Quinoa (Chenopodium quinoa) Purification of
CqMSRA5.1 Prokaryotic Protein, Sulfoxide Reductase
(MSR) Gene CqMSR5.1 in Osmotic Stress Response

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Abstract Located in chloroplast. The vitro enzymatic property verification CqMSRA5.1 can specifically reduce type methionine sulfoxide and belongs to MSRA family. Arabidopsis wild type Col 0, CqMSRA5.1 transformed Arabidopsis overexpression line and msra5 Arabidopsis mutant were used as materials to carryout phenotypic experiments of simulated osmotic stress treatment and soil drought treatment. The above results showed CqMSRA5.1. Enhance the resistance of Arabidopsis to osmotic stress by regulating the balance of ROS. The interaction protein of MSRA5 was predicted and analyzed by bioinformatics technology. Glutathione synthase 2 (GSH2) was predicted as the potential interaction protein of MSRA5. Yeast two hybrid experiment and two molecule fluorescence complementary experiment confirmed that quinoa glutathione synthase 2 gene (GSH2) and CqMSRA5.1 was interacted with each other. Through protein simulation binding analysis, it is found that the specific region of interaction is met residue 193 of CqGSH2. The pharmacological phenotype experiment with GSH specific inhibitor BSO showed that BSO could significantly inhibit CqMSRA5.1. The above CqMSRA5.1 and CqGSH2 interact structurally.

Keywords: protein, quinoa, gene, Abiotic stress, reactive oxygen species, osmotic stress

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1. Introduction

The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be carefully reviewed and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or numerals in square brackets [1-5]. Drought stress, which causes a decline in quality and quantity of crop yields, has become more accentuated these days due to climatic change. It causes many physiological and biochemical changes in plants [6]. Reactive oxygen species (ROS) are produced in plant cells challenged by biotic and abiotic stresses and, if allowed to accumulate excessively, can induce oxidative damage of cells [7]. Oxidative stress, which damages cellular components such as membrane lipids, proteins, and nucleic acids, results in metabolic dysfunction [8]. Methionine (Met), including the free and protein-bound forms, can be easily oxidized in the presence of excess ROS under abiotic stress conditions to form Met sulfoxide (MetO) [4]. Plants possess repair enzymes, known as Met sulfoxide reductases (MSRs) [9]. Based on the pair of MetO diastereomers, MSRs have been classified as MSRA, which show specificity toward Met sulfoxide reductases (MSRs) [9]. Based on the pair of MetO diastereomers, MSRs have been classified as MSRBs, which show specificity toward Met-S-O, and MSRBs, which interact with Met-R-O [10].

Many MSR genes have been characterized and cloned in various species [11]. Plant MSRs are expressed in the leaves, stem, root, and seed, and can be found in the cytosol and chloroplast, or can be secreted from cells [12]. Much research has been performed to determine the physiological context of plant MSRs in mutant and transgenic plants in response to diverse external stimuli,
including pathogen attack [13,14]. All MSR proteins are good antioxidant candidates because cyclic oxidation and reduction of Met residues can serve as an efficient pathway to scavenge ROS in cells [15,16]. Unfortunately, most research on plant MSR function was implemented in the reference plant Arabidopsis [17]. The function of MSR genes in quinoa is not yet known.

Identity of the substrates of plant MSRs will allow to gain further insight into physiological roles of these thiol-dependent reductases. Several specific substrates to different plant MSRs were confirmed. Heat-shock protein 21 (HSP21) was the first specific substrate of plastidial MSRA identified in plants [18,19]. AtMSRB7 interacts with glutathione transferases [20]. Lychee (Litchi chinensis) enzymes LcMSRA1 and LcMSRB1 use CaM as their substrate [21]. Wheat enzyme TaMSRA4.1 can reduce oxidative TaHO1 [22]. TaGSTF3 was the substrate of wheat enzyme TaMSRB5.2 [23]. However, no substrate of the MSRA5 has been identified to date much less in quinoa.

Quinoa (Chenopodium quinoa) Willd. 2n = 4x = 36 was a nutritious crop adapted to thrive in a range of agroecosystems. The plant was presumably first domesticated more than 7,000 years ago by pre-Columbian cultures, and its seed was known as the ‘mother grain’ of the Inca Empire that arose in the 13th century [24,25]. Because quinoa has high genetic diversity and can adapt to harsh conditions (e.g., the highlands of the Andes), it can be grown on marginal soils and is resilient to frost, drought, salinity, and large day-night temperature variations [26,27,28]. Quinoa produces nutritious and gluten-free grains with a fine balance between carbohydrates, essential amino acids, oils, minerals, vitamins, and dietary fibers [29,30]. The draft of the C. quinoa genome has provided insights into the mechanisms underlying agronomically important traits of quinoa and has laid the foundation for accelerating the genetic improvement of other crops [31]. The objectives of this study were to member composition, gene structure, evolutionary relationship and expression pattern of cqmsr gene family were identified and analyzed. Based on the molecular mechanism of its response to osmotic stress was analyzed.

2. Materials and Methods

2.1. Treatments, and Gene Expression Analyses

Quinoa plants were grown in the greenhouse. Seedlings were established under controlled conditions of 60-70% relative humidity, 14-h light, and an average temperature of 23°C until 2 weeks. They were then irrigated with 300 mM NaCl, 25% (w/v) polyethylene glycol (PEG 6000, Sigma-Aldrich, St. Louis, MO, USA) in soil homogeneously, or treated at 4°C and 40°C. Whole plant was expressed in Escherichia coli by cloning it into the MPH vector (-MBP/-His tag; the empty MPH vector is ~40 kDa) using prime rs listed. The recombinant vector was transformed into E. coli BL21 as described [36]. The protein was purified from sonicated cells using a Ni2+-affinity column and was assessed by SDS-PAGE. Total protein and enzyme activity of 10-day-
old Arabidopsis seedlings were evaluated [12,14]. To distinguish between diastereomers, 10 μg purified CqMSRA5.1 recombinant protein was used. The activity of the ROS scavenging enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in leaves was quantified using kits (Beyotime Biotechnology, Shanghai, China) following the supplier’s protocols. All measurements were based on three replicates, and each replicate consisted of three samples.

2.1.4. Generation of transgenic A. thaliana plants

CqMSRA5.1 was introduced into the BamHI and SalI cloning sites of the pSTART vector (Biovector Inc., New York City, NY, USA) to obtain a constitutive expression construct driven by the cauliflower mosaic virus (CaMV) 35S promoter using the primers listed. The construct was then transformed into A. thaliana Col-0 via the Agrobacterium tumefaciens-mediated floral dip method [37]. Transgenic A. thaliana plants were grown in soil under a 16-h photoperiod (22/20°C, 60% relative humidity). Transgenic progeny of the primary transformants was selected by raising seedlings on solidified half-strength Murashige and Skoog [38] medium containing 60 μg/mL kanamycin.

2.1.5. A. thaliana Growth Conditions and Stress Treatments

Arabidopsis seeds were surface-sterilized by immersion in 0.1% (w/v) HgCl2 and plated in 1% agar medium supplemented with half-strength [38] Murashige and Skoog nutrients and 3% (w/v) sucrose kept in the dark at 4°C for 3 days to break dormancy. The plates were then transferred to conditions of a 16-h photoperiod, 22°C, 70% relative humidity, and 170-200 μmol m^-2 s^-1 light intensity. Uniform one-week-old seedlings were transferred to fresh medium containing various concentrations of mannitol, 1.5 mM H2O2, 300 μM L-buthionine sulfoximine (BSO) or 200 mM mannitol + 300 μM BSO for 2 weeks. Experiments were performed in triplicate.

2.1.6. Osmotic Stress Conditions

Four-week-old soil-grown Col-0, CqMSRAS1.1 transgenic A. thaliana, and msra5 (SALK_201557C) plants were exposed to drought stress by withholding water for 2 weeks. Water content was measured to ensure that all genotypes were exposed to equal stress severity. The same amount of water was used for each group. After drought stress treatment, plants were watered and allowed to recover for 3 days before their survival rates were calculated. Each treatment was replicated three times.

2.1.7. Measurement of ROS Levels

ROS levels of Arabidopsis by 3,3′-diaminobenzidine (DAB) staining [39] and nitroblue tetrazolium (NBT) staining [40], in which the samples were immersed in 0.5 mg/mL NBT, dissolved in 10 mM KH2PO3/K2HPO3 (pH 7.6) at 28°C in dark, after which chlorophyll was removed by steeping in 95% ethanol in a boiling water bath.

2.1.8. Yeast-two-hybrid (Y2H) and Bimolecular Fluorescence Complementation (BiFC)

For Y2H, either CqMSRAS1.1 or CqGSH2 was cloned into pGBK7T (Invitrogen) as the bait, and CqMSRAS1.1 or CqGSH2 into pGADT7 (Invitrogen) as the prey. The bait and prey plasmids were co-transformed into yeast strain Y2H GOLD (Clontech), following the manufacturer protocol. Positive yeast transformants were selected on the basis of their ability to grow on SD minimal (-Leu-Trp) and (-Leu-Trp-His) medium and LacZ reporter. The controls chosen were p53-pGBK7T + Larget T-pGADT7 (positive interaction), pGBK7T + pGADT7 (no interaction).

The BiFC protocol closely followed that of Bracha et al. [41]. The CqMSRAS1.5 coding region minus its stop codon was inserted into the YFPN vector, while CqGSH2 was inserted into YFPC using primers listed. The corresponding expression vectors were introduced into tobacco cells. After an overnight incubation in the dark and then in the light for two days, the YFP signal and chlorophyll auto fluorescence were detected by confocal microscopy, using excitation wavelengths of 488 nm and 647 nm, respectively. The OST1-SLAC1 interaction was used as the positive control [42].

2.1.9. Root Length Measurement

The root length of treated and untreated control seedlings was measured using winRHIZO (Régent Instruments Inc., Québec, Canada).

2.1.10. Statistical Analysis

Quantitative data are presented as means ± SE. Means were compared by Student’s two-tailed t-test.

3. Results

3.1. Transcription Profiling of CqMSR Genes

The published MSR gene sequences of Arabidopsis thaliana and rice were used to search and identify quinoa MSR gene family members in quinoa database according to homologous sequence alignment. As shown in total of 8 quinoa MSR gene family members were identified and cloned. The length of the gene sequence ranged from 612 bp to 762 bp. It was divided into MSRA family and MSRB family. MSRA family includes MSRA1.1, MSRA1.2, MSRA3.1 and MSRA5.2 members. The MSRB family consists of MSRB1.1, MSRB1.2, MSRB2.1, and MSRB2.2 members. The basic information of each member of quinoa MSR gene family is shown in (Table 1), and the isoelectric point range of this family protein is 5.46-9.25. The molecular weight was in the range of 25.13kD-28.34 K D. The results of subcellular localization prediction analysis showed that the distribution of MSR in quinoa was diversified, including cytoplasm, mitochondria, chloroplast and nucleus.

3.2. Characteristics of Structure of CqMSR Gene Family

The gene structure analysis of each member of the CqMSR family are shown in (Figure 2). The CqMSRA1.1 gene contains three exons and two introns. CqMSRA1.2 contains two exons and one intron. Both CqMSRAS1.1 and CqMSRAS1.5 contain four exons and three introns. CqMSRB1.1 and CqMSRB1.2 contain 5 exons and 4
introns. *CqMSRB2.1* and *CqMSRB2.2* contain three exons and two introns, and the introns are different in size.

### 3.3. Constitutively Expressed Gene Family Evolution Protein Sequences

MSR protein sequences of Arabidopsis, spinach, sugar beet and Brachium. Results As shown in (Figure 3), quinoa MSR family is divided into two obvious subfamilies, A and B. Four genes of family A were located in two distant branches, namely *CqMSRA1.1* and *CqMSRA1.2*, and *CqMSRA5.1* and *CqMSRA5.2*. Four members of family B were similar to family A and distributed in two collateral branches, respectively. The relative relationship with sugar beet, spinach, Arabidopsis is higher, and other staple crops such as wheat and corn are far away.

![Figure 1. Cloning of members of the quinoa MSR gene family](image)

**Table 1. Summary of member information of quinoa MSR gene family**

| Accession number | Chromosome location | Name       | CDS(aa) | Length | PI   | MW (KD) | Location   | Potential Redox Cys |
|------------------|---------------------|------------|---------|--------|------|---------|------------|---------------------|
| AUR62018817      | Scaffold_1817       | CqMSRA1.1  | 672     | 223    | 5.46 | 25.15   | Cytoplasm  | C45/C80/C86         |
| AUR62007186      | Scaffold_1971       | CqMSRA1.2  | 612     | 203    | 5.66 | 22.93   | Cellmembrane | C45/C80/C86         |
| AUR62044454      | Scaffold_2762       | CqMSRA5.1  | 762     | 253    | 9.32 | 28.34   | chloroplast | C70/C227           |
| AUR62043612      | Scaffold_1798       | CqMSRA5.2  | 762     | 253    | 9.32 | 28.34   | chloroplast | C70/C227           |
| AUR62019677      | Scaffold_2127       | CqMSRB1.1  | 603     | 200    | 9.02 | 22.12   | Nucleus    | C185                |
| AUR62013936      | Scaffold_3035       | CqMSRB1.2  | 603     | 200    | 9.03 | 22.17   | Nucleus    | C185                |
| AUR62027070      | Scaffold_2314       | CqMSRB2.1  | 675     | 224    | 9.04 | 24.74   | cytoplasm  | C154/C207           |
| AUR62030911      | Scaffold_2587       | CqMSRB2.2  | 675     | 224    | 9.25 | 24.78   | cytoplasm  | C154/C207           |

![Figure 2. Structure analysis of exons and introns of CqMSRs](image)
3.4. Heterologous Expression of Promoter Elements of CqMSRs gene

According to the gene sequence of each member of CqMSR, the promoter sequence of 2000bp upstream of CqMSR S was obtained by Phytozome database. The online tool plant CARE was used to analyze the elements of the initiator sequence, and the results are shown in (Table 2) (the number represents the number of elements in the CqMSRS gene promoter contains multiple stress response and hormone response elements. Examples include ABRE elements in response to abscisic acid ABA, TGACG motif in response to methyl jasmonate MeJA, and TCA elements in response to salicylic acid. In addition there are multiple MYB binding elements. The numerous hormone response elements indicate that CqMSR gene family members may be involved in the stress response process and may interact with various hormones, MYB Transcription factor binding elements suggest that CqMSRS may be regulated by upstream transcription factors.

3.5. Amino Acid Sequence Conservation Analysis of CqMSRs

The expression of were used to compare and analyze the MSR protein sequences of quinoa, Arabidopsis thaliana, spinach, sugar beet, wheat, corn, Brachium and millet, and the results were shown in (Figure 4a,b). Quinoa MSR has high similarity with MSR family members of other species, and its domain was conserved in terms of position, size and amino acid sequence. Further analysis revealed that CqMSRA family members contained three different conserved domains, and the active cysteine residue Cyc involved in REDOX reactions was distributed in Domain 1 and Domain 3. CqMSRB family members have four different conserved domains, and the active cysteine residues in these four domains are located in Domain 2 and Domain 4. These results indicated that MSR family genes in quinoa were conserved in evolution.
Table 2. Analysis of CqMSRs promoter elements

| Motif Function | ABRE ABA responsiveness | LTR low-temperature responsive | MBS MYB Binding Site | TC-rich repeats defense and stress responsiveness | TGA-element auxin-responsive element | P-box Gibberellin-responsive element | salicylic acid responsiveness | TGACG-motif MeJA-responsiveness |
|----------------|--------------------------|---------------------------------|----------------------|-----------------------------------------------|-----------------------------------|-----------------------------------|-------------------------------|---------------------------------|
| CqMSRA1.1      | 2                        | 0                               | 0                    | 0                                             | 1                                 | 1                                 | 1                            | 1                               |
| CqMSRA1.2      | 2                        | 1                               | 1                    | 1                                             | 0                                 | 1                                 | 1                            | 1                               |
| CqMSRA5.1      | 3                        | 2                               | 0                    | 1                                             | 1                                 | 0                                 | 0                            | 1                               |
| CqMSRB1.1      | 3                        | 2                               | 0                    | 1                                             | 1                                 | 0                                 | 0                            | 1                               |
| CqMSRB1.2      | 3                        | 1                               | 0                    | 1                                             | 0                                 | 1                                 | 0                            | 2                               |
| CqMSRB2.1      | 2                        | 2                               | 1                    | 1                                             | 1                                 | 0                                 | 0                            | 1                               |
| CqMSRB2.2      | 0                        | 0                               | 2                    | 1                                             | 0                                 | 1                                 | 1                            | 2                               |

Figure 4A. Conserved Motif analysis of amino acid sequence of quinoa MSRA family members (the dark area is the conserved area, the yellow area is the conserved Motif, and the red arrow indicates the active cyce residue)

Figure 4B. Conserved Motif analysis of amino acid sequence of quinoa MSRB family members (the dark area is the conserved area, the yellow area is the conserved Motif, and the red arrow indicates the active cyc residue)
3.6. Prokaryotic Expression and Purification of CqMSRA5.1 were Used for Verification

The enzyme characteristics of CqMSRA5.1, the prokaryotic expression vector of CqMSRA5.1 protein was constructed, and the fusion protein was prokaryotic expression and purification (Figure 5). Among them, BL21 strain manipulated the expression of target protein by lactose operon. IPTG can induce the expression of T7 RNA polymerase. T7 RNA polymerase binds specifically to the T7 initiator sequence to initiate the expression of the target fusion protein. Therefore, BL21 strain was used to explore the induced expression of CqMSRA5.1 in prokaryotic strains under different temperatures and IPTG concentrations. It was found that when IPTG concentration was 0.5 mM and temperature was 16°C, with the extension of induction time, CqMSRA5.1 fusion protein could accumulate in a large amount, and the size of CqMSRA5.1 fusion protein was 69.8kDa. Later, the protein was purified, and a relatively single CqMSRA5.1 MPH fusion protein was purified.

3.7. Agrobacterium Transformed

Agrobacterium GV3101 was transformed to further infect Arabidopsis thaliana to obtain generation positive plants. They were successively passed through and propagated to produce positive plants. The positive plants of generation were obtained by continuous passage and propagation. Generation of positive plants QRTqRT PCR detection and selection of the higher expression level. The expression level detection arabidopsis reference gene was used as the reference gene, and Arabidopsis reference gene was used as the control, total and total MSRMSR enzyme activity was significantly increased (Figure 6A), and the two lines were named AtOE1 and AtOE2, respectively. Selected for subsequent phenotypic experiments. The same time, Arabidopsis thaliana was ordered and selected for subsequent phenotypic experiments. The same time, the Arabidopsis at MSRA5 gene mutation variant MSRA5MSRA5 was ordered and its expression level was detected. The results are shown in
MSRA gene was expressed in the mutant, which could be used for subsequent phenotypic experiments. Because of its expression, it can be used in subsequent phenotypic experiments.

Figure 6. Construction of CqMSRA5.1 Arabidopsis overexpression line and identification of the expression level of mutant msra5 A: Relative expression level in CqMSRA5.1 transgenic Arabidopsis seedlings. B: RT-PCR to detect MSRA5 expression. C: MSR activity in CqMSRA5.1 transgenic Arabidopsis seedlings. Data are presented as means ± SE (n = 3). *P < 0.05, **P < 0.01 by Student’s t test

Figure 7. CqMSRA5.1 can improve the tolerance of Arabidopsis to H2O2. The appearance of seedlings of WT, AtOE1 and AtOE2 lines and the msra5 mutant grown (A) under non-stressed conditions, (B) in the presence of 1.5 mM H2O2. (C) The length of the roots of the seedlings grown under non-stressed conditions or in the presence of 1.5 mM H2O2 for 14 days. Data are presented as means ± SE (n = 3). *P < 0.05, **P < 0.01 by Student’s t-test
3.8. CqMSRA5.1 can Significantly Improve the Tolerance of H$_2$O$_2$ in Arabidopsis Thaliana

When plants are subjected to abiotic stress, excessive ROS will be produced, which will cause oxidative damage to plants. Therefore, studying the antioxidant level of plants can reflect the tolerance of plants to abiotic stress to a certain extent. To this end, on the south H$_2$O$_2$ phenotype was tested in all Chinese Thaliana lines. When different concentrations of H$_2$O$_2$ were added to Arabidopsis thaliana exogenously, phenotypes were observed (Figure 7). Under the condition of not adding H$_2$O$_2$, there were no significant differences in root length and growth status of each line. The primary root length of the two overexpression lines of CqMSRA5.1 was significantly longer than that of Col 0, and the mutant system was significantly. These results indicated that the overexpression lines of Arabidopsis had a strong ability to resist oxidative stress, while the mutant system had a weak ability to resist oxidative stress, that is, the overexpression of CqMSRA5.1 could significantly enhance the resistance of Arabidopsis to oxidative stress.

3.9. Rainfall Distribution on 10-12 CqGSH2

Protein structure analysis and tertiary structure simulation modeling right the results of protein structure analysis of CqGSH2 are shown in (Figure 8A). CqGSH2 possesses two GSH synth domain, that is, the enzyme active functional domain of GSH2. Protein tertiary structure modeling of CqGSH2 was carried out as shown in (Figure 8B). The results showed that CqGSH2 protein contained 13 Met residues, and the Met content was 2.2% higher than the average Met content in the protein 1.7%. And three of them are surface distributed. As reported in the literature, an important characteristic of MSR substrate the content of Met is abundant and distributed on the surface. CqGS H$_2$ satisfies this condition, considering GSH2 and plant bodies internal REDOX balance is closely related to CqMSRA5.1, which is more consistent with CqGSH2 in function there may be a potential substrate for CqMSRA5.1.

4. Discussion

Quinoa, as a new cereal crop, has attracted extensive attention in China and around the world because of its comprehensive and balanced nutritional characteristics, strong adaptability, barren resistance and stress resistance. It is an important direction for quinoa breeding in China in the future to isolate and identify quinoa stress resistance genes, analyze the molecular mechanism of quinoa stress tolerance, and then cultivate new quinoa varieties with stress resistance and high yield. Methionine sulfoxide reductase (MSR), as an important member of maintaining redox balance implants, plays an important role in plant tolerance to abiotic stress, which has not been reported in quinoa. Plant MSRs play a supporting role in defines against oxidative stress triggered by multiple stresses,
including drought stresses, but the functionality in quinoa of members of this family has yet to be ascertained. Flexible protein free amino acid repair system based on the ability of MSRs to reduce oxidized Met to normal reduced Met [32]. Plant response to abiotic stress is undoubtedly a complex process involving a variety of biochemical modifications, including activation or up-regulation of a variety of metabolic pathways related to REDOX balance in the plant genome [33]. In this study, we found that CqMSRs responded to four abiotic stresses to varying degrees, and almost all CqMSRs responded to hot and cold stress. CqMSRA1.2 can be induced by drought and salt stress, while CqMSRA1.1 can only be induced by drought stress; CqMSRA5.5s can be induced by salinity, drought and heat stress. These differential responses suggest that these genes may belong to different MSR populations and differ in the selection of substrates for their products. It has been reported that TaMSRA5 is induced by both drought and salt stress [34]. Although both are members of the same MSR subgroup, TaMSRA1 and TaMSRA5 have distinct differences in active site and structure, leading to their unique stress responses and physiological roles. Here we found that CqMSRB1.1 can be induced by drought and salt stress, whereas CqMSRB1.2 can only be induced by cold stress TaMSRB1.1 constitutively expressed in wheat has been reported to result in increased tolerance to stress exerted by H2O2, MV, or NaCl. When expressed in yeast, the rice homolog of this gene showed enhanced resistance to oxidative stress [35]. CqMSRB2.1 was induced under both drought and heat stress, whereas CqMSRB2.2 was induced only under heat stress. It has been reported that TaMSRB5.1 constitutively expressing TaMSRB5.1, which can be induced by drought and salinity, is better able to tolerate both stresses. Thus, although MSRBs clearly share similarities at the protein structure level, their specific activity stability and/or substrate target specificity may not be identical [36]. The most reactive gene, CqMSRA5.1, located in chloroplasts, showed clear changes after four abiotic stresses in leaves. Given that chloroplasts are a major source of reactive oxygen species, chloroplast proteins may be more sensitive to damage. The presence of MSRs and MSRBs implies that oxygenated Met in proteins is efficiently repaired in chloroplasts [37]. CqMSRA5.2 is less responsive to heat and cold stress than CqMSRA5.1. Although two genes have a high degree of sequence similarity, their specific activity stability and/or their substrate target specificity may not be the same [38]. The function of quinoa MSRA5.1 and its partner together involved in osmotic stress remains unclear. In this paper, we identified CqGSH2 as an interacting partner of CqMSRA5.1, which sheds some light on the mechanism of CqMSRA5.1 gene in quinoa in response to osmotic stress.

4.1. Quinoa MSR Gene Family Responds to Abiotic Stress

Plants have evolved a flexible protein/free amino acid repair system, based on the ability of MSRs to replace MetO with Met [9]. Adaptation to abiotic stress is undoubtedly a complex process involving several biochemical modifications, including the activation or upregulation of MSRs involved in redox balance that are harbored in the plant genome [46]. CqMSRs responded to four abiotic stresses in varying degrees, and almost all CqMSRs were induced by PEG and NaCl stresses (Fig. 1b, c). In comparison, few CqMSRs responded to heat and cold stresses. These differential responses indicated that the genes probably belong to different MSR groups and vary in the choice of substrate of their product. CqMSRA1.2 was found to be inducible by both drought and salinity stress while CqMSRA1.1 can only be induced by drought stress; CqMSRA5.s were found to be inducible by salinity, drought and heat stresses. It was reported that TaMSRA5 was induced by both drought and salinity stress. Despite both being members of the same MSR subgroup, there was a distinct difference between TaMSRA1 and - A5 in terms of site of activity and structure, resulting in their having a distinctive stress response and physiological role. CqMSRB1.1 was found to be inducible by both drought and salinity stress while CqMSRB1.2 can only be induced by cold stress; It was reported that constitutive expression of TaMSRB1.1 in A. thaliana resulted in a heightened tolerance to the stress imposed by either H2O2, MV or NaCl. The rice homolog of this gene, when expressed in yeast, has been shown to enhance resistance to oxidative stress [8]. CqMSRB2.1 was found to be inducible by both drought and heat stress while CqMSRB2.2 can only be induced by heat stress. It was reported that A. thaliana plants constitutively expressing TaMSRB5.1, which was inducible by both drought and salinity, were better able to tolerate both these stresses. Thus, although the MSRBs clearly share similarity at the level of protein structure, their specific activity/stability and/or their substrate/target specificity may well not be identical [9]. The most responsive gene CqMSRA5.1, which is located in the chloroplast, displayed pronounced changes after the four abiotic stresses in leaves. Given that the chloroplast is a major source of ROS, chloroplast proteins may be more sensitive to damage. The presence of both MSRA and MSRB implies that oxidized Met in proteins is efficiently repaired in the chloroplast [18]. Compared with CqMSRA5.1, CqMSRA5.2 has a lower response to heat and cold stresses. Although both genes have high sequence similarity, their specific activity/stability and/or their substrate/target specificity may not be identical.

4.2. CqMSRA5.1 was Involved in Osmotic Stress Resistance

Drought is one of the most detrimental types of abiotic stress in plant agriculture [47]. Adaptation to drought (or osmotic stress) is undoubtedly a highly complex process, involving numerous biochemical modifications, such as the activation or upregulation of MSRs, harbored in plant genome, involved in redox balance [47]. The quinoa MSR member CqMSRA5.1 deduced peptide revealed a C-terminal PMSR domain, characteristic of MSRA sequences and shares a substantial level of homology with MSRA5 proteins in other plant species. The results protein reduced Met-S-SO in vitro function specific to MSRA. Furthermore, CqMSRA5.1overexpression enhanced the total MSR enzyme activity in Arabidopsis. It is known
that two plant MSRA homologs, including TaMSRA2 and TaMSRA5, have been reported to enhance salt tolerance of Arabidopsis [13]. TaMSRA4.1 have been reported to decrease the level of ROS in response to osmotic stress [48]. In the present study, it was found that constitutive expression of CqMSRA5.1 in Arabidopsis had a positive effect on the tolerance to osmotic stress by decreasing ROS level. CqMSRA5.1 also increased Arabidopsis tolerance to osmotic stress in soil. The above data imply that CqMSRA5.1 is a positive component involved in osmotic stress tolerance. This indicates that the plants have greater tolerance ability to be subjected to oxidative stress. Addition of BSO, a glutathione synthesis inhibitor, significantly inhibited the overexpression of Arabidopsis thaliana the tolerance level of osmotic stress. This suggests that osmotic tolerance conferred by CqMSRA5.1 depends on its association with CqGSH2. The interaction between the discovery of CqMSRA5.1 substrate will help to fully explain its effect on osmotic stress tolerance, and Explaining how CqMSRA5.1 acts on the ROS pathway provides additional theoretical support.

4.3. CqMSRA5.1 Promotes Osmotic Stress Tolerance by Regulating ROS Content

At the cellular level, drought induces oxidative stress by overproduction of ROS [49]. Plants have developed a complex ROS scavenging system, containing a series of nonenzymatic compounds and a set of enzymes including SOD, CAT, POD, and ascorbate peroxidase (APX), in which, the latter set could be regulated by many genes including MSR family [14,47,49]. We demonstrated that the constitutive expression of CqMSRA5.1 in Arabidopsis decreased the accumulation of ROS contents which could be beneficial from the enhancement of CAT and SOD activities. Interestingly, the expression of a set of AtRboh genes including Rboh C, D, E and F were significantly down-regulated in AtOE plants compared to WT. Our previous researches have been reported that overexpression TaMSR genes in A. thaliana decreased ROS content by up-regulating SOD, CAT, and POD activities, and down-regulating the expression of Rboh genes [14,45].

4.4. Biological Significance of the CqMSRA5.1/CqGSH2 Interaction in Osmotic Stress

Oxidation of Met causes dysfunction for many proteins, and in certain cases, this can be restored by MSRs. Numerous proteins have been identified as substrates of MSR in animals and microorganisms in vitro [23]. However, few have been verified as MSR targets in higher plants: AtGST2/3 to AtMSRB7, LcCaM1 to LcMsrA1/B1, TaHO1 to TaMSRA4.1 [14], TaHO1 to TaMSRB3.1 [45] and TaGSTF3-A to TaMSRB5.2 [24]. Here, a candidate interactor of CqMSRA5.1, CqGSH2 was screened by STRING database, and identified as one of CqMSRA5.1 interacting partner. It has multiple physiological activities, such as antioxidization, amino acid transport, detoxification and immune, [50]. Ascorbate (AsA) and GSH are the two major antioxidant compounds found in plant cells and are known to play a central role in redox regulation via the AsA-GSH cycle, which maintains cellular redox homeostasis [51]. In the present study, CqGSH2 protein has thirteen Met residues, three of which are distributed on the surface. It has been suggested that MSR substrates tend to be Met-rich proteins the Met content of CqGSH2 (2.1%) is higher than the average 1.7% Met found in proteins (1.7%) [52]. As shown in Figure 5f and g, Met193 in CqGSH2 located in the junction of the CqMSRA5.1-CqGSH2 complex and highly close to the spatial position of two active Cys residues (Cys70 and Cys227) of CqGSH2. We speculate that Met193 is beneficiary from this interaction. CqMSRA5.1 overexpression in arabidopsis not only increased the transcription level of AtGSH2 but also elevated the GSH content. CqMSRA5.1 overexpression in Arabidopsis decreased the level of GSSG leading to increase the GSH/GSSG ratio encoding genes were highest expressed in leaf induced by osmotic stress and positive role in response to abiotic stress and physical interaction between them it suggested the osmotic tolerance conferred by CqMSRA5.1 depends partly on its interaction with CqGSH2. The discovery of substrate of CqMSRA5.1 will help to fully explain its effect on osmotic stress tolerance and pave a way for explaining how CqMSRA5.1 acts on ROS pathway. Flexible protein free amino acid repair system, the basis of the ability of MSRs to reduce oxidized to normal reduced Met [53]. It is undoubtedly a complex process involving a variety of biochemical modifications, including activation or upregulation of REDOX plains in the plant genome in this study, it was found that CqMSRs had different degrees of response to four abiotic stresses. Therefore, almost all CqMSRs responded to PEG and NaCl stress. In contrast, there are few CqMSRs for heat and cold the stress responds. CqMSRA1.2 could be induced by drought and salt stress, while CqMSRA1.1 could only be induced by drought stress. Guide CqMSRA5s can be induced by salinity, drought and heat stress. These differential responses suggest that these genes may belong to different MSR populations and varied in the selection of substrates for their products. TaMSRA5 is reported to be caused by drought. Although both are members of the same MSR subgroup, TaMSRA1, and TaMSRA5. There are obvious differences in active site and structure, resulting in their unique stress response and physiological role. This study found that CqMSRB1.1 could be induced by drought and salt stress, while CqMSRB1.2 could only be induced by cold stress. TaMSRB1.1, constitutively expressed in wheat, has been reported to result in tolerant to stress exerted by H2O2, MV or NaCl Increased receptivity. The rice homolog of this gene, when expressed in yeast, shows enhanced resistance to oxidative stress [54]. CqMSRB2.1 could be induced under both drought and heat stress, while CqMSRB2.2 could only be induced under heat stress. According to the report, TaMSRB5.1 constitutively expressing TamsrB5.1 was better able to tolerate both stresses, and the plant could be affected by drought and induction of salinity. Thus, despite the apparent similarity of MSRBs at the protein structure level, their specific activities stability and/or substrate target specificity may not be identical [53, 54]. Most REACTIVE genes located in CHLORO plasts CqMSRA5.1 showed obvious changes
after four abiotic stresses in leaves. Because chloroplasts are reactive oxygen species the main source, chloroplast proteins may be more sensitive to damage. The presence of MSRAs and MSRBs means oxygen in the protein Chemise Met is efficiently repaired in chloroplasts [52,53,54]. Compared with CqMSRA5.1, CqMSRA5.2 is resistant to heat and cold stress the response is low. Although two genes have high sequence similarity, their specific activity stability and or they substrate target specificity may be different.

5. Conclusions

In this study CqSMRs gene family has an important contribution to quinoa abiotic stress response, including CqMSRA5.1 plays an important role in coping with osmotic stress. Through the interaction with CqGSH2, it regulates the ROS balance in quinoa. This result provides an important theoretical basis for stress resistance breeding of quinoa, and also provides stress resistance gene source for other crop breeding. The protein cluster CqMSR had a typical MSR domain, which was evolutionarily conservative and closely related to spinach and sugar beet. Tissue expression results showed that all the CqMSRhad highest expression in stems except CqMSRA5.1 and CqMSRA5.2.

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