Function and Mechanism of WRKY Transcription Factors in Abiotic Stress Responses of Plants

Weixing Li†, Siyu Pang†, Zhaogeng Lu and Biao Jin*†

College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China; liwx@yzu.edu.cn (W.L.); pangsiyu_0212@163.com (S.P.); luzhaogeng@163.com (Z.L.)
* Correspondence: bjin@yzu.edu.cn
† Contributed equally to this work.

Received: 26 September 2020; Accepted: 4 November 2020; Published: 8 November 2020

Abstract: The WRKY gene family is a plant-specific transcription factor (TF) group, playing important roles in many different response pathways of diverse abiotic stresses (drought, saline, alkali, temperature, and ultraviolet radiation, and so forth). In recent years, many studies have explored the role and mechanism of WRKY family members from model plants to agricultural crops and other species. Abiotic stress adversely affects the growth and development of plants. Thus, a review of WRKY with stress responses is important to increase our understanding of abiotic stress responses in plants. Here, we summarize the structural characteristics and regulatory mechanism of WRKY transcription factors and their responses to abiotic stress. We also discuss current issues and future perspectives of WRKY transcription factor research.

Keywords: WRKY transcription factor; abiotic stress; gene structural characteristics; regulatory mechanism; drought; salinity; heat; cold; ultraviolet radiation

1. Introduction

As a fixed-growth organism, plants are exposed to a variety of environmental conditions and may encounter many abiotic stresses, for example, drought, waterlogging, heat, cold, salinity, and Ultraviolet-B (UV-B) radiation. To adapt and counteract the effects of such abiotic stresses, plants have evolved several molecular mechanisms involving signal transduction and gene expression [1,2]. Transcription factors (TFs) are important regulators involved in the process of signal transduction and gene expression regulation under environmental stresses. TFs can be combined with cis-acting elements to regulate the transcriptional efficiency of target genes by inhibiting or enhancing their transcription [3,4]. Accordingly, plants may show corresponding responses to external stresses via TFs regulating target genes. Although some TF families (MYB, bZIP, AP2/EREBP, NAC) are associated with adversity [2,5], WRKY is the most extensively studied TF family in plant stress responses.

The WRKY family is a unique TF superfamily of higher plants and algae, which play important roles in many life processes, particularly in response against biotic and abiotic stress [6,7]. In 1994, the SWEET POTATO FACTOR1 (SPF1) gene of the WRKY family was first found in Ipomoea batatas [8]. Later, ABF1 and ABF2 were found in wild Avena sativa, and showed regulatory roles in seed germination [9]. A previous study successively cloned WRKY1, WRKY2, and WRKY3 from Petroselinum crispum, named the WRKY TF, and proved for the first time that WRKY protein can regulate plant responses to pathogens [10]. With an increase in available published genomes, many members of the WRKY TF family have been identified in various species, including 104 from Populus [11], 37 from Physcomitrella patens [12], 45 from Hordeum vulgare [13], 55 from Cucumis sativus [14], 74 from Arabidopsis thaliana [15], 83 from Pinus monticola [16], 81 from Solanum lycopersicum [17], and 102 from Oryza sativa [18]. WRKY TFs exist as gene families in plants, and the number of WRKY TFs varies among species. In plants exposed to...
abiotic stresses (salt, drought, temperature, and so forth), WRKY family members play important roles in diverse stress responses. In addition, these TFs affect the growth and development of plants [19,20]. Therefore, WRKY TFs have attracted broad attention. Although some reviews on WRKY TFs are available, in this review we focus on the structural characteristics and regulatory mechanisms of WRKY TFs and summarize recent progress in understanding the roles of WRKY TFs during exposure to abiotic stresses such as drought, temperature, salt, and UV radiation.

2. Structural Characteristics of WRKY TFs

The WRKY structure consists of two parts: the N-terminal DNA binding domain and the C-terminal zinc-finger structure [21]. The DNA binding domain sequence of WRKY is based on the heptapeptide WRKYGQK (Figure 1), but there are some differences, such as WRKYGQK, WRKYGKK, WRKYGMK, WSKYGQK, WKRYGQK, WVKYGQK, and WKKYGQK [17,22]. Zinc-finger structures mainly include C$_2$H$_2$ type and C$_2$HC type [23], whereas some exist in the form of CX$_{29}$HXH and CX$_7$CX$_{24}$HXC [17] (Figure 1). According to the number of WRKY domains and the structure of their zinc-finger motifs, WRKY can be divided into groups I, II, and III [23] (Figure 1). Group I usually contains two WRKY domains and one C$_2$H$_2$ zinc-finger structure. Those in group II and group III contain only one WRKY domain. The difference is that the zinc-finger structure in group II is C$_2$H$_2$ and that in group III is C$_2$HC [19,21,23] (Figure 1). According to the phylogenetic relationship of the amino acid sequence of the primary structure, group II can be further divided into subgroups a–e [7,23,24]. Evolutionary analyses have shown that the WRKY of group II is not generally a single source, mainly including five categories I, IIa + IIb, IIc, IIId + IIe, and III [7,24]. In addition, some WRKY proteins contain a glutamate enrichment domain, a proline enrichment domain, and a leucine zipper structure [25].
3. Regulatory Mechanism of WRKY TFs

WRKY family members have diverse regulatory mechanisms. Briefly, WRKY protein can be effectively combined with W-box elements to activate or inhibit the transcription of downstream target genes. Moreover, it can also bind other acting elements to form protein complexes, which enhances the activity of transcription binding [21].

WRKY TFs can effectively activate the expression of downstream genes by binding conserved W-box cis-acting elements in the downstream gene promoter region [21,26]. There are abundant W-box elements in the self-promoter of most WRKY TFs. Therefore, these WRKY TFs can bind with
their own promoters to achieve self-regulation or cross-regulation networks by combining with other WRKY TFs [27]. For example, CaWRKY6 of Capsicum frutescens can activate CaWRKY40 and make the plant more tolerant to high temperature and humidity. Glycine max GmWRKY27 not only inhibits the activity of downstream GmNAC29 promoter by independent inhibition, but also cooperatively interacts with GmMYB174 to inhibit the expression of GmNAC29, thereby increasing drought and salt stress resistances [28]. Moreover, chromatin immunoprecipitation (ChIP) studies have shown that when Petroselinum crispum is infected by pathogenic bacteria, PcWRKY1 promoter can effectively bind to itself and the W-box of PcWRKY3 promoter, and transcriptional activation can be achieved through self-negative feedback regulation and cross-regulation with other WRKY proteins [29]. In addition, WRKY TFs can interact with non-W-box elements. For example, Oryza sativa OsWRKY13 can interact with PRE4 (TGCGCTT) elements [30]. Hordeum vulgare HvWRKY46 and Nicotiana tabacum NtWRKY12 can effectively combine with the sucrose response element SURE [31,32]. These results indicate that there are multiple binding modes between WRKY TFs and structural genes. Different binding patterns and preferences of binding sites allow for the regulation of downstream target genes, providing WRKY TFs with versatile functions in the plant transcriptional regulation network.

4. WRKY TF Involved in Abiotic Stress Responses

When plants sense stress, the corresponding signaling is activated and transferred to the cell interior. Reactive oxygen species (ROS) and Ca^{2+} ions are usually exchanged as the signal transduction in the cell. Protein kinases such as MPKs are subsequently activated to regulate the activities of related TFs. Consequently, the plant presents a stress response [31,32]. In response to abiotic stresses, some WRKY TFs can be rapidly differentially expressed, promoting signal transduction and regulating the expression of related genes [33]. The expression patterns and functional identifications of WRKys in most studies are generally based on transcriptome analyses, real-time fluorescence quantitative PCR, gene chip analyses, and genetic transformation. Hence, WRKY genes can function effectively in most abiotic stress responses or tolerances in various plants (Table 1, Figure 2).

**Table 1.** WRKY transcription factors (TFs) involved in abiotic stress responses in plants.

| No. | Gene       | Species                | Induced by Factors | Function                                                      | References |
|-----|------------|------------------------|--------------------|--------------------------------------------------------------|------------|
| 1   | AtWRKY25/26| Arabidopsis            | Heat               | Tolerance to heat and NaCl, negative regulator in oxidative stress and abscisic acid (ABA) | [34]       |
| 2   | AtWRKY33   | Arabidopsis            | NaCl, mannitol, H$_2$O$_2$ | Negative regulator in oxidative stress and abscisic acid (ABA) | [33]       |
| 3   | AtWRKY34   | Arabidopsis            | Cold               | Negative regulator in cold stress | [35]       |
| 4   | AtWRKY39   | Arabidopsis            | Heat               | Tolerance to heat | [36]       |
| 5   | AtWRKY53   | Arabidopsis            | Drought, salt      | Reduced drought resistance and H$_2$O$_2$, sensitive to salt | [37,38]   |
| 6   | AtWRKY57   | Arabidopsis            | Drought            | Tolerance to drought | [39]       |
| 7   | AtWRKY63   | Arabidopsis            | ABA                | Tolerance to drought, regulated ABA signaling | [40]       |
| 8   | AtWRKY54   | Arabidopsis            | Heat               | Response to heat stress | [41]       |
| 9   | POWRKY13   | Populus tomentosa      | Heat               | Response to heat stress | [42]       |
| 10  | GhWRKY22   | Gossypium hirsutum     | Drought            | Tolerance to drought | [43]       |
| 11  | GhWRKY25   | Gossypium hirsutum     | Drought            | Tolerance to salt, reduced drought resistance | [44]       |
| 12  | GhWRKY68   | Gossypium hirsutum     | Salt, drought      | Reduced salt tolerance and drought resistance, positive regulator in ABA signaling | [45]       |
| 13  | VoWRKY24   | Vitis vinifera         | Cold               | Upregulated expression at all stages of hypothermia | [46]       |
| No. | Gene       | Species                  | Induced by Factors | Function                                      | References |
|-----|------------|--------------------------|--------------------|-----------------------------------------------|------------|
| 14  | CaWRKY40   | Capsicum annuum          | Heat               | Tolerance to heat                              | [47]       |
| 15  | BtWRKY36   | Brachypodium distachyon   | Drought            | Tolerance to drought                           | [48]       |
| 16  | FeWRKY70   | Fortunella crassifolia    | Salt               | Tolerance to salt                              | [49]       |
| 17  | OsWRKY11   | Oryza sativa             | Heat, drought      | Tolerance to drought and heat                  | [50]       |
| 18  | OsWRKY72   | Oryza sativa             | Drought, NaCl, ABA | Sensitive to salt, drought, sucrose, and ABA   | [51]       |
| 19  | OsWRKY74   | Oryza sativa             | Pi deprivation, cold | Tolerance to cold and Pi deprivation         | [52]       |
| 20  | OsWRKY76   | Oryza sativa             | Cold               | Tolerance to cold                              | [53]       |
| 21  | OsWRKY89   | Oryza sativa             | ABA, UV-B          | Tolerance to UV                                | [54]       |
| 22  | GmWRKY13   | Soybean                  | Salt, drought      | Sensitive to salt and mannitol, negative      | [55]       |
|     |            |                          |                    | regulator in ABA signaling                     |            |
| 23  | GmWRKY17   | Soybean                  | Salt               | Reduced salt tolerance                        | [56]       |
| 24  | GmWRKY54   | Soybean                  | Salt, drought      | Tolerance to salt and drought                 | [55]       |
| 25  | GmWRKY21   | Glycine max              | NaCl, drought, cold | Tolerance to cold                             | [55]       |
| 26  | ZmWRKY17   | Zea mays                 | ABA, salt          | Reduced salt tolerance                        | [57]       |
| 27  | TaWRKY2/19 | Triticum aestivum        | NaCl, drought, ABA | Tolerance to salt and drought                 | [58]       |
| 28  | BeWRKY46   | Brassica campestris      | NaCl, drought, cold | Tolerance to salt and drought                 | [59]       |
| 29  | BiWRKY1    | Boa hygrometrica         | Dehydration, ABA   | Tolerance to drought                          | [60]       |
| 30  | VpWRKY1    | Vitis pseudoreticulata    | NaCl, ABA          | Tolerance to salt                             | [61]       |
| 31  | VpWRKY2    | Vitis pseudoreticulata    | Cold, NaCl, ABA    | Tolerance to salt and cold                    | [61]       |
| 32  | VpWRKY3    | Vitis pseudoreticulata    | Drought, ABA, salicylic acid (SA) | Tolerance to salt                       | [62]       |
| 33  | TcWRKY53   | Thlaspi callistegii      | Cold, PEG, NaCl    | Negative regulator in osmotic stress          | [63]       |
| 34  | NaWRKY3    | Nicotiana attenuata      | Mechanical damage  | Sensitive to mechanical damage                | [64]       |
| 35  | JhWRKY2/7  | Juglans regia           | Drought, cold      | Tolerance to drought and cold                 | [65]       |
| 36  | ShWRKY30   | Sorghum bicolor          | Salt, drought      | Tolerance to salt and drought                 | [66]       |
| 37  | ShWRKY50   | Sorghum bicolor          | Salt               | Tolerance to salt                             | [67]       |
| 38  | HwWRKY47   | Ipomoea batatas          | Salt               | Tolerance to salt                             | [68]       |
| 39  | HwWRKY2    | Ipomoea batatas          | Salt, drought      | Tolerance to salt and drought                 | [69]       |
| 40  | MwWRKY30   | Malus domestica          | Salt, osmotic stress | Tolerance to salt and osmotic stress        | [70]       |
| 41  | MdWRKY100  | Malus domestica          | Salt               | Sensitive to salt                             | [71]       |
| 42  | SiWRKY81   | Solanum lycopersicum     | Drought            | Reduced drought tolerance                     | [72]       |
| 43  | GwWRKY1    | Gossypium barbadense     | Salt               | Tolerance to salt                             | [73]       |
| 44  | VwWRKY32   | Verbena bonariensis      | Cold               | Tolerance to cold                             | [74]       |
| 45  | PwWRKY33/62| Pennisetum glaucum      | Salt, drought      | Tolerance to salt and drought                 | [75]       |
| 46  | PwWRKY75   | Populus alba             | Drought            | Negative regulator in salt and osmotic stress | [76]       |
4.1. WRKY TFs and Drought Stress

Drought has a major impact on plant growth and development, resulting in a significant decrease in grain and other types of crop yield [77]. Under drought stress, drought-tolerant plants can accumulate oligosaccharides through sucrose metabolism to improve drought resistance. For example, when *Arabidopsis* is subjected to drought stress, the expression of *AtWRKY53* combined with the Qua-Quine Starch (QQS) promoter sequence is rapidly induced, hydrogen peroxide content is reduced, and the glucose metabolism pathway is significantly enhanced, thereby regulating stomatal opening and ultimately affecting drought tolerance [37]. In *Boea hygrometrica*, *BhWRKY1* effectively regulates the expression of the *BhGolS1* gene, and the overexpression of *BhGolS1* and *BhWRKY1* induces the accumulation of raffinose family oligosaccharides (RFOs) in transgenic *Nicotiana tabacum*, thus improving the ability of seedlings to resist drought [60].

WRKY protein can directly regulate the expression of drought-resistant genes. For example, in sorghum, *SbWRKY30* regulates the drought stress response gene *SbRD19* by binding with W-box elements of the *SbRD19* promoter, and protects plant cells from the damage of reactive oxygen species by improving ROS scavenging capability, enhancing drought tolerance [66]. *TaWRKY2* of wheat can
bind to STZ and downstream drought-resistant gene RD29B promoter, with a positive regulatory effect on the expression of RD29B [58]. DREB2A regulates the expression of dehydration stress-related genes [78], while TaWRKY19 can bind to DREB2A promoter, ultimately activating the expression of RD29A, RD29B, and Cor6.6 in transgenic Arabidopsis plants [58]. Similarly, Arabidopsis AtWRKY57 positively regulates the expression of RD29A and NCED3 genes by binding their W-box elements in the promoter regions [39]. In addition, WRKY protein can act on other TFs to play regulatory roles in drought tolerance. For example, TeWRKY53 of Thlaspi arvense significantly inhibits the expression of NtERF5 and NterERFp-1 of the AP2/ERF TF family, thus improving plant resistance to drought stress [63].

WRKY TFs also regulate plant tolerance through abscisic acid (ABA) and ROS-related signaling pathways. During drought stress, higher ABA levels were accumulated in plants, and leaf stomata were closed to reduce transpiration rate, thus regulating water balance in plants. ABA accumulation in cells, integrated with a variety of stress signals, regulates the expression of downstream genes, consequently sensing and responding to the adverse environment [40]. Arabidopsis AtWRKY63 has a specific effect on ABA-mediated stomatal closure and other signal transduction pathways, thus affecting the drought response [40]. GhWRKY21 regulates ABA-mediated cotton drought tolerance by promoting the expression of GhHAB [43]. Overexpression of BdWRKY36 in tobacco reduces the accumulation of ROS, activated NtLEA5, NtNCED1, and NtDREB3 in the ABA biosynthetic pathway, and significantly enhances the drought resistance of plants [48]. In Solanum lycopersicum, SiWRKY81 increases the drought tolerance of plants by inhibiting the accumulation of H2O2, playing a negative regulation role of stomatal closure [72].

4.2. WRKY TFs and Salt Stress

Salt stress is an important abiotic stress affecting crop productivity, particularly in arid and semiarid regions. WRKY TFs play essential roles in regulating the response to salt stress. To date, a total of 47 WRKY genes have been found to be expressed under salt stress in the wheat genome [79]. STZ is a protein related to ZPT2, which acts as a transcriptional inhibitor to downregulate the deactivation of other transcription factors. GmWRKY54 of Glycine max inhibits STZ expression and responds to salt stress by positively regulating the DREB2A-mediated pathway [55]. FcWRKY70 promotes the upregulation of arginine decarboxylase (ADC) expression, which is heterologously expressed in tobacco, and the content of lemon putrescine is significantly increased, thus enhancing the salt tolerance of plants [49]. The bwrky47 gene positively regulates stress resistance-related genes and significantly improves the salt tolerance of Ipomoea batatas [68]. MiR156/SPL modulates salt tolerance by upregulation of Malus domestica salt tolerance gene MdWRKY100 [71]. In Sorghum bicolor, SbWRKY50 could directly bind to the upstream promoter of SOS1 and HKT1 and participate in plant salt response by controlling ion homeostasis [67]. In addition, some WRKY genes function as negative regulation factors involved in salt stress resistance. Arabidopsis RPD3-like histone deacetylase HDA9 inhibits salt stress tolerance by regulating the DNA binding and transcriptional activity of WRKY53 [38]. Chrysanthemum CmWRKY17 overexpressed in Arabidopsis allows the plants to be more sensitive to salt stress. The expression level of stress resistance-related genes in transgenic Arabidopsis is lower than that in wild-type plants, indicating that CmWRKY17 may be involved in negatively regulating the salt stress response in Chrysanthemum [80]. The expression of GhWRKY68 is strongly induced in upland cotton and decreases salt tolerance [45]. In contrast, a high expression level of GhWRKY25 enhances the salt tolerance of upland cotton, while transgenic tobacco shows a relatively weaker tolerance to drought stress [44], indicating that the regulatory effects of different WRKY TFs involved in drought response are different.

Plants can also respond to saline–alkali stress through ABA, H2O2, and other signal pathways. In Glycine max, the negative regulatory factor ABI1 in the ABA pathway may be the downstream target gene of GmWRKY13. Genetic transformation experiments in Arabidopsis have shown that overexpression of GmWRKY13 significantly increases the expression of ABI1, but plants show a low tolerance to salt stress [55]. Overexpression of ZmWRKY17 has an inhibitory effect on the sensitivity
of exogenous ABA treatment, resulting in a relatively lower tolerance to high levels of salinity [57]. Under salt-induced H$_2$O$_2$ and cytosolic Ca$^{2+}$ stimulation, the activity of antioxidant enzymes increases, thus improving the tolerance to high-salinity environments [81]. ABA-induced WRKY gene expression is largely related to salt stress. Exogenous application of ABA and NaCl also induce AtWRKY25 and AtWRKY33 in Arabidopsis [33], OsWRKY72 in rice [51], GbWRKY1 in Verbena bonariensis [73], and VpWRKY12 [61] and VpWRKY3 [62] in grape.

4.3. WRKY TFs and Temperature Stress

Both low- and high-temperature stress can reduce crop yield and quality in plants. WRKY TFs play a role in the stress response through different signal transduction pathways. For example, in Verbena bonariensis, VbWRKY32 as a positive regulator, upregulates the transcriptional level of cold response genes, which increases the antioxidant activity, maintains membrane stability, and enhances osmotic regulation ability, thereby improving the survival ability under cold stress [74]. The BcWRKY46 gene of Brassica campestris is strongly induced by low temperature and ABA, activating related genes in the ABA signaling pathway to improve the low-temperature tolerance of plants [39]. CBF TFs regulate the expression of COR, and the overexpressed transgenic lines of CBF1, CBF2, and CBF3 show stronger cold resistance [82]. AtWRKY34 has a negative regulatory effect on the CBF-mediated cold response pathway; it is specifically expressed in mature pollen grains after exposure to low temperatures, resulting in resistance to low temperatures [35]. In addition, plants respond to temperature changes by coordinating organ development in an adverse environment. At low temperatures, rice MADS-Box TF OsMADS57 and its interacting protein OsTB1 synergistically activate the transcriptional regulation of OsWRKY94, preventing tillering by inhibiting transcription of the organ development gene D14 [83].

Due to global climate change, high-temperature stress has attracted significant attention. There is evidence that, to a certain extent, high temperatures will lead to biochemical changes in plants [84]. Thermal stimulation can activate Ca$^{2+}$ channels to maintain a higher intracellular Ca$^{2+}$ concentration, thereby activating calmodulin protein expression and inducing thermal-shock protein transcriptional expression [85]. In Arabidopsis, AtWRKY54 significantly responds to heat shock whereas basic leucine zipper factors (bZIPS) respond to prolonged warming [41]. Overexpression of AtWRKY39 can make plants more heat-sensitive. AtWRKY39 is highly homologous to AtWRKY7, and both of them can effectively bind calmodulin in plants, indicating a similar function [36]. In addition, AtWRKY25, AtWRKY26, and AtWRKY33 can improve tolerance to high-temperature stress in transgenic Arabidopsis by regulating the Hsp101 and Zat10 genes [34]. Plants subjected to heat stress can also activate the oxidative stress response through ethylene [86]. Under high-temperature stress, the expressions of AtWRKY25, AtWRKY26, and AtWRKY33 in Arabidopsis are induced by ethylene, the feedback factor EIN2 is transcriptionally regulated, and the effective activation of ethylene signal transduction contribute to relatively stronger heat resistance. In Oryza sativa, HSP101 promoter can activate the expression of the OsWRKY11 gene. Under heat treatment, the leaves wilted more slowly and the green part of the plant was less damaged, which makes it more heat-resistant [50]. In addition, some noncoding RNAs, such as miR396, play a role in the response to heat stress by regulating its target WRKY6 [87].

4.4. WRKY TFs and Other Abiotic Stresses

WRKY TFs are also involved in oxidative stress, mechanical damage, UV radiation, and other abiotic stresses (Figure 3). FcWRKY40 overexpression can significantly enhance the resistance of transgenic tobacco to oxidative stress [88]. When Arabidopsis is treated with ROS, the expressions of AtWRKY30, AtWRKY40, AtWRKY75, AtWRKY6, AtWRKY26, and AtWRKY45 are significantly upregulated [89]. After mechanical injury, the expression levels of AtWRKY11, AtWRKY15, AtWRKY22, AtWRKY33, AtWRKY40, AtWRKY53 [90] and AtWRKY6 [64] are upregulated. Similarly, NaWRKY3 is strongly expressed in tobacco. By contrast, the sensitivity of transgenic plants is increased when NaWRKY3 is knocked out [64]. In two previous studies, UV-B radiation treatment induced three WRKY
genes in *Arabidopsis* and the *OsWRKY89* gene in rice, resulting in a thick waxy substance on the leaf surface and improved tolerance to heat [54,91].

Figure 3. WRKY transcription factors in response to abiotic stresses.

In addition, a single WRKY TF can play multiple roles in different stress responses via various signal pathways and regulatory networks. For example, *TaWRKY44* expression in tobacco can improve resistance to drought, salt stress, and osmotic stress [92], while *PgWRKY62* and *PgWRKY33* in *Pennisetum glaucum* respond to salt and drought simultaneously [75]. *BhWRKY1* protein in *Boea hygrometrica* binds to the promoter of *BhGolS1* and is associated with both low-temperature resistance and drought tolerance [60]. *IbWRKY2* can interact with *IbVQ4*, and drought and salt treatment can induce the expression of *IbVQ4*, thus improving the tolerance of plants to drought and salt stress [69]. *MdWRKY30* overexpression enhances tolerance to salt and osmotic stress in transgenic apple callus through transcriptional regulation of stress-related genes [70]. *PagWRKY75* negatively regulates the tolerance of 84 K poplar (*Populus alba × P. glandulosa*) to salt and osmotic stress by reducing the scavenging capacity of ROS and the accumulation of proline, thus actively regulates the rate of leaf water loss [76].

5. Conclusions and Perspectives

As one of the largest TF families, WRKY plays an important and indispensable role in normal life activities of plants. Over the years, it has been shown that WRKY TFs not only participate in plant growth and development, but also show complex regulatory mechanisms and networks involved in external abiotic stresses. A large number of WRKYs have been functionally characterized in model plants, providing abundant functional references for other plants. Given that crops usually face various stresses and WRKYs play important roles in stress responses, further in-depth studies on WRKY genes in more crops are required. As increasing plant genomes have been sequenced, particularly of economically important crops, the genome-wide identification of WRKY genes will facilitate screening for stress resistance-related functional genes in plants. Moreover, previous studies...
of WRKY gene functions were largely dependent on transcriptomics and functional predictions, whereas more applications of genetic verification combined with new technologies are accelerating the research progress of WRKY’s novel functions. In addition, characterization of the downstream genes regulated by WRKY TFs or WRKY TF self-regulation will help clarify the regulatory network of abiotic stress responses. Furthermore, noncoding RNAs and epigenetic modifications involved in the regulation of WRKY TFs should be explored in future studies. Ultimately, using WRKY TFs to screen for stress-resistant plant cultivars and improve plant stress resistance will significantly benefit agricultural crop yield and quality in the context of aggravated climate change.

Author Contributions: Conceptualization, W.L. and B.J.; methodology, B.J.; software, S.P.; formal analysis, S.P. and Z.L.; investigation, S.P. and Z.L.; resources, S.P.; writing—original draft preparation, S.P. and W.L.; writing—review and editing, B.J., S.P., Z.L. and W.L.; visualization, B.J. and S.P.; supervision, B.J. and W.L.; project administration, W.L.; funding acquisition, W.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded by the Natural Science Foundation of China (grant numbers 31971408, 31670695 and 31971686), and Forestry Technology Innovation and Extension of Jiangsu Province (grant number LYKJ [2019]48).

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

References
1. Yoon, Y.; Seo, D.H.; Shin, H.; Kim, H.J.; Kim, C.M.; Jang, G. The role of stress-responsive transcription factors in modulating abiotic stress tolerance in plants. *Agronomy* 2020, 10, 788. [CrossRef]
2. Ma, Q.; Xia, Z.; Cai, Z.; Li, L.; Cheng, Y.; Liu, J.; Nian, H. *GmWRKY16* enhances drought and salt tolerance through an ABA-mediated pathway in *Arabidopsis thaliana*. *Front. Plant Sci.* 2019, 9, 9. [CrossRef]
3. Liu, Y.; Yang, T.; Lin, Z.; Guo, B.; Xing, C.; Zhao, L.; Dong, H.; Gao, J.; Xie, Z.; Zhang, S.-L.; et al. A WRKY transcription factor *PbrWRKY53* from *Pyrus betulaefolia* is involved in drought tolerance and AsA accumulation. *Plant Biotechnol. J.* 2019, 17, 1770–1787. [CrossRef] [PubMed]
4. Shrestha, A.; Khan, A.; Dey, N. cis-trans Engineering: Advances and perspectives on customized transcriptional regulation in plants. *Mol. Plant* 2018, 11, 886–898. [CrossRef] [PubMed]
5. Yamasaki, K.; Kigawa, T.; Inoue, M.; Watanabe, S.; Tateno, M.; Seki, M.; Shinozaki, K.; Yokoyama, S. Structures and evolutionary origins of plant-specific transcription factor DNA-binding domains. *Plant Physiol. Biochem.* 2008, 46, 394–401. [CrossRef] [PubMed]
6. Zhang, Y.; Wang, L. The WRKY transcription factor superfamily: Its origin in eukaryotes and expansion in plants. *BMC Evol. Biol.* 2005, 5, 1. [CrossRef]
7. Pandey, S.P.; Somssich, I.E. The role of WRKY transcription factors in plant immunity. *Plant Physiol.* 2009, 150, 1648–1655. [CrossRef] [PubMed]
8. Ishiguro, S.; Nakamura, K. Characterization of a cDNA encoding a novel DNA-binding protein, *SPF1*, that recognizes SP8 sequences in the 5′ upstream regions of genes coding for sporamin and beta-amylase from sweet potato. *Mol Gen Genet* 1994, 244, 563–571. [CrossRef]
9. Rushton, P.J.; Macdonald, H.; Hutty, A.K.; Lazarus, C.M.; Hooley, R. Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of Amy2 genes. *Plant Mol. Biol.* 1995, 29, 691–702. [CrossRef]
10. Rushton, P.J.; Torres, J.T.; Parniske, M.; Wernert, P.; Hahlbrock, K.; Somssich, I.E. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *EMBO J.* 1996, 15, 5690–5700. [CrossRef] [PubMed]
11. He, H.; Dong, Q.; Shao, Y.; Jiang, H.; Zhu, S.; Cheng, B.; Xiang, Y. Genome-wide survey and characterization of the WRKY gene family in *Populus trichocarpa*. *Plant Cell Rep.* 2012, 31, 1199–1217. [CrossRef] [PubMed]
12. Rensing, S.A.; Lang, D.; Zimmer, A.D.; Terry, A.; Salamov, A.A.; Shapiro, H.; Nishiyama, T.; Perroud, P.-F.; Lindquist, E.; Kamisugi, Y.; et al. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* 2007, 319, 64–69. [CrossRef] [PubMed]
13. Mangelsen, E.; Kilian, J.; Berendzen, K.W.; Kolukisaoglu, Ü.; Harter, K.; Jansson, C.; Wanke, D. Phylogenetic and comparative gene expression analysis of barley (Hordeum vulgare) WRKY transcription factor family reveals putatively retained functions between monocots and dicots. *BMC Genom.* 2008, 9, 194. [CrossRef]

14. Ling, J.; Jiang, W.; Zhang, Y.; Yu, H.; Mao, Z.; Gu, X.; Huang, S.; Xie, B. Genome-wide analysis of WRKY gene family in *Cucumis sativus*. *BMC Genom.* 2011, 12, 1–20. [CrossRef]

15. Berri, S.; Abbruscato, P.; Fairev-Rampant, O.; Brasileiro, A.C.M.; Fumasoni, I.; Satoh, K.; Kikuchi, S.; Mizzi, L.; Morandini, P.; Pè, M.E.; et al. Characterization of WRKY co-regulatory networks in rice and *Arabidopsis*. *BMC Plant Biol.* 2009, 9, 1–22. [CrossRef]

16. Liu, J.-J.; Ekramoddoullah, A.K. Identification and characterization of the WRKY transcription factor family in *Pinus monticola*. *Genome* 2009, 52, 77–88. [CrossRef] [PubMed]

17. Huang, S.; Gao, Y.; Liu, J.; Peng, X.; Niu, X.; Fei, Z.; Cao, S.; Liu, Y. Genome-wide analysis of WRKY transcription factors in *Solanum lycopersicum*. *Mol. Genet. Genom.* 2012, 287, 495–513. [CrossRef]

18. Ross, C.A.; Liu, Y.; Shen, Q.J. The WRKY gene family in rice (*Oryza sativa*). *J. Integr. Plant Biol.* 2007, 49, 827–842. [CrossRef]

19. Rushton, P.J.; Somssich, I.E.; Ringler, P.; Shen, Q.J. WRKY transcription factors. *Trends Plant Sci.* 2010, 15, 247–258. [CrossRef]

20. Ülker, B.; Somssich, I.E. WRKY transcription factors: From DNA binding towards biological function. *Curr. Opin. Plant Biol.* 2004, 7, 491–498. [CrossRef]

21. Phukan, U.J.; Jeena, G.S.; Shukla, R.K. WRKY transcription factors: Molecular regulation and stress responses in plants. *Front. Plant Sci.* 2016, 7, 760. [CrossRef] [PubMed]

22. Xie, Z.; Zhang, Z.-L.; Zou, X.; Huang, J.; Ruas, P.; Thompson, D.; Shen, Q.J. Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. *Plant Physiol.* 2004, 137, 176–189. [CrossRef]

23. Eulgem, T.; Rushton, P.J.; Robatzek, S.; Somssich, I.E. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* 2000, 5, 199–206. [CrossRef]

24. Rushton, P.J.; Bokowiec, M.T.; Han, S.; Zhang, H.; Brannock, J.F.; Chen, X.; Laudeman, T.W.; Timko, M.P. Tobacco transcription factors: Novel insights into transcriptional regulation in the *Solanaceae*. *Plant Physiol.* 2008, 147, 280–295. [CrossRef]

25. Chen, L.; Song, Y.; Li, S.; Zhang, L.; Zou, C.; Yu, D. The role of WRKY transcription factors in plant abiotic stresses. *Biochim. Biophys. Acta BBA Bioenerg.* 2012, 1819, 120–128. [CrossRef]

26. Ciolkowski, I.; Wanke, D.; Birkenbihl, R.P.; Somssich, I.E. Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. *Plant Mol. Biol.* 2008, 68, 81–92. [CrossRef]

27. Ezentgraf, U.; Laun, T.; Miao, Y. The complex regulation of WRKY53 during leaf senescence of *Arabidopsis thaliana*. *Eur. J. Cell Biol.* 2010, 89, 133–137. [CrossRef]

28. Cai, H.; Yang, S.; Yan, Y.; Xiao, Z.; Cheng, J.; Wu, J.; Qiu, A.; Mou, S.; Guan, D.; et al. CaWRKY6 transcriptionally activates CaWRKY40, regulatesRalstonia solanacearum resistance, and confers high-temperature and high-humidity tolerance in pepper. *J. Exp. Bot.* 2015, 66, 3163–3174. [CrossRef]

29. Turck, F.; Zhou, A.; Somssich, I.E. Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to its native promoter and the defense-related gene *PcPR1-1* in *Parsley*. *Plant Cell* 2004, 16, 2573–2585. [CrossRef]

30. Cai, T.; Flanagan, L.B.; Jassal, R.S.; Black, T.A. Modelling environmental controls on ecosystem photosynthesis and the carbon isotope composition of ecosystem-respired CO₂ in a coastal Douglas-fir forest. *Plant Cell Environ.* 2008, 31, 435–453. [CrossRef]

31. Grierson, C.; Du, J.-S.; Zabala, M.D.T.; Beggs, K.; Smith, C.; Holdsworth, M.; Bevan, M.W. Separate cis sequences and trans factors direct metabolic and developmental regulation of a potato tuber storage protein gene. *Plant J.* 1994, 5, 815–826. [CrossRef] [PubMed]

32. Sun, C.; Palmqvist, S.; Olsson, H.; Borén, M.; Ahlandsberg, S.; Jansson, C. A novel WRKY transcription factor, SUSIBAZ, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *Plant Cell* 2003, 15, 2076–2092. [CrossRef] [PubMed]
33. Jiang, Y.; Deyholos, M.K. Functional characterization of *Arabidopsis* NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses. *Plant Mol. Biol.* 2008, 69, 91–105. [CrossRef]

34. Li, S.; Fu, Q.; Chen, L.; Huang, W.-D.; Yu, D. *Arabidopsis thaliana* WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. *Planta* 2011, 233, 1237–1252. [CrossRef]

35. Zou, C.; Jiang, W.; Yu, D. Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in *Arabidopsis*. *J. Exp. Bot.* 2010, 61, 3901–3914. [CrossRef]

36. Park, C.Y.; Lee, J.H.; Yoo, J.H.; Moon, B.C.; Choi, M.S.; Kang, Y.H.; Lee, S.M.; Kim, H.S.; Kang, K.Y.; Chung, W.S.; et al. WRKY group IId transcription factors interact with calmodulin. *FEBS Lett.* 2005, 579, 1545–1550. [CrossRef] [PubMed]

37. Sun, Y.; Yu, D. Activated expression of AtWRKY53 negatively regulates drought tolerance by mediating stomatal movement. *Plant Cell Rep.* 2015, 34, 1295–1306. [CrossRef] [PubMed]

38. Zheng, Y.; Ge, J.; Bao, C.; Chang, W.; Liu, J.; Shao, J.; Liu, X.; Su, L.; Pan, L.; Zhou, D.-X. Histone deacetylase HDA9 and WRKY53 transcription factor are mutual antagonists in regulation of plant stress response. *Mol. Plant* 2020, 13, 598–611. [CrossRef]

39. Jiang, Y.; Liang, G.; Yu, D. Activated expression of WRKY57 confers drought tolerance in *Arabidopsis*. *Mol. Plant Mol. Biol.* 2012, 5, 1375–1388. [CrossRef]

40. Ren, S.; Ma, K.; Lu, Z.-G.; Ren, S.; Jiang, H.-R.; Cui, J.; Chen, G.; Teng, N.-J.; Lam, H.-M.; Jin, B. Differential physiological, transcriptomic and metabolomic responses of *Arabidopsis* leaves under prolonged warming and heat shock. *BMC Plant Biol.* 2020, 20, 1–15. [CrossRef]

41. Wang, L.; Ma, K.-B.; Lu, Z.-G.; Ren, S.-X.; Jiang, H.-R.; Cui, J.; Chen, G.; Teng, N.-J.; Lam, H.-M.; Jin, B. Differential physiological, transcriptomic and metabolomic responses of *Arabidopsis* leaves under prolonged warming and heat shock. *BMC Plant Biol.* 2020, 20, 1–15. [CrossRef]

42. Ren, S.; Ma, K.; Lu, Z.; Chen, G.; Cui, J.; Tong, P.; Wang, L.; Teng, N.; Jin, B. Transcriptomic and metabolomic analysis of the heat-stress response of *Populus tomentosa*. *Carr. For.* 2019, 10, 383. [CrossRef]

43. Wang, J.; Wang, L.; Yan, Y.; Zhang, S.; Li, H.; Gao, Z.; Wang, C.; Guo, X. GhWRKY21 regulates ABA-mediated drought tolerance by fine-tuning the expression of GhHAB in cotton. *Plant Cell Rep.* 2020, 39. [CrossRef]

44. Liu, X.; Song, Y.; Xing, F.; Wang, N.; Wen, F.; Zhu, C. GhWRKY25, a group I WRKY gene from cotton, confers differential tolerance to abiotic and biotic stresses in transgenic *Nicotiana benthamiana*. *Protoplasma* 2015, 253, 1265–1281. [CrossRef]

45. Jia, H.; Wang, C.; Wang, F.; Liu, S.; Li, G.; Guo, X. GhWRKY68 reduces resistance to salt and drought in transgenic *Nicotiana benthamiana*. *PLoS ONE* 2015, 10, e0120646. [CrossRef]

46. Wang, M.; Vannozzi, A.; Wang, G.; Liang, Y.-H.; Tornielli, G.B.; Zenoni, S.; Cavallini, E.; Pezzotti, M.; Cheng, Z.-M. Genome and transcriptome analysis of the grapevine (*Vitis vinifera* L.) WRKY gene family. *Hortic. Res.* 2014, 1, 14016. [CrossRef] [PubMed]

47. Dang, F.F.; Wang, Y.N.; Yu, L.; Eulgem, T.; Lai, Y.; Liu, Z.Q.; Wang, X.; Qiu, A.L.; Zhang, T.X.; Lin, J.; et al. CaWRKY40, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to *Ralstonia solanacearum* infection. *Plant Cell Environ.* 2013, 36, 757–774. [CrossRef]

48. Sun, J.; Hu, W.; Zhou, R.; Wang, L.; Wang, X.; Wang, Q.; Feng, Z.-J.; Yu, H.; Qiu, D.; He, G.; et al. The *Brachypodium distachyon* BdWRKY36 gene confers tolerance to drought stress in transgenic tobacco plants. *Plant Cell Rep.* 2014, 34, 23–35. [CrossRef]

49. Gong, X.; Zhang, J.; Hu, J.; Wang, W.; Wu, H.; Zhang, Q.; Liu, J.-H. *FcWRKY70*, a WRKY protein of *Fortunella crassifolia*, functions in drought tolerance and modulates putrescine synthesis by regulating arginine decarboxylase gene. *Plant Cell Environ.* 2015, 38, 2248–2262. [CrossRef]

50. Wu, X.; Shirotos, Y.; Kishitani, S.; Ito, Y.; Toriyama, K. Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing *OsWRKY11* under the control of *HSP101* promoter. *Plant Cell Rep.* 2009, 28, 21–30. [CrossRef] [PubMed]

51. Song, Y.; Chen, L.; Zhang, L.; Yu, D. Overexpression of *OsWRKY72* gene interferes in the abscisic acid signal and auxin transport pathway of *Arabidopsis*. *J. Biosci.* 2010, 35, 459–471. [CrossRef]

52. Dai, X.; Wang, Y.; Zhang, W.-H. *OsWRKY74*, a WRKY transcription factor, modulates tolerance to phosphate starvation in rice. *J. Exp. Bot.* 2016, 67, 947–960. [CrossRef]
Plants 2020, 9, 1515

53. Yokotani, N.; Sato, Y.; Tanabe, S.; Chuo, T.; Shimizu, T.; Okada, K.; Yamane, H.; Shimono, M.; Sugano, S.; Takatsuji, H.; et al. WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. *J. Exp. Bot.* 2013, 64, 5085–5097. [CrossRef]

54. Wang, H.; Hao, J.; Chen, X.; Hao, Z.; Wang, X.; Lou, Y.; Peng, Y.; Guo, Z. Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. *Plant Mol. Biol.* 2007, 65, 799–815. [CrossRef]

55. Zhou, Q.-Y.; Tian, A.-G.; Zou, H.-F.; Xie, Z.-M.; Lei, G.; Huang, J.; Wang, C.-M.; Wang, H.-W.; Zhang, J.-S.; Chen, S.-Y. Soybean WRKY-type transcription factor genes, *GmWRKY13, GmWRKY21,* and *GmWRKY54,* confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. *Plant Biotechnol. J.* 2008, 6, 486–503. [CrossRef]

56. Wang, F.; Hou, X.; Tang, J.; Wang, Z.; Wang, S.; Jiang, F.; Li, Y. A novel cold-inducible gene from Pak-choi (*Brassica campestris* ssp. chinensis), *BeWRKY46,* enhances the cold, salt and dehydration stress tolerance in transgenic *Arabidopsis* plants. *Plant Sci.* 2012, 186, 1215–1231. [CrossRef]

57. Wang, Z.; Zhu, Y.; Wang, L.; Liu, X.; Liu, Y.; Phillips, J.; Deng, X. A WRKY transcription factor participates in dehydration tolerance in *Boea hygrometrica* by binding to the W-box elements of the galactinol synthase (*BhGols1*) promoter. *Planta* 2009, 230, 1155–1166. [CrossRef]

58. Li, H.; Xu, Y.; Xiao, Y.; Zhu, Z.; Xie, X.; Zhao, H.; Wang, Y. Expression and functional analysis of two genes encoding transcription factors, *VpWRKY1* and *VpWRKY2,* isolated from Chinese wild *Vitis pseudoreticulata.* *Planta* 2010, 232, 1325–1337. [CrossRef]

59. Zhu, Z.; Shi, J.; Cao, J.; He, M.; Wang, Y. *VpWRKY3,* a biotic and abiotic stress-related transcription factor from the Chinese wild *Vitis pseudoreticulata.* *Plant Cell Rep.* 2012, 31, 2109–2120. [CrossRef] [PubMed]

60. Wei, W.; Zhang, Y.; Han, L.; Guo, X. The cotton WRKY transcription factor *GhWRKY17* functions in drought and salt stress in transgenic *Nicotiana benthamiana* through ABA signaling and the modulation of reactive oxygen species production. *Plant Cell Physiol.* 2014, 55, 2060–2076. [CrossRef]

61. Cai, R.; Dai, W.; Zhang, C.; Wang, Y.; Wu, M.; Zhao, Y.; Ma, Q.; Xiang, Y.; Cheng, B. The maize WRKY transcription factor *ZmWRKY17* negatively regulates salt stress tolerance in transgenic *Arabidopsis* plants. *Plant Cell Environ.* 2017, 40, 1245–1251. [CrossRef]

62. Li, H.; Xu, Y.; Xiao, Y.; Zhu, Z.; Xie, X.; Zhao, H.; Wang, Y. Expression and functional analysis of two genes encoding transcription factors, *VpWRKY1* and *VpWRKY2,* isolated from Chinese wild *Vitis pseudoreticulata.* *Planta* 2010, 232, 1325–1337. [CrossRef]

63. Skibbe, M.; Qu, N.; Galis, I.; Baldwin, I.T. Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell* 2008, 20, 1984–2000. [CrossRef]

64. Yang, G.; Zhang, W.; Liu, Z.; Yi-Maer, A.Y.; Zhai, M.; Xu, Z. Both *JrWRKY2* and *JrWRKY7* of *Juglas regia* mediate responses to abiotic stresses and abscisic acid through formation of homodimers and interaction. *Plant Biol.* 2017, 19, 268–278. [CrossRef]

65. Yang, Z.; Chi, X.; Guo, F.; Jin, X.; Luo, H.; Hawar, A.; Chen, Y.; Feng, K.; Wang, B.; Qi, J.; et al. *ShWRKY30* enhances the drought tolerance of plants and regulates a drought stress-responsive gene, *SbrD19,* in sorghum. *J. Plant Physiol.* 2020, 246, 153142. [CrossRef]

66. Song, Y.; Li, J.; Sui, Y.; Han, G.; Zhang, Y.; Guo, S.; Sui, N. The sweet sorghum *SbWRKY5* is negatively involved in salt response by regulating ion homeostasis. *Plant Mol. Biol.* 2020, 102, 603–614. [CrossRef]

67. Qin, Z.; Hou, F.; Li, A.; Dong, S.; Wang, Q.; Zhang, L. Transcriptome-wide identification of WRKY transcription factor and their expression profiles under salt stress in sweetpotato (*Ipomoea batatas* L.). *Plant Biotechnol. Rep.* 2020, 14, 599–611. [CrossRef]

68. Zou, H.; Zhou, Y.; Zhai, H.; He, S.; Zhao, N.; Liu, Q. A novel sweetpotato WRKY transcription factor, *IpWRKY2,* positively regulates drought and salt tolerance in transgenic. *Arabidopsis* 2020, *10*, 506. [CrossRef]

69. Dong, Q.; Zheng, W.; Duan, D.; Huang, D.; Wang, Q.; Liu, C.; Li, C.; Gong, X.; Li, C.; Mao, K.; et al. *MdWRKY30,* a group IIA WRKY gene from apple, confers tolerance to salinity and osmotic stresses in transgenic apple callus and *Arabidopsis* seedlings. *Plant Sci.* 2020, 299, 110611. [CrossRef] [PubMed]
71. Ma, Y.; Xue, H.; Zhang, F.; Jiang, Q.; Yang, S.; Yue, P.; Wang, F.; Zhang, Y.; Li, L.; He, P.; et al. The miR156/SPL module regulates apple salt stress tolerance by activating MdWRKY100 expression. *Plant Biotechnol. J.* 2020, 18. [CrossRef]

72. Ahammed, G.J.; Li, X.; Yang, Y.; Liu, C.; Zhou, G.; Wan, H.; Cheng, Y. Tomato WRKY81 acts as a negative regulator for drought tolerance by modulating guard cell H$_2$O$_2$-mediated stomatal closure. *Environ. Exp. Bot.* 2020, 171, 103960. [CrossRef]

73. Luo, X.; Li, C.; He, X.; Zhang, X.; Zhu, L.-F. ABA signaling is negatively regulated by GbWRKY1 through JAZ1 and AB1 to affect salt and drought tolerance. *Plant Cell Rep.* 2019, 39, 181–194. [CrossRef]

74. Wang, M.-Q.; Huang, Q.-X.; Lin, P.; Zeng, Q.-H.; Li, Y.; Liu, Q.-L.; Zhang, L.; Pan, Y.-Z.; Jiang, B.-B.; Zhang, F. The overexpression of a transcription factor gene VbWRKY32 enhances the cold tolerance in *Verbena bonariensis*. *Front. Plant Sci.* 2020, 10, 1746. [CrossRef]

75. Chanwala, J.; Satpati, S.; Dixit, A.; Parida, A.; Giri, M.K.; Dey, N. Genome-wide identification and expression analysis of WRKY transcription factors in pearl millet (*Pennisetum glaucum*) under dehydration and salinity stress. *BMC Genom.* 2020, 21, 1–16. [CrossRef]

76. Zhao, K.; Zhang, D.; Lv, K.; Zhang, X.; Cheng, Z.; Li, R.; Zhou, B.; Jiang, T. Functional characterization of poplar WRKY75 in salt and osmotic tolerance. *Plant Sci.* 2019, 289, 110259. [CrossRef]

77. Anjum, S.A.; Xie, X.-Y.; Wang, L.-C.; Saleem, M.F.; Man, C.; Lei, W. Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agr. Res.* 2011, 6, 2026–2032. [CrossRef]

78. Sakuma, Y.; Maruyama, K.; Osakabe, Y.; Qin, F.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell* 2006, 18, 1292–1309. [CrossRef] [PubMed]

79. Hassan, S.; Lethin, J.; Blomberg, R.; Mousavi, H.; Aronsson, H. In silico based screening of WRKY genes for identifying functional genes regulated by WRKY under salt stress. *Comput. Biol. Chem.* 2019, 83, 107131. [CrossRef]

80. Linxiao, W.; Song, A.; Gao, C.; Wang, L.; Wang, Y.; Sun, J.; Jiang, J.; Chen, F.; Chen, S. *Chrysanthemum* WRKY gene CmWRKY17 negatively regulates salt stress tolerance in transgenic chrysanthemum and *Arabidopsis* plants. *Plant Cell Rep.* 2015, 34, 1365–1378. [CrossRef]

81. Shen, Z.; Yao, J.; Sun, J.; Chang, L.; Wang, S.; Ding, M.; Qian, Z.; Zhang, H.; Zhao, N.; Sa, G.; et al. *Populus euphratica* HSF binds the promoter of WRKY1 to enhance salt tolerance. *Plant Sci.* 2015, 235, 89–100. [CrossRef] [PubMed]

82. Jaglo-Ottosen, K.R. *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* 1998, 280, 104–106. [CrossRef]

83. Chen, L.; Zhao, Y.; Xu, S.; Zhang, Z.; Xu, Y.; Zhang, J.; Chong, K. OsMADS57 together with OsTB1 coordinates transcription of its target OsWRKY94 and D14 to switch its organogenesis to defense for cold adaptation in rice. *New Phytol.* 2018, 218, 219–231. [CrossRef]

84. Jin, B.; Wang, L.; Wang, J.; Jiang, K.-Z.; Wang, Y.; Jiang, X.X.; Ni, C.-Y.; Wang, Y.; Teng, N.-J. The effect of experimental warming on leaf functional traits, leaf structure and leaf biochemistry in *Arabidopsis thaliana*. *BMC Plant Biol.* 2011, 11, 12–35. [CrossRef] [PubMed]

85. Liu, H.-T.; Li, G.-L.; Chang, H.; Sun, D.-Y.; Zhou, R.-G.; Li, B. Calmodulin-binding protein phosphatase PP7 is involved in thermotolerance in *Arabidopsis*. *Plant Cell Environ.* 2007, 30, 156–164. [CrossRef] [PubMed]

86. Larkindale, J.; Huang, B. Effects of abscisic acid, salicylic acid, ethylene and hydrogen peroxide in thermotolerance and recovery for creeping bentgrass. *Plant Growth Regul.* 2005, 47, 17–28. [CrossRef]

87. Zhao, J.; He, Q.; Chen, G.; Wang, L.; Jin, B. Regulation of non-coding RNAs in heat stress responses of plants. *Front. Plant Sci.* 2016, 7, 1213. [CrossRef]

88. Gong, X.-Q.; Hu, J.-B.; Liu, J.-H. Cloning and characterization of *FcWRKY40*, A WRKY transcription factor from *Fortunella crassifolia* linked to oxidative stress tolerance. *Plant Cell Tissue Organ Cult.* PCTOC 2014, 119, 197–210. [CrossRef]

89. Cheong, Y.H.; Chang, H.-S.; Gupta, R.; Wang, X.; Zhu, T.; Luan, S. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. *Plant Physiol.* 2002, 129, 661–677. [CrossRef]

90. Robatzeck, S.; Somssich, I.E. A new member of the *Arabidopsis* WRKY transcription factor family, *AtWRKY6*, is associated with both senescence- and defence-related processes. *Plant J.* 2001, 28, 123–133. [CrossRef]
91. Kilian, J.; Whitehead, D.; Horak, J.; Wanke, D.; Weinl, S.; Batistic, O.; D’Angelo, C.; Bornberg-Bauer, E.; Kudla, J.; Harter, K. The AtGenExpress global stress expression data set: Protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *Plant J.* 2007, 50, 347–363. [CrossRef]

92. Han, Y.; Zhang, X.; Wang, Y.; Ming, F. The suppression of WRKY44 by GIGANTEA-miR172 pathway is involved in drought response of *Arabidopsis thaliana*. *PLoS ONE* 2013, 8, e73541. [CrossRef] [PubMed]

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).