of SOS1 to cope with salt stress. Salt tolerant plants as S. brachiate and S. dolichostachya have two W-box in SOS1 promoter. Considering these halophytes have small genome as compared to T. aestivum and O. sativa, we predict that the two WRKY TFs would significantly contribute to plant high salinity stress response by maintaining Na+/K+ ion potential and activating salt stress responsive genes [14-15]. WRKYs 25 and WRKY33 were characterized using sos1-1, sos2-1 and sos3-1 mutants and their result suggested that the NaCl-induced expression of WRKY25 and WRKY33 is independent of SOS-signaling pathway [23]. It seems interesting as most of the salt stress responses in plants, involve typical SOS pathway. These results indicate that WRKY25/33 may have different downstream target gene to play their role in salt stress. The exceptional six cis-element WRKY motifs found in C. hollandica needs to be explored for their role in salinity tolerance. The transcriptional network of WRKY proteins forms an intricate signaling complex that plays an important role in plant response to cope with salt stress by excluding excessive Na+ and maintaining ion homeostasis. Since many members of the WRKY family act as important node of convergence for abiotic stress during transcriptional reprogramming, understanding the complex mechanism of WRKY TFs will pave way for improved agriculture production for important crop plants in salt tolerance.

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00212

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