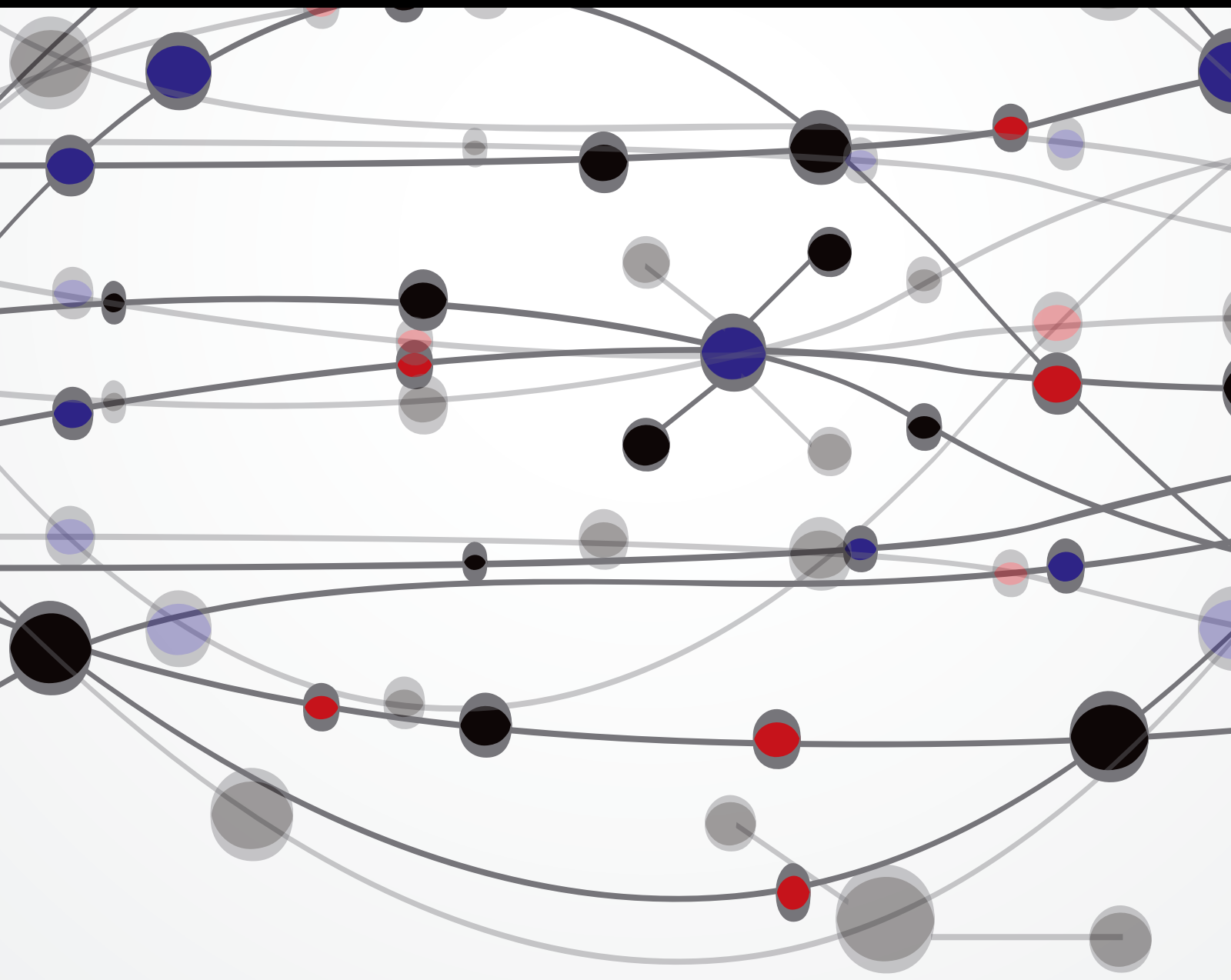
The Scientific World Journal

# Plant Abiotic Stress

Guest Editors: Ji Huang, Alexander Levine, and Zhoufei Wang



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# *Editorial*
# Plant Abiotic Stress

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Abiotic stress such as cold, drought, salt, and heavy metals largely influences plant development and crop productivity. Abiotic stress has been becoming a major threat to food security due to the constant changes of climate and deterioration of environment caused by human activity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses. Better understanding of the plant responsiveness to abiotic stress will aid in both traditional and modern breeding applications towards improving stress tolerance. Studies on some special wild plant species with high stress tolerance also greatly contribute to our understanding of stress tolerance.

With the development of modern molecular biology, genomics approaches have been applied in crop breeding while not popularly in practice. In this special issue, B. A. Akpınar et al. present a compressive review on genomic approaches, including structure genomics, comparative genomics, and functional genomics, for crop improvement against abiotic stress. Some traditional and modern genomics approaches of crop improvement, such as expressed sequence tag (EST) profiling, microarray, Targeting Induced Local Lesions IN Genomes (TILLING), and next generation sequencing (NGS), are summarized in this review. In the other review paper, H. Budak et al. focused on the studies of drought tolerance in modern and wild wheat. They reviewed recent advances on drought related gene/QTL identification, studies on drought related molecular pathways, and current efforts on the improvement of wheat cultivars for drought tolerance.

The exploitation and utilization of crop germplasm resources are the basis of crop breeding. However, with continuous collection of germplasm resources, the size of populations has been becoming bigger, which hinders the evaluation and utilization of the germplasm resources. Constructing core collection is an efficient way to solve the problem. A core collection is a representative sample of the whole collection which has minimum repetitiveness and maximum genetic diversity of a plant species. J. Wang et al. suggest a strategy to construct a core collection based on drought resistant germplasm resources in wheat. The results showed that the strategy was effective and was valuable in another crop's core collection construction.

The wild plant species with high tolerance to abiotic stress have attracted more and more attentions of plant biologists, especially when the studies within a crop species on improving the stress tolerance show the limitation. Understanding the mechanisms for stress tolerance achieved by these wild species will help in crop improvement; even some relatives can be directly or indirectly applied in crop breeding by cytological ways. H. Budak et al. introduce the research advances on wild emmer wheat which is important for its high drought tolerance. W. Liu et al. present cold induced physiological responses of naked oats (*Avena nuda* L.), a cold-tolerant plant species, and show that the cold tolerance involves the increase of antioxidant activities of several reactive oxygen species (ROS) savaging enzymes. The heavy metal contamination is an environmental problem in the margin sea. H.-p. Jiang et al. studied the responses of green algae species *Ulva prolifera* and *Ulva linza* to $Cd^{2+}$ and concluded the major physiological parameters involved in the $Cd^{2+}$ adaptation.

It is necessary to further clarify the mechanisms underlying plant stress responses through modern biological technologies, especially to understand stress responses of some wild plant species with extremely high stress tolerance, which

will be eventually applied in developing crops with high stress tolerance.

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*Research Article*

# GmNAC5, a NAC Transcription Factor, Is a Transient Response Regulator Induced by Abiotic Stress in Soybean

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GmNAC5 is a member of NAM subfamily belonging to NAC transcription factors in soybean (*Glycine max* (L.) Merr.). Studies on NAC transcription factors have shown that this family functioned in the regulation of shoot apical meristem (SAM), hormone signalling, and stress responses. In this study, we examined the expression levels of *GmNAC5*. *GmNAC5* was highly expressed in the roots and immature seeds, especially strongly in immature seeds of 40 days after flowering. In addition, we found that *GmNAC5* was induced by mechanical wounding, high salinity, and cold treatments but was not induced by abscisic acid (ABA). The subcellular localization assay suggested that GmNAC5 was targeted at nucleus. Together, it was suggested that GmNAC5 might be involved in seed development and abiotic stress responses in soybean.

## 1. Introduction

Environmental stresses such as drought, salinity, and cold are major factors that significantly limit agricultural productivity. NAC transcription factors play essential roles in response to various abiotic stresses [1]. The N-terminal region of NAC proteins contains a highly conserved NAC domain, which can be divided into five subdomains based on sequence similarities and may function as DNA-binding region. The C-terminal regions of NAC proteins, which exhibit the transactivation activity, are highly divergent in both sequence and length [2–4]. This family of transcription factors is involved in a lot of plant developmental processes, including shoot apical meristem formation [5], hormone signaling [2, 6], regulation of cell division and cell expansion [7], control of secondary wall formation [8–10], and responses to various stresses [11–14].

The NAC family consists of several subfamilies [15]. The NAM subfamily is the best studied NAC subfamily. *CUC1* and *CUC2*, encoding NAM subfamily proteins, are a pair of functionally redundant genes, expressed in *Arabidopsis* meristem and organ primordia boundary [1, 16]. The cotyledons of the transgenic seedlings overexpressing *CUC1* (*35S::CUC1*) regularly had two basal lobes, small and round epidermal cells between the sinuses, and adventitious SAMs on the adaxial surface of this region [17]. It has been reported that CUC2 is essential for dissecting the leaves of a wide range of lobed/serrated *Arabidopsis* lines. Inactivation of *CUC3* leads to a partial suppression of the serrations, indicating a role for this gene in leaf shaping. Morphometric analysis of leaf development and genetic analysis provide evidences for different temporal contributions of CUC2 and CUC3 [18]. The *CUP* played an important role in the lateral organ boundary forming snapdragon. Cupuliformis mutants are defective in shoot apical meristem formation, but cup plants overcome this early barrier to development to reach maturity. *CUP* encodes a NAM protein, homologous to the petunia NAM and *Arabidopsis* CUC proteins. The phenotype of *cup* mutants differs from the phenotype of *NAM* and *CUC1 CUC2* in that dramatic organ fusion is observed throughout development [19]. Phloem transport of *CmNACP* mRNA was proved directly by heterograft studies between pumpkin and cucumber plants, in which *CmNACP* transcripts were shown to accumulate in cucumber scion phloem and apical tissues [20]. Petunia NAM proteins were mainly expressed in the meristem and primordia boundaries, which might be required by embryo and flower pattern formation [5]. For abiotic stress, it was observed that *Arabidopsis AtNAC2*

expression was induced by salt stress and this induction was reduced in magnitude in the transgenic *Arabidopsis* plants overexpressing tobacco ethylene receptor gene *NTHK1*. *AtNAC2* was localized in the nucleus and had transcriptional activation activity. It can form a homodimer in yeast. *AtNAC2* was highly expressed in roots and flowers but less expressed in other organs examined. In addition to the salt induction, *AtNAC2* can be induced by abscisic acid (ABA), ACC, and NAA [21]. These showed that the NAM subfamily members not only play a regulatory role in plant development but also participate in stress responses. *GmNAC5*, which is a member of NAM subfamily belonging to NAC transcription factor in soybean, was cloned and analysed [22]. In order to further study the physiological and biochemical processes that *GmNAC5* gene may be involved in, the soybean organ expression patterns of the gene and the relationship between *GmNAC5* gene and abiotic stress were examined.

## 2. Materials and Methods

*2.1. Plant Materials.* Soybean cv. Ludou 10th was used in this study. Plants were field-grown under normal conditions in Nanjing Agricultural University. Vegetable tissues such as roots, stems, and leaves were collected from 4-week-old seedlings, while floral buds at R1 stage [23], young pods at R3 stage, and developing seeds from 15 to 50 days after flowering (DAF) were collected and frozen immediately in liquid nitrogen and stored at −80°C until use.

*2.2. RNA Isolation, cDNA Synthesis, and Quantitative Real-Time PCR.* Total RNA was extracted using a Total RNA Plant Extraction Kit (Tiangen, Beijing, China), according to the manufacturer's protocol. First-strand cDNA was synthesized using the TaKaRa PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa, Dalian, China), according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted using the SYBR Green Real-Time PCR Master Mix (TOYOBO, Osaka, Japan) on an ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene expression was quantified using the comparative method Ct: $2^{-\Delta\Delta Ct}$ method as previously described [24].

*2.3. Stress Treatments.* The soybean seedlings cultured with sand were moved to Hoagland nutrient solution, when growing to two true leaves. After the first cluster of fronds grew, the plants were applied with stress treatments with three replicates. For hormone treatments, the seedlings were treated with 100 $\mu$M JA and 100 $\mu$M of ABA, respectively. For salt stress, the seedlings were treated with 200 mM NaCl. For dehydration stress, the seedlings were placed on filter paper, respectively. For cold stress, the seedlings were placed in 4°C light incubator. For mechanical wounding, the seedling leaves were cut into pieces with a sharp and clean scissor. After each treatment, the leaves were harvested and frozen in liquid nitrogen immediately.


Figure 1: Schematic diagram of gene structures of *GmNAC5* and its homologous genes in *Arabidopsis thaliana* (*ANAC08*), *Zea mays* (*GRMZM5G898290*), and *Linum usitatissimum* (*Lus10021659*). The black and white boxes indicate exons and introns, respectively.

*2.4. Subcellular Localization of NAC Proteins.* The full-length cDNA of *GmNAC5* was cloned in pBI121-GFP vector, in frame fusing with GFP reporter gene and producing the plasmid pBI-GmNAC5-GFP. After transient expression of the fusion plasmid in onion epidermal cells, the cells were observed under florescence microscope.

## 3. Results

*3.1. Genomic Structure of GmNAC5.* NAC transcription factors have been considered one of the largest families of transcription factors so far discovered in the plant genomes. *GmNAC5* encodes a NAC transcription factor belonging to the NAM subfamily. It was found that the exon-intron structures were conserved among *GmNAC5* homologous genes in three common species, including *Arabidopsis thaliana*, *Zea mays,* and *Linum usitatissimum* (Figure 1).

*3.2. Subcellular Localization.* *GmNAC5* encoding product is presumed to act as a transcription factor. If transcription factors achieve the precise adjustment of the target genes, this specific transcription factor should be located in the nucleus. Interestingly, GmNAC5 lacks the traditional nuclear localization signal (NLS); even some researchers have found that some NAC domain proteins have the nuclear localization signals [16, 25, 26]. To clarify whether soybean NAC protein GmNAC5 is located in the nucleus, the subcellular localization assay was performed (Figure 2). Despite the transient expression in the onion epidermal cells, it was observed that the GmNAC5-GFP fusion protein was located predominantly in the nucleus whereas GFP alone was localized throughout the cells (Figure 2(b)).

*3.3. Tissue-Specific Expression of GmNAC5.* In order to analyze the physiological and biochemical processes that *GmNAC5* gene may involve, qRT-PCR approach was used to analyze *GmNAC5* gene expression in soybean in different tissues and organs. *GmNAC5* was mainly expressed in the roots and seeds in soybean development and weakly expressed in the other organs (Figure 3). *GmNAC5* has the lowest expression level in the stems, but the highest expression level

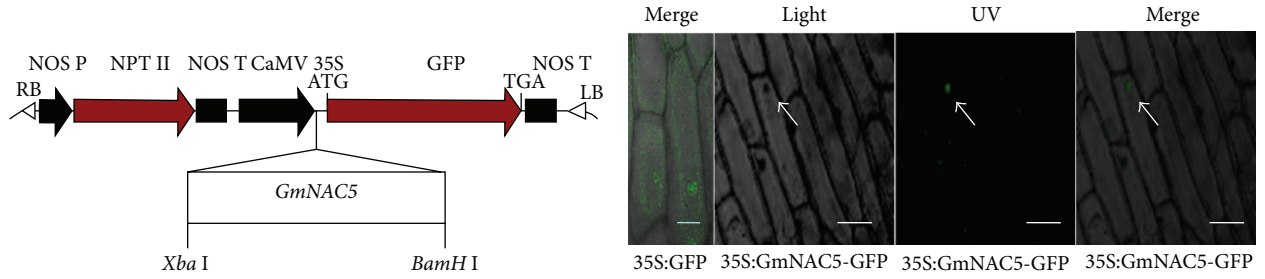


Figure 2: Subcellular localization of GmNAC5. (a) The structure of 35S:GmNAC5-GFP vector. (b) Subcellular localization of GmNAC5-GFP fusion protein. The arrow indicates the location of the nucleus. Bars: 40 $\mu$m in 35S:GFP; 80 $\mu$m in 35S:GmNAC5-GFP.

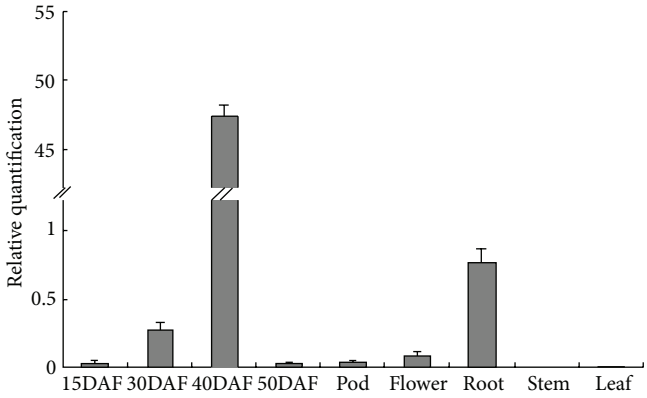


Figure 3: Real-time RT-PCR analysis of *GmNAC5* expression in various soybean tissues. DAF: days after flowering.

in soybean seeds of 40 days after flowering. The difference in *GmNAC5* expression level of each period in soybean seed development is obvious. The highest expression level was found 40 days after flowering (DAF), but only weak expression in the seeds of 15 days and 50 days after flowering, which indicates that the *GmNAC5* may participate in the middle stage of soybean seed development. We found that *GmNAC5* has strong expression in roots, but expression levels in stems, leaves, and pods are weak.

*3.4. Expression of GmNAC5 in Soybean under Various Stresses.* *GmNAC5* was weakly expressed in leaves in soybean under normal growth condition. The real-time qRT-PCR was performed to detect the expression of *GmNAC5* in soybean under various stresses (Figure 4). For jasmonic acid treatment, *GmNAC5* was significantly induced after 3 h of JA treatment (Figure 4(a)). For mechanical wounding, expression of *GmNAC5* was sharply induced after 1 h of treatment (Figure 4(b)). For NaCl treatment, *GmNAC5* expression was markedly upregulated by 8-fold after 3 h of treatment and then decreased (Figure 4(c)). Under drought treatment, expression of *GmNAC5* showed a weak increase and then declined (Figure 4(d)). For cold stress, it was found that *GmNAC5* expression was gradually increased and reached the maximum after 12 h of treatment (Figure 4(e)). In order to reveal whether stress responsive expression of *GmNAC5* was involved in ABA pathway, we studied expression of *GmNAC5* under ABA treatment (Figure 4(f)). The qRT-PCR assay suggested that expression of GmNAC5 was not markedly affected by ABA, suggesting that GmNAC5 may participate in ABA-independent signaling pathway in soybean under abiotic stresses.

## 4. Concluding Remarks

It has been documented that the plant-specific NAC (for NAM, ATAF1, 2, and CUC2) transcription factors play an important role in plant development and stress responses [27]. GmNAC5 belongs to the NAM subgroup and is most closely related to CUC1, CUC2, and NAM, which are involved in developmental events, maintenance of shoot meristem, and cotyledons separation [28]. In this study, we observed some new clues involved in the functions of GmNAC5. Tissue-specific expression analysis indicated that *GmNAC5* was highly expressed in immature seeds at 40 DAF and in the roots, suggesting the involvements of GmNAC5 in seed development and root development. It was also found that transcripts of *Arabidopsis AtNAC2* were accumulated at the late stages of seed development [29].

It is also possible that higher expression of *GmNAC5* in soybean roots is associated with abiotic stress responses. *Arabidopsis* AtNAC2 expression was highly in roots and induced by salt stress [21]. Further studies suggested that AtNAC2 functioned downstream of ethylene and auxin signaling pathways and regulated lateral root development under salt stress. Expression of *GmNAC5* was significantly induced by multiple abiotic stresses but not by ABA, suggesting that GmNAC5 may be involved in ABA-independent stress responses in soybean under abiotic stresses. It was previously reported that NAC transcription factor involves the control of plant senescence and transient expression of *GmNAC5* in tobacco leaves induced senescence and necrosis, suggesting that GmNAC5 may play a role in the regulation of stress promoted senescence. Through microarray analysis, it was found that *Arabidopsis* AtNAC2 regulated many senescence-related genes and the majority of them are also regulated by salt stress, a major promoter of plant senescence [29]. Whether GmNAC5 plays a regulatory role in stress regulated

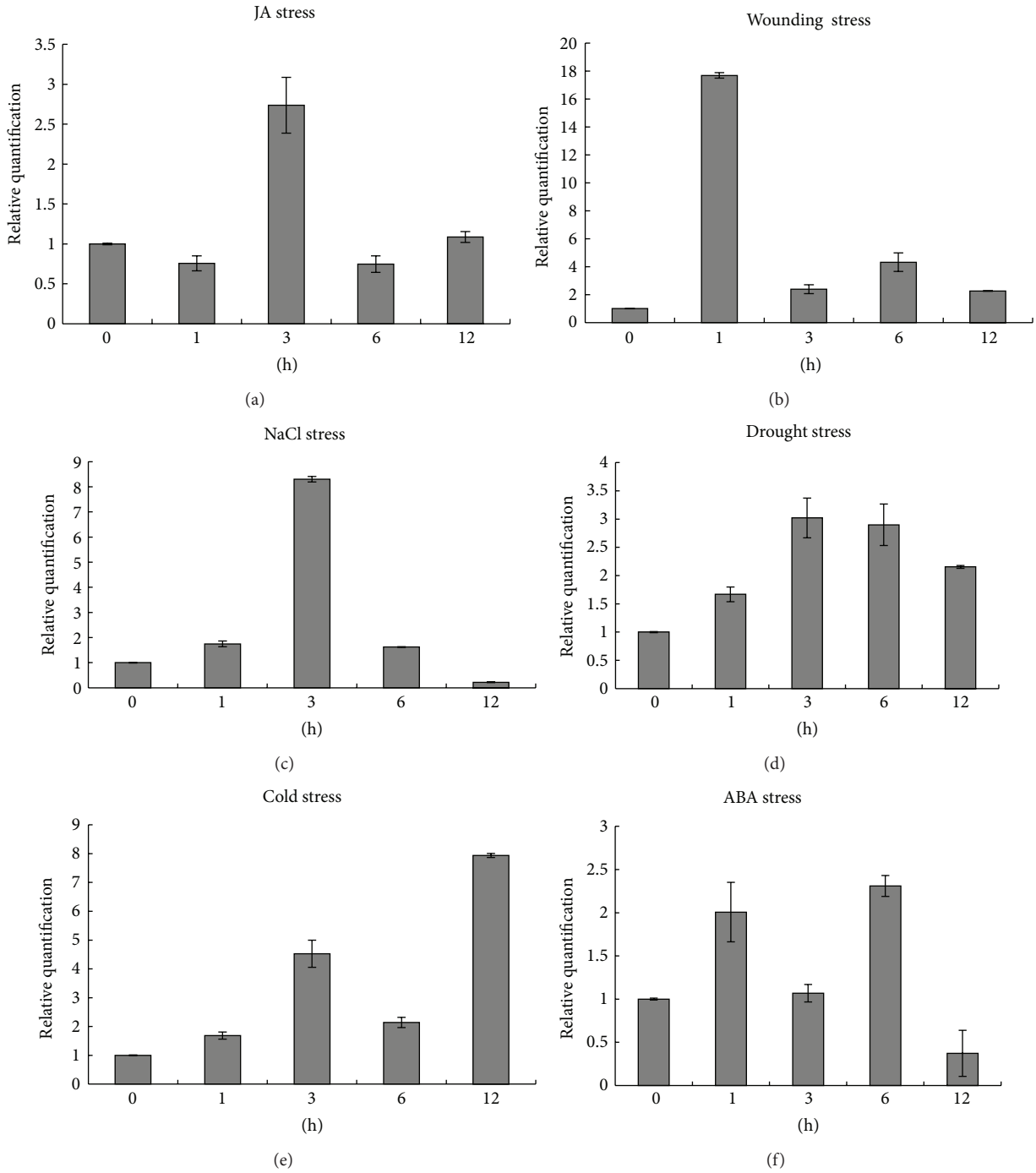


Figure 4: Expression of *GmNAC5* in soybean seedlings under various stresses. The soybean seedlings were stressed with 100 $\mu$M JA (a), wounding (b), 200 mM NaCl (c), drought (d), 4°C (e), and 100 $\mu$M ABA (f).

root development or stress promoted senescence still needs to be further analyzed.

## Conflict of Interests

The authors declare no conflict of interests. The authors do not have a direct financial relation with the commercial identity mentioned in the current paper.

## Authors' Contribution

Hangxia Jin and Guangli Xu contributed equally to this work.

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## References


[1] X. Mao, H. Zhang, X. Qian, A. Li, G. Zhao, and R. Jing, "TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in Arabidopsis," *Journal of Experimental Botany*, vol. 63, no. 8, pp. 2933–2946, 2012.

[2] Q. Xie, G. Frugis, D. Colgan, and N.-H. Chua, "Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development," *Genes and Development*, vol. 14, no. 23, pp. 3024–3036, 2000.

[3] S. Takada, K.-I. Hibara, T. Ishida, and M. Tasaka, "The CUP-SHAPED *COTYLEDON1* gene of Arabidopsis regulates shoot apical meristem formation," *Development*, vol. 128, no. 7, pp. 1127–1135, 2001.

[4] M. Duval, T.-F. Hsieh, S. Y. Kim, and T. L. Thomas, "Molecular characterization of AtNAM: a member of the Arabidopsis NAC domain superfamily," *Plant Molecular Biology*, vol. 50, no. 2, pp. 237–248, 2002.

[5] E. Souer, A. Van Houwelingen, D. Kloos, J. Mol, and R. Koes, "The no apical Meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries," *Cell*, vol. 85, no. 2, pp. 159–170, 1996.

[6] M. Fujita, Y. Fujita, K. Maruyama et al., "A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway," *Plant Journal*, vol. 39, no. 6, pp. 863–876, 2004.

[7] R. W. M. Sablowski and E. M. Meyerowitz, "A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*," *Cell*, vol. 92, no. 1, pp. 93–103, 1998.

[8] A. Iwase, A. Hideno, K. Watanabe, N. Mitsuda, and M. Ohme-Takagi, "A chimeric NST repressor has the potential to improve glucose productivity from plant cell walls," *Journal of Biotechnology*, vol. 142, no. 3-4, pp. 279–284, 2009.

[9] N. Mitsuda, A. Iwase, H. Yamamoto et al., "NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of Arabidopsis," *Plant Cell*, vol. 19, no. 1, pp. 270–280, 2007.

[10] N. Mitsuda and M. Ohme-Takagi, "NAC transcription factors NST1 and NST3 regulate pod shattering in a partially redundant manner by promoting secondary wall formation after the establishment of tissue identity," *Plant Journal*, vol. 56, no. 5, pp. 768–778, 2008.

[11] F. T. S. Nogueira, P. S. Schlögl, S. R. Camargo et al., "SsNAC23, a member of the NAC domain protein family, is associated with cold, herbivory and water stress in sugarcane," *Plant Science*, vol. 169, no. 1, pp. 93–106, 2005.

[12] T. Ohnishi, S. Sugahara, T. Yamada et al., "OsNAC6, a member of the NAC gene family, is induced by various stresses in rice," *Genes and Genetic Systems*, vol. 80, no. 2, pp. 135–139, 2005.

[13] H. Hu, M. Dai, J. Yao et al., "Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 35, pp. 12987–12992, 2006.

[14] X. Han, G. He, S. Zhao, C. Guo, and M. Lu, "Expression analysis of two NAC transcription factors *PtNAC068* and *PtNAC154* from poplar," *Plant Molecular Biology Reporter*, vol. 30, no. 2, pp. 370–378, 2012.

[15] H. Ooka, K. Satoh, K. Doi et al., "Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*," *DNA Research*, vol. 10, no. 6, pp. 239–247, 2003.

[16] K.-I. Taoka, Y. Yanagimoto, Y. Daimon, K.-I. Hibara, M. Aida, and M. Tasaka, "The NAC domain mediates functional specificity of CUP-SHAPED COTYLEDON proteins," *Plant Journal*, vol. 40, no. 4, pp. 462–473, 2004.

[17] K.-I. Hibara, S. Takada, and M. Tasaka, "CUC1 gene activates the expression of SAM-related genes to induce adventitious shoot formation," *Plant Journal*, vol. 36, no. 5, pp. 687–696, 2003.

[18] A. Hasson, A. Plessis, T. Blein et al., "Evolution and diverse roles of the CUP-SHAPED COTYLEDON genes in Arabidopsis leaf development," *Plant Cell*, vol. 23, no. 1, pp. 54–68, 2011.

[19] I. Weir, J. Lu, H. Cook, B. Causier, Z. Schwarz-Sommer, and B. Davies, "Cupuliformis establishes lateral organ boundaries in *Antirrhinum*," *Development*, vol. 131, no. 4, pp. 915–922, 2004.

[20] R. Ruiz-Medrano, B. Xoconostle-Cázares, and W. J. Lucas, "Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants," *Development*, vol. 126, no. 20, pp. 4405–4419, 1999.

[21] X.-J. He, R.-L. Mu, W.-H. Cao, Z.-G. Zhang, J.-S. Zhang, and S.-Y. Chen, "AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development," *Plant Journal*, vol. 44, no. 6, pp. 903–916, 2005.

[22] Q. Meng, C. Zhang, J. Gai, and D. Yu, "Molecular cloning, sequence characterization and tissue-specific expression of six NAC-like genes in soybean (*Glycine max* (L.) Merr.)," *Journal of Plant Physiology*, vol. 164, no. 8, pp. 1002–1012, 2007.

[23] W. R. Fehr, C. E. Caviness, D. T. Burmood, and J. S. Pennington, "Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill," *Crop Science*, vol. 11, no. 6, pp. 929–931, 1971.

[24] F. Huang, Y. Chi, J. Gai, and D. Yu, "Identification of transcription factors predominantly expressed in soybean flowers and characterization of *GmSEP1* encoding a SEPALLATA1-like protein," *Gene*, vol. 438, no. 1-2, pp. 40–48, 2009.

[25] H. A. Ernst, A. N. Olsen, K. Skriver, S. Larsen, and L. Lo Leggio, "Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors," *EMBO Reports*, vol. 5, no. 3, pp. 297–303, 2004.

[26] L.-S. P. Tran, K. Nakashima, Y. Sakuma et al., "Isolation and functional analysis of arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter," *Plant Cell*, vol. 16, no. 9, pp. 2481–2498, 2004.

[27] H. Peng, H.-Y. Cheng, X.-W. Yu et al., "Characterization of a chickpea (*Cicer arietinum* L.) NAC family gene, CarNAC5, which is both developmentally- and stress-regulated," *Plant Physiology and Biochemistry*, vol. 47, no. 11-12, pp. 1037–1045, 2009.

[28] A. N. Olsen, H. A. Ernst, L. L. Leggio, and K. Skriver, "NAC transcription factors: structurally distinct, functionally diverse," *Trends in Plant Science*, vol. 10, no. 2, pp. 79–87, 2005.

[29] S. Balazadeh, H. Siddiqui, A. D. Allu et al., "A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence," *Plant Journal*, vol. 62, no. 2, pp. 250–264, 2010.

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*Research Article*

# The Low Temperature Induced Physiological Responses of *Avena nuda* L., a Cold-Tolerant Plant Species

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The paperaim of the was to study the effect of low temperature stress on *Avena nuda* L. seedlings. Cold stress leads to many changes of physiological indices, such as membrane permeability, free proline content, malondialdehyde (MDA) content, and chlorophyll content. Cold stress also leads to changes of some protected enzymes such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). We have measured and compared these indices of seedling leaves under low temperature and normal temperature. The proline and MDA contents were increased compared with control; the chlorophyll content gradually decreased with the prolongation of low temperature stress. The activities of SOD, POD, and CAT were increased under low temperature. The study was designated to explore the physiological mechanism of cold tolerance in naked oats for the first time and also provided theoretical basis for cultivation and antibiotic breeding in *Avena nuda* L.

## 1. Introduction

*Avena nuda* L., also named naked oats, is originated and widely separated in north and high altitude region in China. It belongs to herb of gramineous plant with annual growing. Naked oats has great value in nutrition and medicine. It contains abundant proteins with 18 kinds of amino acid, and lots of unsaturated fatty acids. Naked oats likes to grow under cool weather and has tolerance to low temperature. Naked oats is a typical temperate crop adapted to cool climates.

Low temperature is a major abiotic stress that limits the growth, productivity, and geographical distribution of agricultural crops and can lead to significant crop loss [1, 2]. To cope with low temperature, plants have evolved a variety of efficient mechanisms that allow them to adapt to the adverse conditions [3, 4]. This adaptive process involves a number of biochemical and physiological changes, including increased levels of proline, soluble sugars, and MDA, as well as enzyme activities [5].

Understanding the mechanisms of low temperature adaptation is crucial to the development of cold-tolerant crops. The study was designated to explore the physiological mechanism of cold tolerance in naked oats. The responses of the *Avena nuda* L. seedlings to low temperature stress were also evaluated by measuring electrolyte leakage (EL), chlorophyll content, and the concentration of MDA. We measured and compared these indices of seedlings leaves under low temperature and normal temperature. The study provided theoretical basis for cultivator and antibiotic breeding in *Avena nuda* L.

## 2. Materials and Methods

*2.1. Materials and Cold Treatment.* Naked oats cultivar Jinyan 14 (*Avena nuda* L.) was used in the experiment. Seeds were sterilized by incubation for 1 min in 75% ethanol and then washed thoroughly with sterile water. The seeds were germinated in soil in pots at 20°C under long-day conditions (16 h of cool white fluorescent light, photon flux of 70 umol $m^{-2}$ $s^{-1}$).

Seedlings at the four-leaf stage were subjected to cold stress. Plants were divided into three groups, one group was

under normal temperature as control, the other two were subjected to low temperature processing, and each grouphad the stress repeated three times. Seedlings of the control group were grown at 20°C continuously. For low temperature treatments, seedlings were transferred to a temperature of 1°C and −10°C in an artificial climate box under the same light and photoperiodic conditions for 7 days.

The leaves were sampled after 0, 1, 3, 5 and, 7 d of treatment for next measurement. The leaf samples were immediately frozen in liquid nitrogen and stored at −80°C until use. Three independent biological samples for each treatment were harvested, and each replicate contained 10 plants.

*2.2. Determination of Relative Electrolyte Leakage.* For electrolyte leakage measurement, protocol was used as described [6]. Briefly, 100 mg leaves were placed in 25 mL distilled water, shaken on a gyratory shaker (200 rpm) at room temperature for 2 h, and the initial conductivity ($C1$) was measured with a conductivity instrument. The samples were then boiled for 10 min to induce maximum leakage. After cooling down at room temperature, electrolyte conductivity ($C2$) was measured, and the relative electrical conductivity ($C\%$) was calculated based on $(C1/C2) \times 100$. All low temperature testing experiments were repeated three times. A paired $t$-test was used to determine the difference between the cold treatment and normal condition.

*2.3. Determination of Chlorophyll Content.* For estimation of total chlorophyll, protocol was followed as described [7]. About 100 mg of fine powder of leaf tissue was homogenized in 1 mL of 80% acetone and kept for 15 min at room temperature in dark. The crude extract was centrifuged for 20 min at 10,000 rpm at room temperature, and the resultant supernatant was used for assessing absorbance at 633 and 645 nm with a spectrophotometer. Total chlorophyll content was computed in terms of fresh weight (FW).

*2.4. Determination of Proline Content.* Proline concentrations in naked oats leaves were measured by the sulfosalicylic acid-acid ninhydrin method with slight modifications [8]. Around 100 mg of tissues were used and extracted in 5 mL of 3% sulphosalicylic acid at 95°C for 15 min. After filtration, 2 mL of supernatant was transferred to a new tube containing 2 mL of acetic acid and 2 mL of acidified ninhydrin reagent. After 30 min of incubation at 95°C, samples were kept at room temperature for 30 min and 5 mL of toluene was added to the tube with shaking at 150 rpm to extract red products. The absorbance of the toluene layer was determined at 532 nm using spectrophotometer.

*2.5. Determination of Malondialdehyde (MDA) Content.* Malondialdehyde (MDA) content in naked oats leaves was determined following the protocols as described [9]. Briefly, leaves were homogenized in 5 mL of 10% trichloroacetic acid containing 0.25% thiobarbituric acid. The mixture was incubated in water at 95°C for 30 min, and the reaction was stopped in an ice bath. The mixture was centrifuged at 10,000 g for 20 min, and the absorbance of the supernatant was measured at 450, 532, and 600 nm.

*2.6. Determination of Peroxidase, Superoxide Dismutase, and Catalase Activity.* Naked oats leaves (0.5 g) were ground thoroughly with a cold mortar and pestle in 50 mmol potassium phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 20 min at 4°C. The supernatant was crude enzyme extraction. The activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were measured using the protocols described [10].

## 3. Results and Discussion

*3.1. Phenotypic Changes under Cold Stress.* We investigated the phenotypic response to low temperature. During the cold treatment of 1°C, naked oats grew well as usual. Until 5 days later, the seedlings were always strong only except some leaf apexes began to get yellow. In the 7th day, most parts of seedling remained green as normal temperature as shown in Figure 1. The seedling got curl after 3-4 hours after exposure to −10°C cold stress. Some leaf began to get yellow and curled seriously in the third day, while the seedling grew slowly. Most leaves showed severe rolling and wilting in the 7th day.

No obvious differences have been found in growth and developed between normal temperature and at 1°C, indicating that the naked oats have cold tolerance at the low temperature of 1°C.

*3.2. Changes of Relative Electrolyte Leakage under Cold Stress.* Cold stress often causes damage to cell membranes, so, we tested the cell-membrane penetrability. Cell membrane penetrability was evaluated by the relative conductance of the cell membrane under cold stress [11, 12]. The electrolyte leakage test was performed to compare membrane integrity [13]. For such experiment, plants were subjected to low temperature of 1°C and −10°C. The relative electrolyte leakage of seedling leaves was increased greatly with cold treatment as shown in Figure 2. There was no significant difference under normal temperature; the average electrolyte leakage was about 9.7%. During the cold stress, the relative electrolyte leakage value gradually increased with the prolongation of low temperature stress. The electrolyte leakage of −10°C was significantly higher than that of 1°C growing plants. In the 7th day, elative electrolyte leakage increased to 66.0% under cold stress of −10°C, while 37.3% at 1°C and the normal condition still 11.0%.

When the plants were under low temperature stress, the structure of cellular membrane was damaged. The degree of cell membrane injury induced by cold stress can be reflected by intracellular electrolyte leakage rate. The relative conductance value is one of the effective indicators to indirectly evaluate plant response ability to low temperature stress [14]. The damage degree of cellular membrane was aggravated with the continuity of low temperature stress [12]. In the experiment, the electrolyte leakage in leaves of naked oats seedlings gradually increased under low temperature of 1°C


Figure 1: The seedlings of naked oats at normal temperature (a) and low temperature of 1°C (b) and −10°C (c) after 7 days.

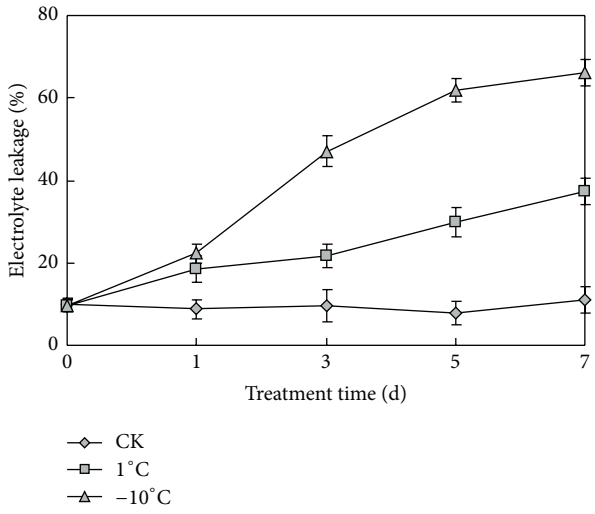


Figure 2: Relative electrolyte leakage of seedling leaves under normal and low temperatures. CK represents normal growth condition. Each value is the average ± standard error (±SE) result of three independent measurements.

stress, indicating that the damage of low temperature on cell membrane increased gradually. The electrolyte leakage increased more significantly at −10°C than 1°C, indicating that cell membrane was damaged seriously at −10°C than 1°C. At 1°C, the naked oats had some tolerance to protect the cell membraneavoiding cold damage.

*3.3. Changes of Total Chlorophyll Content under Cold Stress.* Chlorophyll is an extremely important and critical biomolecule in photosynthesis with function of light absorbance and light energy transformation [15]. Low temperature stress can influent plant photosynthesis and decrease the utilization of light [16, 17]. We examined the content of chlorophyll under low temperature of 1°C and −10°C. Compared with the control, the chlorophyll content in seedling leaves under low temperature was lower than that under room temperature. As shown in Figure 3, the change range of chlorophyll content in leaves of naked oats was 6.4–6.9 mg/g

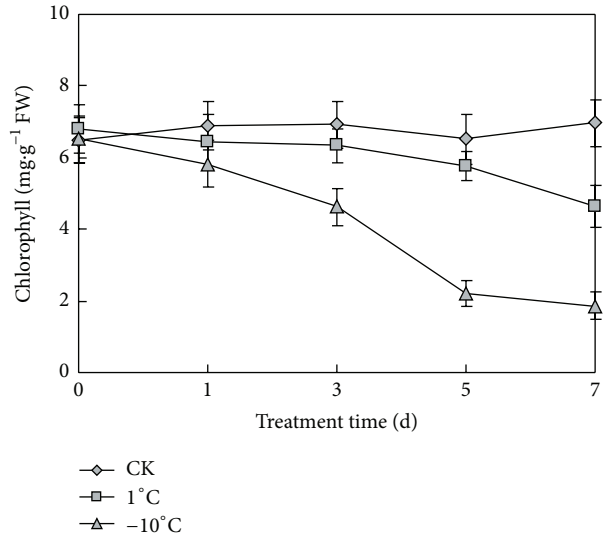


Figure 3: Chlorophyll content of seedling leaves under normal and low temperatures. CK represents normal growth condition. Each value is the average ± standard error (±SE) result of three independent measurements.

under room temperature. At 1°C, the chlorophyll content was decreased with the prolongation of low temperature stress. The chlorophyll decreased slightly from the 1st day to the 5th day, and the content was onlyslightly less than control, but in 7th day the chlorophyll decreased greatly to 4.6 mg/g, while at −10°C, the chlorophyll content decreased seriously. Especially in the 5th day, chlorophyll content decreased to 2.2 mg/g while 5.8 mg/g at 1°C and 6.5 mg/g at normal temperature. The results indicated that naked oats was damaged less at 1°C than at −10°C. Low temperature inhibits chlorophyll accumulations in actively growing leaves. Naked oats has some degree of cold tolerance at 1°C.

*3.4. Changes of Free Proline Content under Cold Stress.* Proline is widely distributed in plants as protection material, which is an organic osmolyte [18]. It plays a vital role in maintaining osmotic balance and stabilizing cellular structures in plants. Many plants accumulate free proline in response to abiotic stress of low temperature. Increased free proline content protects the plant against the stress [19].

The effect of cold on content of proline was investigated. There was no significant difference in proline contents without cold stress as shown in Figure 4. An increase in proline content was observed upon exposure to cold stress. Compared with the control, the free proline content in seedling leaves under low temperature was obviously higher than that under room temperature. At 1°C, the proline content increased with the prolongation of low temperature stress. At −10°C, the proline content was increased in the first five days and reached the max of 601 $\mu$g/g in the 5th day, about 6 times of control. Then proline content decreased to 404 $\mu$g/g in the 7th day, which was still much higher than control. At −10°C, the plant accumulated more amounts of proline than at 1°C.

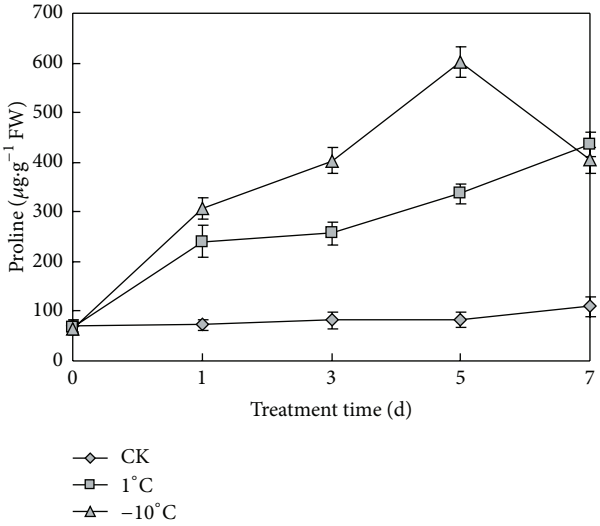


Figure 4: Free proline content of seedling leaves under normal and low temperatures. CK represents normal growth condition. Each value is the average ± standard error (±SE) result of three independent measurements.

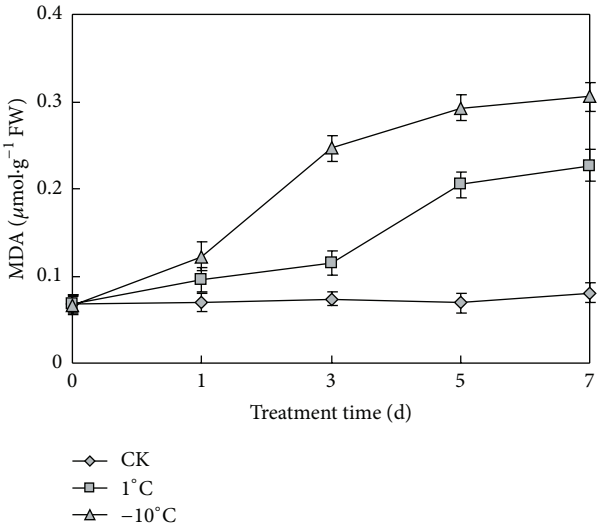


Figure 5: MDA content of seedling leaves under normal and low temperatures. CK represents normal growth condition. Each value is the average ± standard error (±SE) result of three independent measurements.

This indicated that the lower the temperature was, the more proline could be accumulated.

Proline plays a vital role in maintaining osmotic balance in plants. The accumulation of proline may function in preventing plants from being damaged by stress. The free proline acts as osmolytes to facilitate osmoregulation, thus protecting plants from dehydration resulting from cold stress by reducing water potential of plant cells [20]. In addition, proline can also function as a molecular chaperone to stabilize the structure of proteins as well as play a role in regulation of the antioxidant system [21, 22]. So increased free proline content protects the plant against the stress. The study found greater accumulation of free proline under cold stress, which may partially account for the higher tolerance of plants to cold stress. Accumulation of proline to facilitate osmo-regulation is a common adaptive mechanism for tolerance of plants to abiotic stress. The results were consistent with previous studies that proline accumulated in leaves exposed to cold, salt, and other stresses [23].

*3.5. Changes of Malondialdehyde (MDA) Content under Cold Stress.* Cold stress often causes damage to cell membranes. Malondialdehyde (MDA) is an important indicator of membrane system injuries and cellular metabolism deterioration [24]. So, we further measured the cell membrane penetrability. The effects of MDA contents were investigated in the seedling of naked oats. In our experiment, naked oats had a significantly higher MDA level under low temperature stress compared to the control level. As shown in Figure 5, the MDA concentrations increased with the prolongation of low temperature stress. In the seventh day, the MDA content reached to the max nearly 3 times of control at 1°C and about 4 times at −10°C. The increase in MDA content at −10°C was significantly higher than 1°C all the time.

MDA has been well recognized as a parameter reflecting damage by cold stress. Cell membrane systems are the primary sites of freezing injury in plants [25]. Plants subjected to low temperatures frequently suffer membrane damage, which can be evaluated by relative electrolyte leakage and MDA production. MDA is considered to be the final product of lipid peroxidation in the plant cell membrane [26]. MDA is also an important intermediate in ROS scavenging, and a high level of MDA is toxic to plant cells. In this experiment, in the first day the MDA only increased 0.095 $\mu$mol·g$^{-1}$ at 1°C, while the MDA concentrations increased to 0.122 $\mu$mol·g$^{-1}$ at −10°C. In the third day, MDA increased slightly to 0.115 $\mu$mol·g$^{-1}$ at 1°C, while MDA increased rapidly to 0.247 $\mu$mol·g$^{-1}$ at −10°C, which was 4 times of control. The increase of MDA at −10°C was greater than at 1°C. These results suggested that cell membrane was little damaged at 1°C at the beginning of cold stress, but was hurt seriously at −10°C. It may have contributed to the different phenotypes under cold stress. Prolonged treatment finally led toa great cell damaged and MDA accumulation. The MDA content increased slowly in first three days at 1°C, but increased rapidly to 0.20 $\mu$mol·g$^{-1}$ in the fifth day. It indicated that cell membranes of seedlings were injured by cold seriously in 3–5 days at 1°C.

*3.6. Changes of Antioxidant Enzymes under Cold Stress.* Cold stress induces the accumulation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyradicals [27, 28]. The elevated concentrations of ROS can damage cellular structures and macromolecules, leading to cell death [29, 30]. Plants under abiotic stress have evolved a defense system against oxidative stress by increasing the activity of ROS-scavenging enzyme. ROS can be scavenged by superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) [31]. SOD plays an important role in eliminating ROS induced by cold [32]. POD and CAT can scavenge $H_2O_2$.

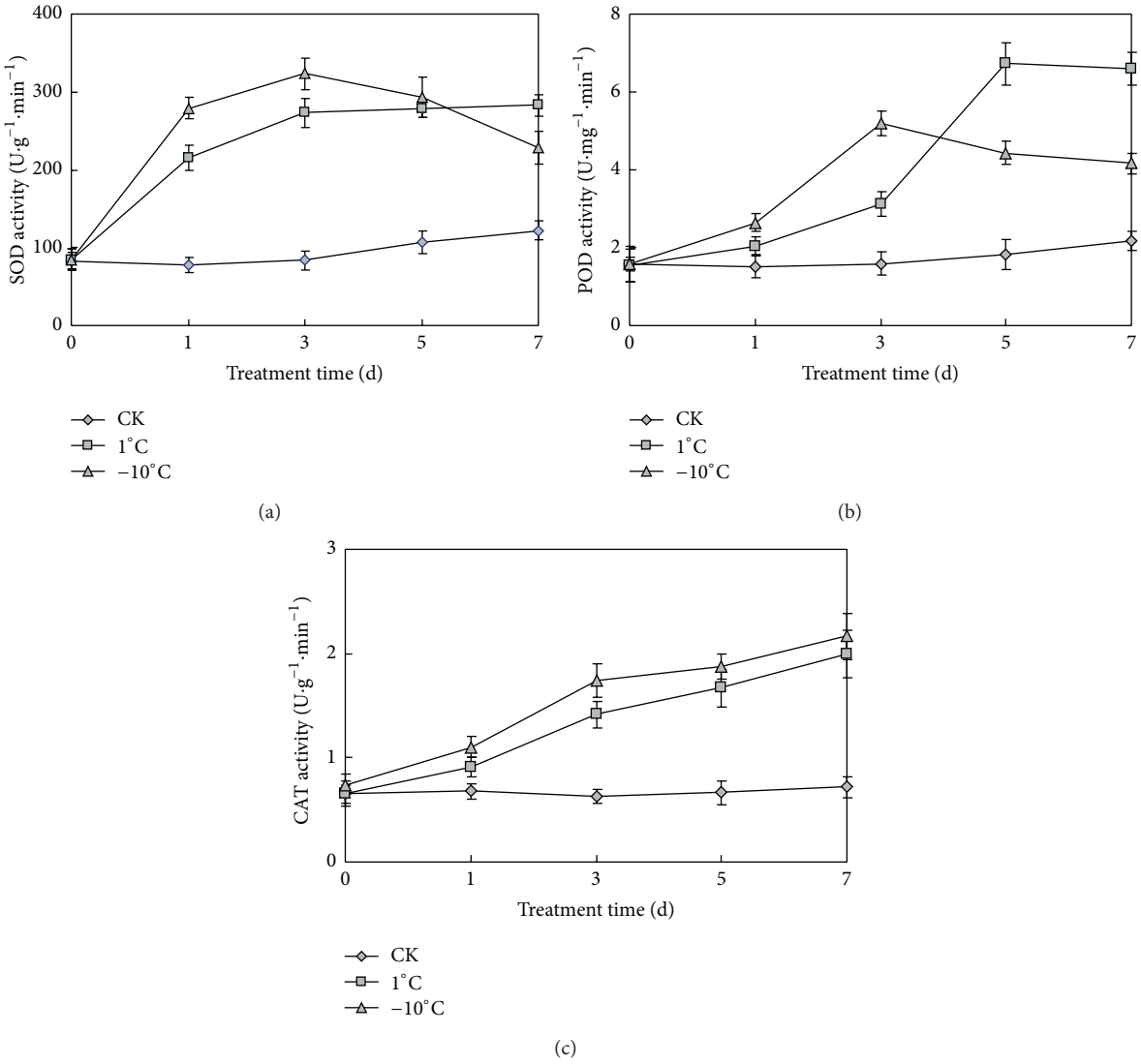


Figure 6: Activities of SOD (a), POD (b), and CAT (c) of seedling leaves under normal and low temperatures. CK represents normal growth condition. Each value is the average ± standard error (±SE) result of three independent measurements.

SOD, POD, and CAT are important enzymes protecting membrane system. Many kinds of antioxidant enzyme activities have been changed under low stress. Changes of enzyme activities are relvant to cold tolerance.

We measured the activities of these enzymes under normal and cold stress. Compared with normal, the SOD activity was higher under the cold treatment, as shown in Figure 6(a). At 1°C, the SOD activity was increased rapidly in the first three days and remained unchanged in later days. At −10°C, the SOD activity was increased first and decreased later. In the third day, the SOD activity was increased to the max value 323 U·g$^{-1}$·min$^{-1}$, which was nearly 4 times of normal temperature. Then, the SOD activity was decreased rapidly to a low value of 229 U·g$^{-1}$·min$^{-1}$ in the seventh day. At this time, the seedlings were damaged seriously by low temperature. Compared with normal, the SOD activity was higher than normal under the cold treatment. Increased SOD activity contributed for cold tolerance of naked oats under cold stress. Especially in the first day, the SOD activities had increasedgreatly, indicated that naked oats is resisting and adapting to low temperature, and contributed to reduce the damage by cold. The SOD activity decreased later at −10°C, which may be due to the greater damage by temperature of −10°C affected the synthesis of SOD in plant.

POD activities were higher under low temperature than normaltemperature as shown in Figure 6(b). At 1°C, POD activity was slowly increased in the first three days, rapidly increased in the third to fifth days, and reached the max of 6.7 U·min$^{-1}$·mg$^{-1}$ in the fifth day, more than 4 times of control. Then POD activity slightly decreased to 6.6 U·mg$^{-1}$·min$^{-1}$ in the 7th day, which was almost nearly the max value. At −10°C, POD activity was rapidly increased in the first three days and reached the max of 5.2 U·mg$^{-1}$·min$^{-1}$ in the third day, more than 3 times of

control. Then POD activity decreased in the third to seventh days. The POD activity decreased to 4.16 $U \cdot mg^{-1} \cdot min^{-1}$ in the 7th day, which was still 2.5 timesmore than that of control. PODs are a large family of enzymes that typically catalyze peroxide. The increased POD activities under low temperature had improved cold tolerance in some degree. POD activities decreased greatly in later days, indicating that low temperature had affected POD enzyme. It maybe due to low temperature affected RNA transcription and translation, reducing the synthesis of POD. At the same time proline content also increased under low temperature, which can degrade peroxidase. It can also decrease the POD activity.

Compared with normal, the CAT activity was higher under the cold treatment than normal temperature as shown in Figure 6(c). At 1°C, the CAT activity was increased with the prolongation of low temperature stress. CAT activity was 0.66 $U \cdot g^{-1} \cdot min^{-1}$ before of cold treatment and increased to 1.99 $U \cdot g^{-1} \cdot min^{-1}$ after the seven days cold treatment. At −10°C, the CAT activity was increased rapidly in the first three days and increased slowly in later days. In the third day, the CAT activity was increased to the 1.74 $U \cdot g^{-1} \cdot min^{-1}$, which was nearly 2.5 times of normal temperature. Then, the CAT activity was increased to 2.16 $U \cdot g^{-1} \cdot min^{-1}$ in the seventh day. Compared with normal, the CAT activity was higher than normal under the cold treatment. Increased CAT activity contributed to cold tolerance of naked oats under cold stress.

In this experiment, the activities of these enzymes were increased in different degrees under cold stress. The results imply that higher SOD, POD, and CAT activities enhanced the capacity for scavenging ROS and contributed to enhanced tolerance of plant to cold stress. The result was similar to the SOD changes in other cold treatment plants.

## 4. Conclusions

*Avena nuda* L. is an important crop and has tolerance to low temperature. In the long time of evolution, it should have some cold resistant mechanism. The cold-stress leads to complex cellular and biochemical changes such as altered membrane composition and accumulation of proline, as well as the activities of antioxidant enzymes. In the present study, we have, for the first time, investigated the physiological changes in naked oats under low temperature. As results showed, low temperature stress caused a certain degree of physiological impairment in naked oats leaves. Here, we showed that cold increased the content of proline, MDA, electrolyte leakage, SOD, CAT, and POD activities. The content of chlorophyll was decreased. The study provided theoretical basis for cultivation and antibiotic breeding in *Avena nuda* L.

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## References

[1] C. Guy, F. Kaplan, J. Kopka, J. Selbig, and D. K. Hincha, "Metabolomics of temperature stress," *Physiologia Plantarum*, vol. 132, no. 2, pp. 220–235, 2008.

[2] H. J. Bohnert, D. E. Nelson, and R. G. Jensen, "Adaptations to environmental stresses," *Plant Cell*, vol. 7, no. 7, pp. 1099–1111, 1995.

[3] R. Bressan, H. Bohnert, and J.-K. Zhu, "Perspective: Abiotic stress tolerance: from gene discovery in model organisms to crop improvement," *Molecular Plant*, vol. 2, no. 1, pp. 1–2, 2009.

[4] W. Y. Liu, Y. F. Zhang, and D. X. Zhang, "Study on cold resistant genes in plants," *Journal of Shanxi Datong Unirersity*, vol. 28, no. 6, pp. 52–55, 2012.

[5] A. S. Holaday, W. Martindale, R. Aired, A. L. Brooks, and R. C. Leegood, "Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature," *Plant Physiology*, vol. 98, no. 3, pp. 1105–1114, 1992.

[6] M. Bajji, P. Bertin, S. Lutts, and J.-M. Kinet, "Evaluation of drought resistance-related traits in durum wheat somaclonal lines selected in vitro," *Australian Journal of Experimental Agriculture*, vol. 44, no. 1, pp. 27–35, 2004.

[7] D. Arnon, "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris," *Plant Physioligacal*, vol. 24, no. 1, pp. 1–15, 1949.

[8] L. S. Bates, R. P. Waldren, and I. D. Teare, "Rapid determination of free proline for water-stress studies," *Plant and Soil*, vol. 39, no. 1, pp. 205–207, 1973.

[9] S.-Y. Song, Y. Chen, J. Chen, X.-Y. Dai, and W.-H. Zhang, "Physiological mechanisms underlying *OsNAC5*-dependent tolerance of rice plants to abiotic stress," *Planta*, vol. 234, no. 2, pp. 331–345, 2011.

[10] B.-H. Miao, X.-G. Han, and W.-H. Zhang, "The ameliorative effect of silicon on soybean seedlings grown in potassium-deficient medium," *Annals of Botany*, vol. 105, no. 6, pp. 967–973, 2010.

[11] P. Rohde, D. K. Hincha, and A. G. Heyer, "Heterosis in the freezing tolerance of crosses between two Arabidopsis thaliana accessions (Columbia-0 and C24) that show differences in non-acclimated and acclimated freezing tolerance," *Plant Journal*, vol. 38, no. 5, pp. 790–799, 2004.

[12] P. L. Steponkus, D. V. Lynch, and M. Uemura, "The influence of cold-acclimation on the lipid-composition and cryobehavior of the plasma-membrane of isolated rye protoplasts," *Philosophical Transactions B*, vol. 326, no. 1237, pp. 571–583, 1990.

[13] M. B. Murray, J. N. Cape, and D. Fowler, "Quantification of frost damage in plant-tissues by rates of electrolyte leakage," *New Phytologist*, vol. 113, no. 3, pp. 307–311, 1989.

[14] P. L. Steponkus, "Role of the plasma membrane in freezing-injury and cold acclimation," *Annual Review of Plant Physiology*, vol. 35, pp. 543–584, 1984.

[15] W. Xu, D. T. Rosenow, and H. T. Nguyen, "Stay green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration," *Plant Breeding*, vol. 119, no. 4, pp. 365–367, 2000.

[16] J. C. Glaszmann, R. N. Kaw, and G. S. Khush, "Genetic divergence among cold tolerant rices (*Oryza sativa* L.)," *Euphytica*, vol. 45, no. 2, pp. 95–104, 1990.

[17] J. Wu, J. Lightner, N. Warwick, and J. Browse, "Low-temperature damage and subsequent recovery of fab1 mutant arabidopsis exposed to 2°C," *Plant Physiology*, vol. 113, no. 2, pp. 347–356, 1997.

[18] N. Verbruggen and C. Hermans, "Proline accumulation in plants: a review," *Amino Acids*, vol. 35, no. 4, pp. 753–759, 2008.

[19] Y. Igarashi, Y. Yoshiba, Y. Sanada, K. Yamaguchi-Shinozaki, K. Wada, and K. Shinozaki, "Characterization of the gene for Δ1-pyrroline-5-carboxylate synthetase and correlation between the expression of the gene and salt tolerance in *Oryza sativa* L.," *Plant Molecular Biology*, vol. 33, no. 5, pp. 857–865, 1997.

[20] G. Székely, E. Ábrahám, Á. Cséplo et al., "Duplicated *P5CS* genes of arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis," *Plant Journal*, vol. 53, no. 1, pp. 11–28, 2008.

[21] P. Armengaud, L. Thiery, N. Buhot, G. G.-D. March, and A. Savouré, "Transcriptional regulation of proline biosynthesis in Medicago truncatula reveals developmental and environmental specific features," *Physiologia Plantarum*, vol. 120, no. 3, pp. 442–450, 2004.

[22] Z. Xin and J. Browse, "*eskimo1* mutants of Arabidopsis are constitutively freezing-tolerant," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7799–7804, 1998.

[23] W.-Y. Liu, M.-M. Wang, J. Huang, H.-J. Tang, H.-X. Lan, and H.-S. Zhang, "The *OsDHODH1* gene is involved in salt and drought tolerance in rice," *Journal of Integrative Plant Biology*, vol. 51, no. 9, pp. 825–833, 2009.

[24] W. J. Fan, M. Zhang, H. X. Zhang, and P. Zhang, "Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betaine aldehyde dehydrogenase," *PLoS One*, vol. 7, no. 5, Article ID e37344, 2012.

[25] R. A. J. Hodgson and J. K. Raison, "Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light," *Planta*, vol. 185, no. 2, pp. 215–219, 1991.

[26] D. M. Hodges, J. M. DeLong, C. F. Forney, and R. K. Prange, "Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds," *Planta*, vol. 207, no. 4, pp. 604–611, 1999.

[27] R. Mittler, "Oxidative stress, antioxidants and stress tolerance," *Trends in Plant Science*, vol. 7, no. 9, pp. 405–410, 2002.

[28] N. Suzuki and R. Mittler, "Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction," *Physiologia Plantarum*, vol. 126, no. 1, pp. 45–51, 2006.

[29] K. Apel and H. Hirt, "Reactive oxygen species: metabolism, oxidative stress, and signal transduction," *Annual Review of Plant Biology*, vol. 55, pp. 373–399, 2004.

[30] J. Krasensky and C. Jonak, "Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks," *Journal of Experimental Botany*, vol. 63, no. 4, pp. 1593–1608, 2012.

[31] G. Miller, N. Suzuki, S. Ciftci-Yilmaz, and R. Mittler, "Reactive oxygen species homeostasis and signalling during drought and salinity stresses," *Plant, Cell and Environment*, vol. 33, no. 4, pp. 453–467, 2010.

[32] R. G. Alscher, N. Erturk, and L. S. Heath, "Role of superoxide dismutases (SODs) in controlling oxidative stress in plants," *Journal of Experimental Botany*, vol. 53, no. 372, pp. 1331–1341, 2002.

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*Review Article*

# Genomics Approaches for Crop Improvement against Abiotic Stress

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As sessile organisms, plants are inevitably exposed to one or a combination of stress factors every now and then throughout their growth and development. Stress responses vary considerably even in the same plant species; stress-susceptible genotypes are at one extreme, and stress-tolerant ones are at the other. Elucidation of the stress responses of crop plants is of extreme relevance, considering the central role of crops in food and biofuel production. Crop improvement has been a traditional issue to increase yields and enhance stress tolerance; however, crop improvement against abiotic stresses has been particularly compelling, given the complex nature of these stresses. As traditional strategies for crop improvement approach their limits, the era of genomics research has arisen with new and promising perspectives in breeding improved varieties against abiotic stresses.

## 1. Introduction

Abiotic stresses are the most significant causes of yield losses in plants, implicated to reduce yields by as much as 50% [1]. Among abiotic stresses, drought is the most prominent and widespread; consequently the drought stress response has been dissected into its components and extensively studied in order to understand tolerance mechanisms thoroughly [2]. To improve abiotic stress, particularly drought, tolerance of cereals is of extreme importance, as cereals, including wheat and barley, are the main constituents of the world food supply. However, many abiotic stresses are complex in nature, controlled by networks of genetic and environmental factors that hamper breeding strategies [3]. As traditional approaches for crop improvement reach their limits, agriculture has to adopt novel approaches to meet the demands of an ever-growing world population.

Recent technological advances and the aforementioned agricultural challenges have led to the emergence of high-throughput tools to explore and exploit plant genomes for crop improvement. These genomics-based approaches aim to decipher the entire genome, including genic and intergenic regions, to gain insights into plant molecular responses which will in turn provide specific strategies for crop improvement. In this paper, genomics approaches for crop improvement against abiotic stresses will be discussed under three generalized classes, functional, structural, and comparative genomics. However, it should be noted that genomics approaches are highly intermingled, in terms of both the methodologies and the outcome (Figure 1).

## 2. Functional Genomics

Genomics research is frequently realized by functional studies, which produce perhaps the most readily applicable information for crop improvement. Functional genomics techniques have long been adopted to unravel gene functions and the interactions between genes in regulatory networks, which can be exploited to generate improved varieties. Functional genomics approaches predominantly employ sequence or hybridization based methodologies which are discussed below.

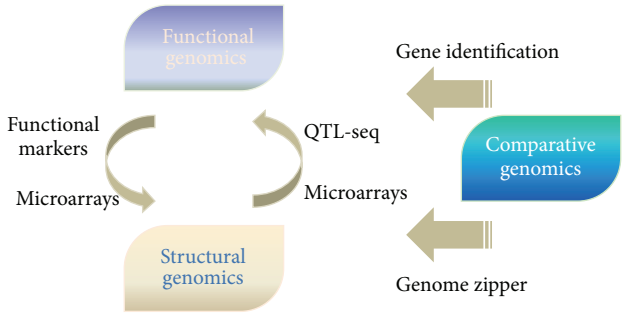


Figure 1: Functional, structural, and comparative genomics approaches are highly interrelated. For example, microarrays can be used either to anchor markers to genome maps or to analyze gene expression; functional markers indicate both phenotypes and genetic locations; QTL-seq utilizes a reference genome sequence to isolate QTLs based on phenotypic variation. As more structural genomics information becomes available, comparative genomics tools such as genome zippers can be used both to elucidate the structure of unsequenced genomes and as a shortcut to design targeted functional studies.

*2.1. Sequencing-Based Approaches.* One way to explore the expressed gene catalogue of a species is to analyze Expressed Sequence Tags (ESTs). ESTs are partial genic sequences that are generated by single-pass sequencing of cDNA clones [4]. Despite the concerns over the quality of ESTs as well as the representation of the parental cDNA [5], ESTs have been shown to identify corresponding genes unambiguously in a rapid and cost-effective fashion [4]; therefore, ESTs have been a major focus on functional studies.

Large-scale EST sequencing has been one of the earliest strategies for gene discovery and genome annotation [5, 6]. Currently, over a million ESTs are deposited in the EST database at National Center for Biotechnological Information (NCBI) for important crops such as maize, soybean, wheat, and rice, along with several thousands of ESTs for other plants (http://www.ncbi.nlm.nih.gov/dbEST/). cDNA libraries from various tissues, developmental stages, or treatments generally serve as the sources for EST sequencing to reveal differentially expressed genes [7]. These approaches can successfully identify tissue or developmental stage-specific and treatment-responsive transcripts. However, such cDNA libraries may underrepresent rare transcripts or transcripts that are not expressed under certain conditions. In addition, ESTs are usually much shorter in length than the cDNAs from which they are obtained. Assembly of overlapping EST sequences into consensus contigs is likely to be more informative on the structure of the parental cDNA, which may reveal polymorphisms. However, assembly and interpretation must be handled cautiously, as paralogous genes may lead to misassemblies of sequences, particularly in polyploid species such as wheat [5]. EST sequencing is utilized extensively in the absence of whole genome sequences, particularly in crops with large and repetitive genomes, although the entire transcriptome is unlikely to be fully represented and resolved. Even so, EST sequencing is still a valid approach, and a recent study has demonstrated its potential in gene discovery via the comparison of different genotypes under control and stress conditions [8].

An alternative approach, Serial Analysis of Gene Expression (SAGE), has been developed to quantitate the abundance of thousands of transcripts simultaneously. In this approach, short sequence tags from transcripts are concatenated and sequenced, giving an absolute measure of gene expression [9, 10]. The ability of these short tags to identify genes unambiguously depends on the existence of comprehensive EST databases for the respective species [11]. Although SAGE is not widely applied in plants, there are a number of examples, including modifications to the original methodology, such as SuperSAGE and DeepSAGE [12–17]. The first report of SAGE in plants not only identified novel genes but also implied novel functions for known genes in rice seedlings [12]. SAGE has also been used to investigate stress-responsive genes [12, 15]. A similar tag-based approach, Massively Parallel Signature Sequencing (MPSS), where longer sequence tags are ligated to microbeads and sequenced in parallel, enables analysis of millions of transcripts simultaneously [18]. Due to longer tags and high-throughput analysis, MPSS is likely to identify genes with greater specificity and sensitivity. The ability of MPSS to capture rare transcripts is particularly beneficial in species that lack a whole genome sequence [19]. In plants, besides mRNA transcripts, MPSS has been employed in the expression studies of small RNAs [20, 21], which are increasingly implicated in abiotic stress responses [22]. Currently, plant MPSS databases (http://mpss.udel.edu/) contain publicly available MPSS expression data for a number of plant species, including important crops such as rice, maize, and soybean [23]. These MPSS data can be extracted, compiled, and compared with newly generated MPSS data for functional analysis of gene expression, as demonstrated by Jain et al. [24].

*2.2. Hybridization-Based Approaches.* In contrast to sequence-based approaches, array-based techniques utilize hybridization of the target DNA with cDNA or oligonucleotide probes attached to a surface to assess expression [25, 26]. These array-based methods are targeted; that is, prior knowledge of the transcript to be analyzed, either sequence or clone, is a prerequisite to design probes [27]. Extensive microarray expression data exists for *Arabidopsis thaliana* and rice [28–31], model species with fully sequenced genomes. In addition, microarray studies have been widely employed in crop species such as wheat [32], barley [33], and maize [34], as well as less emphasized but still industrially and agriculturally important plant species, such as cotton [35], cassava [36], and tomato [37] to unravel stress responses.

Besides inherent limitations such as cross-hybridization and background noise, microarray studies investigating stress-responsive genes suffer from technical considerations that may limit their usefulness. Isolation of total RNA from complex tissues that are composed of different types of cells may obscure transcript changes occurring in cell types that are particularly relevant to the stress response. Subtle transcriptional changes may be diluted in the overall stress response of the whole tissue and, thus, remain unnoticed.

Similarly, the choice of tissue or genotype that is sampled in a microarray study is closely related to the relevance of results. Reproductive tissues and stress-tolerant genotypes are most relevant in terms of agricultural gain and stress adaptation mechanisms, respectively [38]. In addition, laboratory-based stress treatments rarely represent field conditions, where multiple stresses usually act together. Interestingly, a comparison of microarray studies carried out using different water deficit stress conditions revealed only a small number of commonly regulated genes [39]. Abiotic stresses are generally complex in nature, eliciting intricate mechanisms of responses in plants. Consequently, slight differences in the experimental application of stress conditions may produce significant differences in stress responses. A further caveat when interpreting microarray studies is that many transcripts are known to undergo posttranscriptional and posttranslational modifications, which results in uncorrelated transcriptomic and proteomic data in some cases.

For species with an available whole genome sequence, a successful expansion of array-based transcript profiling is whole genome tiling arrays [27]. Tiling arrays can identify novel transcriptional units on chromosomes and alternative splice sites and can map transcripts and methylation sites [40, 41]. Tiling arrays have already been applied in model species to investigate abiotic stress responses [42–44].

*2.3. Expansions to Functional Genomics Approaches.* Genome wide expression profiles are most useful in the detection of candidate genes for desired traits, such as stress tolerance. A fraction of functional studies then adopt inactivation or overexpression of such candidate genes for further characterization and utilization. Of these, Targeting Induced Local Lesions IN Genomes (TILLING) enables high-throughput analysis of large number of mutants [45]. TILLING is applicable to virtually all genes in all species where mutations can be induced and has been reported in several crop species, including hexaploid wheat [46]. TILLING mutants are reported in sorghum [47], maize [48], barley [49], soybean [50], rice [51], and other crops. Although TILLING populations are conventionally screened by phenotypic or genotypic variations, further use of certain TILLING mutants in elucidation of stress responses has been demonstrated. In such a study, TILLING mutants for a specific kinase were used to assess salt stress response in legume species [52].

Importantly, a modified strategy, called EcoTILLING, has been developed to identify natural polymorphisms, analogous to TILLING-assisted identification of induced mutations. Polymorphisms demonstrating natural variation in germplasms are valuable tools in genetic mapping. Furthermore, via the discovery of polymorphisms among individuals, EcoTILLING is able to implicate favorable haplotypes for further analyses, such as sequencing. Similar to TILLING, EcoTILLING is applicable to polyploid species, where it can be utilized to differentiate between alleles of homologous and paralogous genes [53]. In a recent study, EcoTILLING not only provided allelic variants of a number of genes involved in salt stress response but also emphasized the complex nature of salt stress; salt-tolerant genotypes were revealed to harbor different combinations of favorable alleles indicating the presence of multiple pathways conferring salt stress tolerance [54]. Transcription factors, diversifying stress responses, have also been targeted via EcoTILLING to examine natural rice variants exposed to drought stress [55].

The availability of comprehensive EST databases is central to the success of the above-mentioned approaches to identify genes accurately and unambiguously. Besides their utility in genome annotation and expression profiling, ESTs also provide a source of sequences for designing "functional markers." Functional markers refer to polymorphic sites on genes that are attributed to phenotypic variation of traits among individuals of a species. Functional marker design requires the knowledge of the allelic sequences of functionally characterized genes [56]. In contrast to random DNA markers, functional markers are completely linked to the trait of interest; hence, these markers are also called "perfect markers." The use of random DNA markers in breeding studies necessitates validation and revalidation of linkage between the marker and the trait over generations, since genetic recombination may break the linkage [56, 57]. In addition, functional markers may explore natural variation and biodiversity better, particularly compared to random DNA markers with absence/presence polymorphisms, where allelic variations of a trait exceed that of the linked DNA marker. In the case of such random DNA markers, the locus tested during genotyping will only exhibit biallelic variation, whereas the linked gene may actually have more variants [56]. The importance of functional markers has been highlighted in stress tolerance studies as well [58, 59].

## 3. Structural Genomics

While functional genomics focus on the functions of genes and gene networks, structural genomics focus on the physical structure of the genome, aiming to identify, locate, and order genomic features along chromosomes. Together, structural genomics and functional genomics can characterize a genome to its full extent.

*3.1. Genome Sequencing and Mapping.* In the last decade, advances in DNA sequencing technologies have enabled the generation of a wealth of sequence information including whole genome sequences. Next-generation sequencing (NGS) platforms such as Roche 454 GS FLX Titanium (http://www.454.com/) or Illumina Solexa Genome Analyzer (http://www.illumina.com/) can carry out high capacity sequencing at reduced costs and increased rates compared to conventional Sanger sequencing [60]. These advances have paved the way for the exploitation of plant genomics studies for breeding improved varieties. Through NGS technologies, sequencing and resequencing of even large genomes have become feasible. Accordingly, reference or draft genome sequences for a number of species, including the model species *Arabidopsis thaliana* and *Brachypodium distachyon*, along with important crop species such as rice, sorghum, soybean, and maize, have been published [61]. Whole genome sequences provide remarkably detailed information

on genomic features including coding and noncoding genes, regulatory sequences, repetitive elements, and GC content which can be exploited in functional studies such as microarray or tiling arrays [41]. A high-quality reference genome sequence is considered pivotal to crop improvement via molecular breeding, particularly for complex traits. Despite their usefulness, producing such reference genomes requires a major investment of resources, and currently they are only available for species with relatively small genomes of low repetitive content [61].

*Triticeae* genomics, including that of the staple crops barley and wheat, has lagged behind recent advances primarily due to their large and complex genomes (~5 Gb for barley and ~17 Gb for wheat) [62]. As pointed out by Morrell et al. [61], 25x coverage sequencing of *Drosophila* is equivalent to approximately 1x coverage of wheat genome in terms of sequence read counts, demonstrating the challenging genome size of wheat. The high content of repetitive elements is another major challenge, causing ambiguities in sequence assembly. In polyploid species such as wheat, the sequence assembly problem is further exacerbated due to the presence of homoeologous genomes and paralogous loci [61]. For such genomes, construction of a reference sequence has been considered unattainable until recently.

Over the last few years, advances in chromosome sorting technologies have enabled construction of chromosome-specific Bacterial Artificial Chromosome (BAC) libraries to tackle the challenges of complex genomes. Physical mapping of the 1 Gb chromosome 3B of hexaploid wheat has proven the feasibility of a chromosome-by-chromosome approach to explore and exploit complex genomes [63]. Physical maps not only compile genetic mapping data into physical contigs but also serve as scaffolds for sequence assembly into a reference genome. The physical mapping and reference genome sequencing of wheat and barley are ongoing with combined efforts from a number of consortia [62].

In the absence of reference genome sequences, whole genome or BAC-end shotgun sequences provide valuable insights into genome structure and evolution [64–69]. Intriguingly, whole genome shotgun sequences have also been proposed for Quantitative Trait Loci (QTL) detection via a very recently developed methodology named QTL-seq. In this method, extremes of a population exhibiting a normal distribution with respect to a trait of interest are bulked, sequenced, and compared to detect putative QTLs [70].

*3.2. Molecular Markers.* Genomics applications involving molecular markers are largely dominated by Single Nucleotide Polymorphisms (SNPs) [71] as reflected in the predominance of software related to SNP discovery [60]. The high abundance of SNPs in genomes is particularly beneficial for their use in genomics. SNPs are readily identified by genome or transcript resequencing and by comparison of different genotypes in species where reference genome sequences or extensive transcript databases are available. Transcriptome resequencing not only avoids repetitive sequences of complex genome but also identifies SNPs within transcripts that may serve as functional markers [72]. However, due to low-quality sequences obtained by most NGS platforms, over-sampling may be required to differentiate SNPs from sequencing errors [71]. In addition, the presence of homoeologous and paralogous loci must be taken into account in SNP identification in polyploid species [72]. Despite the challenges of SNP discovery on the repetitive portion of genomes, efforts are underway to improve SNP identification even in gene-poor regions [73]. In fact, these regions are of functional importance as well; for example, an important vernalization gene *Vrn-D4* has recently been mapped to the centromeric region of chromosome 5D of hexaploid wheat [62, 74].

A recently developed molecular marker type, Insertion Site-Based Polymorphisms (ISBPs), utilizes the insertional polymorphisms observed in the repeat junctions of complex genomes [75]. ISBP markers are readily designed from low coverage shotgun sequences, such as BAC-end sequences [64, 69]. Typically, 50–60% of ISBP markers tested are specific for the locus from which they were designed, and in one study which these ~70% contained SNPs in at least some members of a panel of 14 wheat genotypes [75]. This approach may break the ground for genome saturation particularly for crops with highly repetitive genomes that are impractical to exploit otherwise.

*3.3. Applications of Structural Genomics in Crop Improvement.* A major impact of NGS-mediated shotgun sequences has been their substantial contribution to the development of molecular markers. These markers indicate diagnostic polymorphisms at the DNA sequence level, and in contrast to morphological markers which once had been the focus of traditional breeding studies, they are not affected by the environment [76]. In general terms, Marker-Assisted Selection (MAS) refers to the utilization of molecular markers in breeding improved varieties with respect to desired traits, such pathogen resistance, abiotic stress tolerance, or high yield [77]. Through MAS, phenotype can be predicted from genotype [71]. For efficient and accurate MAS, the trait of interest should be tightly linked to a molecular marker [78] or more preferably flanked by two close markers. Recombination between both flanking markers and the trait is less likely to occur compared to a single marker, due to the low frequency of double crossovers. In both cases, a genetic distance of less than 5 cM for each marker from the trait is crucial to the success of MAS [77].

Additionally, for efficient MAS, markers should be highly polymorphic in the germplasm used for breeding. MAS can make use of molecular markers at multiple levels. Plant breeding depends on genetic diversity to improve crops [57]. Molecular markers may aid in the exploration of the variation among the germplasm to select the best candidate parental lines. Similarly, molecular markers may identify heterotic groups or ensure genomic purity of cultivars to achieve heterosis. In addition, molecular markers also assist in backcrossing. Plant breeding conventionally involves several backcrossing steps to enable transfer of one or a few traits to an elite cultivar while retaining most of the recurrent genomes. In general, at least six rounds of backcrossing are required to achieve the desired homozygosity, particularly

for the selection of traits with low heritability. In contrast, MAS can greatly accelerate this process by utilizing both the flanking markers linked to the trait for selecting the trait and a set of unlinked markers for tracking the recurrent genome. Flanking markers and selection for recombination also reduces "linkage drag," which is the reduction in crop performance due to the cotransfer of undesirable traits that are located in the vicinity of the trait of interest [77]. Typically, a conventional QTL analysis can provide a resolution of approximately 15 cM intervals which may contain hundreds of genes [79]. The availability of a saturated map can potentially reduce this interval to less than 1 cM by backcrossing [78]. Furthermore, MAS enables early selection of traits that are labor and/or cost-intensive to score phenotypically, that are under complex genetic control, or that are manifested late in development. In cases where genotyping by MAS is affordable, this dramatically reduces the number of the plants to be screened in further steps [77, 78].

The major drawbacks of MAS in breeding are high costs of implementation, typically requiring specialized equipment, and the risk of recombination between the marker and the trait that reduces the reliability of MAS to predict phenotype via genotype. The high cost of MAS is particularly relevant in cases where an effective phenotyping method is already established through conventional breeding. Additionally, MAS usually requires the validation of QTLs when applied in different genetic backgrounds. Functional markers, however, may overcome the issue of QTL validation [78]. Despite its drawbacks, MAS has been successfully utilized to improve crops for abiotic stress tolerance, including drought [80], salinity [81], and waterlogging [82] given that the genetic element responsible for the high tolerance is accurately defined and delineated.

Another use of molecular markers is Map-Based Cloning (MBC) where the gene or a QTL linked to a desired trait is isolated via a "mini" physical map. Such a local physical map flanking the gene must be saturated with molecular markers for efficient MBC [6]. Prior to the construction of high-density physical maps, MBC approaches were inefficient, particularly due to the difficulty of finding unique probes in repetitive sequences for chromosome walking. Importantly, repeat contents of barley and wheat genomes, two staple crops, are estimated to exceed 80% of the whole genome [83, 84], potentiating the utility of physical maps. Accordingly, the physical map of chromosome 3B provided sufficient data to enable fine mapping of 16 genes and QTLs in chromosome 3B, none of which had been previously cloned [64].

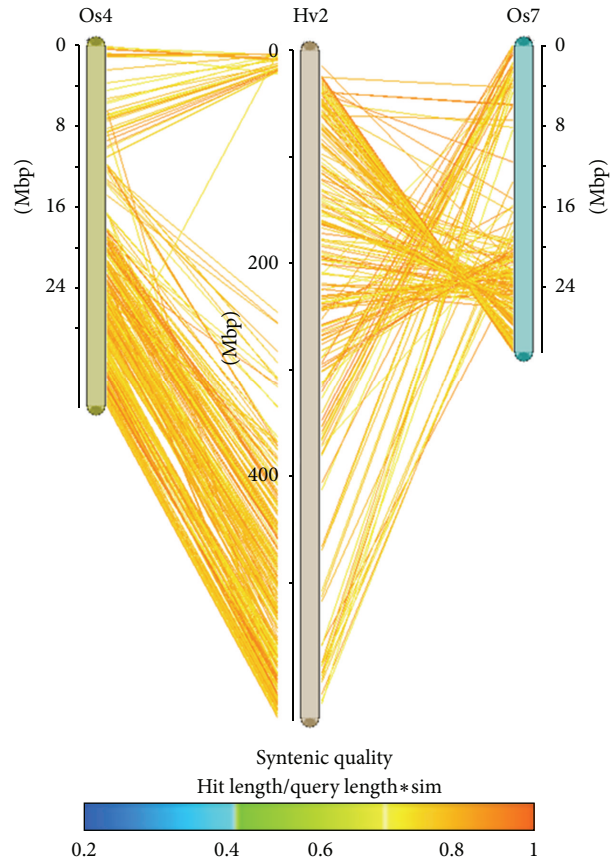


Figure 2: Example of colinearity between grass genomes. Analysis of conserved gene sequences between barley (*H. vulgare*) and rice (*O. sativa*) shows that many genes are found in colinear (syntenic) order. In this example, chromosome 2 of barley (Hv2, centre) is compared with chromosomes 4 and 7 of rice (Os4 and Os7). Each colored line represents a gene conserved between the two chromosomes, with the color indicating the strength of the syntenic relationship. It is clear that many genes from both ends of Hv2 are colinear with the ends of Os4, while the centre of the chromosome is largely colinear with Os7, but in the reverse order. Image was generated using the CrowsNest Comparative Map Viewer at MIPS (http://mips.helmholtz-muenchen.de/plant/genomes.jsp).

## 4. Comparative Genomics

For species with largely unexplored genomes, comparative genomics is a promising tool to gain information by utilizing the conservation between closely related plant species. In fact, plant genomes share extensive similarities even between distantly related species (Figure 2, [85]). Among the plant kingdom, grasses have been the focus of comparative genomics analyses due to their high agronomic importance. The extent of genome conservation first became evident by comparative genome mapping studies, which suggested a colinear order of genes and markers shared by genomes of different species. It is noteworthy that plant genomes differ by several orders of magnitude in size; yet these differences generally correspond to intergenic regions. Although detailed analyses have revealed notable rearrangements such as inversions, deletions, and translocations at the molecular level, large-scale colinearity across grass genomes has been exploited for gene discovery and isolation [86–88].

Comparative genomics has contributed significantly to the emergence of the "genome zipper" concept, which enables the determination of a virtual gene order in a partially sequenced genome. Genome zippers compare the fully sequenced and annotated genomes of *Brachypodium*, sorghum, and rice with various sources of data from less well-studied species, such as genomic survey sequences and

genetically mapped markers, to predict the gene order and organization in these species [65, 89]. These genome zippers indicate evolutionary relationships and medium-scale rearrangements, and for the *Triticeae* provide the closest approximation to a reference genome sequence currently available [65]. However, its reliance on synteny means that recently evolved genes and small-scale rearrangements cannot be explored by this approach.

In addition to syntenic genes that are found in colinear blocks of conserved genes, nonsyntenic genes that are found outside their syntenic location in other genomes also provide valuable insight into genome evolution and speciation. Intriguingly, a recent study focused on the nonconserved portion of wheat and barley genome that suggested novel mechanisms, besides transposable element-driven expansion, have driven the evolution and size of these genomes. Many of these non-syntenic genes exhibited pseudogene characteristics, which may have implications for gene content estimate of these large genomes based on survey sequences [90].

Despite the utility of comparative genomics and genome zippers, it is evident that species-specific genomic features can only be accessed through a fully annotated reference genome sequences. Homoeologous genes with different orthologous relationships are examples of such species-specific features [91]. Species-specific rearrangements are also implicated in the formation of gene islands, containing mainly non-syntenic genes, in large crop genomes [92]. Through comparative genomics, Mayer et al. [65] concluded that genomic models can represent the barley genome to a limited extent. Thus, it can be argued that for maximal exploitation of crop genomes, such as wheat and barley, the construction of reference genome sequences scaffolded by highly-saturated physical and genetic maps is indispensable. Accordingly, efforts to accomplish this goal are currently underway (International Wheat Genome Sequencing Consortium for wheat; International Barley Sequencing Consortium for barley).

## References


[1] F. Qin, K. Shinozaki, and K. Yamaguchi-Shinozaki, "Achievements and challenges in understanding plant abiotic stress responses and tolerance," *Plant and Cell Physiology*, vol. 52, no. 9, pp. 1569–1582, 2011.

[2] M. Kantar, S. J. Lucas, and H. Budak, "Drought stress: molecular genetics and genomics approaches," in *Advances in Botanical Research*, I. Turkan, Ed., pp. 445–493, Elsevier, Burlington, Mass, USA, 2011.

[3] T. R. Sinclair, "Challenges in breeding for yield increase for drought," *Trends in Plant Science*, vol. 16, no. 6, pp. 289–293, 2011.

[4] D. Bouchez and H. Höfte, "Functional genomics in plants," *Plant Physiology*, vol. 118, no. 3, pp. 725–732, 1998.

[5] S. Rudd, "Expressed sequence tags: alternative or complement to whole genome sequences?" *Trends in Plant Science*, vol. 8, no. 7, pp. 321–329, 2003.

[6] R. K. Varshney, D. A. Hoisington, and A. K. Tyagi, "Advances in cereal genomics and applications in crop breeding," *Trends in Biotechnology*, vol. 24, no. 11, pp. 490–499, 2006.

[7] K. Yamamoto and T. Sasaki, "Large-scale EST sequencing in rice," *Plant Molecular Biology*, vol. 35, no. 1-2, pp. 135–144, 1997.

[8] N. Z. Ergen and H. Budak, "Sequencing over 13 000 expressed sequence tags from six subtractive cDNA libraries of wild and modern wheats following slow drought stress," *Plant, Cell and Environment*, vol. 32, no. 3, pp. 220–236, 2009.

[9] V. E. Velculescu, L. Zhang, B. Vogelstein, and K. W. Kinzler, "Serial analysis of gene expression," *Science*, vol. 270, no. 5235, pp. 484–487, 1995.

[10] M. E. Vega-Sánchez, M. Gowda, and G. L. Wang, "Tag-based approaches for deep transcriptome analysis in plants," *Plant Science*, vol. 173, no. 4, pp. 371–380, 2007.

[11] P. Breyne and M. Zabeau, "Genome-wide expression analysis of plant cell cycle modulated genes," *Current Opinion in Plant Biology*, vol. 4, no. 2, pp. 136–142, 2001.

[12] H. Matsumura, S. Nirasawa, and R. Terauchi, "Transcript profiling in rice (*Oryza sativa* L.) seedlings using serial analysis of gene expression (SAGE)," *Plant Journal*, vol. 20, no. 6, pp. 719–726, 1999.

[13] W. W. Lorenz and J. F. D. Dean, "SAGE profiling and demonstration of differential gene expression along the axial developmental gradient of lignifying xylem in loblolly pine (*Pinus taeda*)," *Tree Physiology*, vol. 22, no. 5, pp. 301–310, 2002.

[14] J. G. Gibbings, B. P. Cook, M. R. Dufault et al., "Global transcript analysis of rice leaf and seed using SAGE technology," *Plant Biotechnology Journal*, vol. 1, no. 4, pp. 271–285, 2003.

[15] J. Y. Lee and D. H. Lee, "Use of serial analysis of gene expression technology to reveal changes in gene expression in Arabidopsis pollen undergoing cold stress," *Plant Physiology*, vol. 132, no. 2, pp. 517–529, 2003.

[16] H. Matsumura, S. Reich, A. Ito et al., "Gene expression analysis of plant host-pathogen interactions by SuperSAGE," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15718–15723, 2003.

[17] K. L. Nielsen, A. L. Høgh, and J. Emmersen, "DeepSAGE—digital transcriptomics with high sensitivity, simple experimental protocol and multiplexing of samples," *Nucleic Acids Research*, vol. 34, no. 19, article e133, 2006.

[18] S. Brenner, M. Johnson, J. Bridgham et al., "Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays," *Nature Biotechnology*, vol. 18, no. 6, pp. 630–634, 2000.

[19] J. Reinartz, E. Bruyns, J. Z. Lin et al., "Massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms," *Briefings in Functional Genomics and Proteomics*, vol. 1, no. 1, pp. 95–104, 2002.

[20] B. C. Meyers, F. F. Souret, C. Lu, and P. J. Green, "Sweating the small stuff: microRNA discovery in plants," *Current Opinion in Biotechnology*, vol. 17, no. 2, pp. 139–146, 2006.

[21] K. Nobuta, R. C. Venu, C. Lu et al., "An expression atlas of rice mRNAs and small RNAs," *Nature Biotechnology*, vol. 25, no. 4, pp. 473–477, 2007.

[22] R. Sunkar, V. Chinnusamy, J. Zhu, and J. K. Zhu, "Small RNAs as big players in plant abiotic stress responses and nutrient deprivation," *Trends in Plant Science*, vol. 12, no. 7, pp. 301–309, 2007.

[23] M. Nakano, K. Nobuta, K. Vemaraju, S. S. Tej, J. W. Skogen, and B. C. Meyers, "Plant MPSS databases: signature-based transcriptional resources for analyses of mRNA and small RNA," *Nucleic Acids Research*, vol. 34, pp. D731–D735, 2006.

[24] M. Jain, A. Nijhawan, R. Arora et al., "F-Box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress," *Plant Physiology*, vol. 143, no. 4, pp. 1467–1483, 2007.

[25] M. Schena, D. Shalon, R. W. Davis, and P. O. Brown, "Quantitative monitoring of gene expression patterns with a complementary DNA microarray," *Science*, vol. 270, no. 5235, pp. 467–470, 1995.

[26] D. J. Lockhart, H. Dong, M. C. Byrne et al., "Expression monitoring by hybridization to high-density oligonucleotide arrays," *Nature Biotechnology*, vol. 14, no. 13, pp. 1675–1680, 1996.

[27] W. A. Rensink and C. R. Buell, "Microarray expression profiling resources for plant genomics," *Trends in Plant Science*, vol. 10, no. 12, pp. 603–609, 2005.

[28] P. Zimmermann, M. Hirsch-Hoffmann, L. Hennig, and W. Gruissem, "GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox," *Plant Physiology*, vol. 136, no. 1, pp. 2621–2632, 2004.

[29] D. Wang, Y. Pan, X. Zhao, L. Zhu, B. Fu, and Z. Li, "Genome-wide temporal-spatial gene expression profiling of drought responsiveness in rice," *BMC Genomics*, vol. 12, article 149, 2011.

[30] R. Kumar, A. Mustafiz, K. K. Sahoo et al., "Functional screening of cDNA library from a salt tolerant rice genotype Pokkali identifies mannose-1-phosphate guanyl transferase gene (OsMPG1) as a key member of salinity stress response," *Plant Molecular Biology*, vol. 79, no. 6, pp. 555–568, 2012.

[31] A. Singh, A. Pandey, V. Baranwal, S. Kapoor, and G. K. Pandey, "Comprehensive expression analysis of rice phospholipase D gene family during abiotic stresses and development," *Plant Signaling and Behavior*, vol. 7, no. 7, pp. 847–855, 2012.

[32] N. Z. Ergen, J. Thimmapuram, H. J. Bohnert, and H. Budak, "Transcriptome pathways unique to dehydration tolerant relatives of modern wheat," *Functional and Integrative Genomics*, vol. 9, no. 3, pp. 377–396, 2009.

[33] T. J. Close, S. I. Wanamaker, R. A. Caldo et al., "A new resource for cereal genomics: 22K Barley GeneChip comes of age," *Plant Physiology*, vol. 134, no. 3, pp. 960–968, 2004.

[34] M. Luo, J. Liu, R. D. Lee, B. T. Scully, and B. Guo, "Monitoring the expression of maize genes in developing Kernels under drought stress using oligo-microarray," *Journal of Integrative Plant Biology*, vol. 52, no. 12, pp. 1059–1074, 2010.

[35] A. Ranjan, N. Pandey, D. Lakhwani, N. K. Dubey, U. V. Pathre, and S. V. Sawant, "Comparative transcriptomic analysis of roots of contrasting *Gossypium herbaceum* genotypes revealing adaptation to drought," *BMC Genomics*, vol. 13, article 680, 2012.

[36] Y. Utsumi, M. Tanaka, T. Morosawa et al., "Transcriptome analysis using a high-density oligomicroarray under drought stress in various genotypes of cassava: an important tropical crop," *DNA Research*, vol. 19, no. 4, pp. 335–345, 2012.

[37] R. Loukehaich, T. Wang, B. Ouyang et al., "SpUSP, an annexin-interacting universal stress protein, enhances drought tolerance in tomato," *Journal of Experimental Botany*, vol. 63, no. 15, pp. 5593–5606, 2012.

[38] M. K. Deyholos, "Making the most of drought and salinity transcriptomics," *Plant, Cell and Environment*, vol. 33, no. 4, pp. 648–654, 2010.

[39] E. A. Bray, "Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*," *Journal of Experimental Botany*, vol. 55, no. 407, pp. 2331–2341, 2004.

[40] J. Yazaki, B. D. Gregory, and J. R. Ecker, "Mapping the genome landscape using tiling array technology," *Current Opinion in Plant Biology*, vol. 10, no. 5, pp. 534–542, 2007.

[41] K. Mochida and K. Shinozaki, "Genomics and bioinformatics resources for crop improvement," *Plant and Cell Physiology*, vol. 51, no. 4, pp. 497–523, 2010.

[42] G. Zeller, S. R. Henz, C. K. Widmer et al., "Stress-induced changes in the *Arabidopsis thaliana* transcriptome analyzed using whole-genome tiling arrays," *Plant Journal*, vol. 58, no. 6, pp. 1068–1082, 2009.

[43] A. Matsui, J. Ishida, T. Morosawa et al., "Arabidopsis tiling array analysis to identify the stress-responsive genes," *Methods in Molecular Biology*, vol. 639, pp. 141–155, 2010.

[44] W. Verelst, E. Bertolini, S. de Bodt et al., "Molecular and physiological analysis of growth-limiting drought stress in *Brachypodium distachyon* leaves," *Molecular Plant*, vol. 6, no. 2, pp. 311–322, 2013.

[45] C. M. McCallum, L. Comai, E. A. Greene, and S. Henikoff, "Targeting induced local lesions IN genomes (TILLING) for plant functional genomics," *Plant Physiology*, vol. 123, no. 2, pp. 439–442, 2000.

[46] L. Chen, L. Huang, D. Min et al., "Development and characterization of a new TILLING population of common bread wheat (*Triticum aestivum* L.)," *PLoS ONE*, vol. 7, no. 7, Article ID e41570, 2012.

[47] Z. Xin, M. Li Wang, N. A. Barkley et al., "Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population," *BMC Plant Biology*, vol. 8, article 103, 2008.

[48] B. J. Till, S. H. Reynolds, C. Weil et al., "Discovery of induced point mutations in maize genes by TILLING," *BMC Plant Biology*, vol. 4, article 12, 2004.

[49] D. G. Caldwell, N. McCallum, P. Shaw, G. J. Muehlbauer, D. F. Marshall, and R. Waugh, "A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.)," *Plant Journal*, vol. 40, no. 1, pp. 143–150, 2004.

[50] J. L. Cooper, B. J. Till, R. G. Lapor et al., "TILLING to detect induced mutations in soybean," *BMC Plant Biology*, vol. 8, article 9, 2008.

[51] J. L. Cooper, S. Henikoff, L. Comai, and B. J. Till, "TILLING and ecotilling for rice," *Methods in Molecular Biology*, vol. 956, pp. 39–56, 2013.

[52] L. de Lorenzo, F. Merchan, P. Laporte et al., "A novel plant leucine-rich repeat receptor kinase regulates the response of *Medicago truncatula* roots to salt stress," *Plant Cell*, vol. 21, no. 2, pp. 668–680, 2009.

[53] L. Comai, K. Young, B. J. Till et al., "Efficient discovery of DNA polymorphisms in natural populations by Ecotilling," *Plant Journal*, vol. 37, no. 5, pp. 778–786, 2004.

[54] S. Negrão, M. C. Almadanim, I. S. Pires et al., "New allelic variants found in key rice salt-tolerance genes: an association study," *Plant Biotechnology Journal*, vol. 11, no. 1, pp. 87–100, 2013.

[55] S. Yu, F. Liao, F. Wang et al., "Identification of rice transcription factors associated with drought tolerance using the Ecotilling method," *PLoS ONE*, vol. 7, no. 2, Article ID e30765, 2012.

[56] J. R. Andersen and T. Lübberstedt, "Functional markers in plants," *Trends in Plant Science*, vol. 8, no. 11, pp. 554–560, 2003.

[57] R. K. Varshney, A. Graner, and M. E. Sorrells, "Genomics-assisted breeding for crop improvement," *Trends in Plant Science*, vol. 10, no. 12, pp. 621–630, 2005.

[58] M. Bagge, X. Xia, and T. Lubberstedt, "Functional markers in wheat," *Current Opinion in Plant Biology*, vol. 10, no. 2, pp. 211–216, 2007.

[59] B. Garg, C. Lata, and M. Prasad, "A study of the role of gene *TaMYB2* and an associated SNP in dehydration tolerance in common wheat," *Molecular Biology Reports*, vol. 39, no. 12, pp. 10865–10871, 2012.

[60] R. K. Varshney, S. N. Nayak, G. D. May, and S. A. Jackson, "Next-generation sequencing technologies and their implications for crop genetics and breeding," *Trends in Biotechnology*, vol. 27, no. 9, pp. 522–530, 2009.

[61] P. L. Morrell, E. S. Buckler, and J. Ross-Ibarra, "Crop genomics: advances and applications," *Nature Reviews Genetics*, vol. 13, no. 2, pp. 85–96, 2011.

[62] C. Feuillet, N. Stein, L. Rossini et al., "Integrating cereal genomics to support innovation in the Triticeae," *Functional and Integrative Genomics*, vol. 12, no. 4, pp. 573–583, 2012.

[63] E. Paux, P. Sourdille, J. Salse et al., "A physical map of the 1-gigabase bread wheat chromosome 3B," *Science*, vol. 322, no. 5898, pp. 101–104, 2008.

[64] E. Paux, D. Roger, E. Badaeva et al., "Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B," *Plant Journal*, vol. 48, no. 3, pp. 463–474, 2006.

[65] K. F. X. Mayer, M. Martis, P. E. Hedley et al., "Unlocking the barley genome by chromosomal and comparative genomics," *Plant Cell*, vol. 23, no. 4, pp. 1249–1263, 2011.

[66] N. Vitulo, A. Albiero, C. Forcato et al., "First survey of the wheat chromosome 5A composition through a next generation sequencing approach," *PLoS ONE*, vol. 6, no. 10, Article ID e26421, 2011.

[67] S. Fluch, D. Kopecky, K. Burg et al., "Sequence composition and gene content of the short arm of rye (*Secale cereale*) chromosome 1," *PLoS ONE*, vol. 7, no. 2, Article ID e30784, 2012.

[68] P. Hernandez, M. Martis, G. Dorado et al., "Next-generation sequencing and syntenic integration of flow-sorted arms of wheat chromosome 4A exposes the chromosome structure and gene content," *Plant Journal*, vol. 69, no. 3, pp. 377–386, 2012.

[69] S. J. Lucas, H. Šimková, J. Šafář et al., "Functional features of a single chromosome arm in wheat (1AL) determined from its structure," *Functional and Integrative Genomics*, vol. 12, no. 1, pp. 173–182, 2012.

[70] H. Takagi, A. Abe, K. Yoshida et al., "QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations," *Plant Journal*, vol. 74, no. 1, pp. 174–183, 2013.

[71] D. Edwards and J. Batley, "Plant genome sequencing: applications for crop improvement," *Plant Biotechnology Journal*, vol. 8, no. 1, pp. 2–9, 2010.

[72] J. Mammadov, R. Aggarwal, R. Buyyarapu, and S. Kumpatla, "SNP markers and their impact on plant breeding," *International Journal of Plant Genomics*, vol. 2012, Article ID 728398, 11 pages, 2012.

[73] D. F. Simola and J. Kim, "Sniper: improved SNP discovery by multiply mapping deep sequenced reads," *Genome Biology*, vol. 12, no. 6, article 55, 2011.

[74] T. Yoshida, H. Nishida, J. Zhu et al., "Vrn-D4 is a vernalization gene located on the centromeric region of chromosome 5D in hexaploid wheat," *Theoretical and Applied Genetics*, vol. 120, no. 3, pp. 543–552, 2010.

[75] E. Paux, S. Faure, F. Choulet et al., "Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat," *Plant Biotechnology Journal*, vol. 8, no. 2, pp. 196–210, 2010.

[76] M. Mohan, S. Nair, A. Bhagwat et al., "Genome mapping, molecular markers and marker-assisted selection in crop plants," *Molecular Breeding*, vol. 3, no. 2, pp. 87–103, 1997.

[77] B. C. Y. Collard and D. J. Mackill, "Marker-assisted selection: an approach for precision plant breeding in the twenty-first century," *Philosophical Transactions of the Royal Society B*, vol. 363, no. 1491, pp. 557–572, 2008.

[78] G. O. Edmeades, G. S. McMaster, J. W. White, and H. Campos, "Genomics and the physiologist: bridging the gap between genes and crop response," *Field Crops Research*, vol. 90, no. 1, pp. 5–18, 2004.

[79] R. Tuberosa, S. Salvi, M. C. Sanguineti, P. Landi, M. Maccaferri, and S. Conti, "Mapping QTLS regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize," *Annals of Botany*, vol. 89, pp. 941–963, 2002.

[80] M. Ashraf, "Inducing drought tolerance in plants: recent advances," *Biotechnology Advances*, vol. 28, no. 1, pp. 169–183, 2010.

[81] T. Yamaguchi and E. Blumwald, "Developing salt-tolerant crop plants: challenges and opportunities," *Trends in Plant Science*, vol. 10, no. 12, pp. 615–620, 2005.

[82] F. Ahmed, M. Y. Rafii, M. R. Ismail et al., "Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects," *BioMed Research International*, vol. 2013, Article ID 963525, 10 pages, 2013.

[83] T. Wicker, S. Taudien, A. Houben et al., "A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley," *Plant Journal*, vol. 59, no. 5, pp. 712–722, 2009.

[84] F. Choulet, T. Wicker, C. Rustenholz et al., "Megabase level sequencing reveals contrasted organization and evolution patterns of the wheat gene and transposable element spaces," *Plant Cell*, vol. 22, no. 6, pp. 1686–1701, 2010.

[85] R. Guyot, F. Lefebvre-Pautigny, C. Tranchant-Dubreuil et al., "Ancestral synteny shared between distantly-related plant species from the asterid (*Coffea canephora* and *Solanum* Sp.) and rosid (*Vitis vinifera*) clades," *BMC Genomics*, vol. 13, article 103, 2012.

[86] C. Feuillet and B. Keller, "Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution," *Annals of Botany*, vol. 89, no. 1, pp. 3–10, 2002.

[87] B. Keller and C. Feuillet, "Colinearity and gene density in grass genomes," *Trends in Plant Science*, vol. 5, no. 6, pp. 246–251, 2000.

[88] F. Li, C. Ma, Q. Chen et al., "Comparative mapping reveals similar linkage of functional genes to QTL of yield-related traits between *Brassica napus* and *Oryza sativa*," *Journal of Genetics*, vol. 91, no. 2, pp. 163–170, 2012.

[89] K. F. X. Mayer, S. Taudien, M. Martis et al., "Gene content and virtual gene order of barley chromosome 1H," *Plant Physiology*, vol. 151, no. 2, pp. 496–505, 2009.

[90] T. Wicker, K. F. X. Mayer, H. Gundlach et al., "Frequent gene movement and pseudogene evolution is common to the large and complex genomes of wheat, barley, and their relatives," *Plant Cell*, vol. 23, no. 5, pp. 1706–1718, 2011.

[91] C. Rustenholz, P. E. Hedley, J. Morris et al., "Specific patterns of gene space organisation revealed in wheat by using the combination of barley and wheat genomic resources," *BMC Genomics*, vol. 11, no. 1, article 714, 2010.

[92] C. Rustenholz, F. Choulet, C. Laugier et al., "A 3,000-loci transcription map of chromosome 3B unravels the structural and functional features of gene islands in hexaploid wheat," *Plant Physiology*, vol. 157, no. 4, pp. 1596–1608, 2011.

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*Review Article*

# Drought Tolerance in Modern and Wild Wheat

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The genus *Triticum* includes bread (*Triticum aestivum*) and durum wheat (*Triticum durum*) and constitutes a major source for human food consumption. Drought is currently the leading threat on world's food supply, limiting crop yield, and is complicated since drought tolerance is a quantitative trait with a complex phenotype affected by the plant's developmental stage. Drought tolerance is crucial to stabilize and increase food production since domestication has limited the genetic diversity of crops including wild wheat, leading to cultivated species, adapted to artificial environments, and lost tolerance to drought stress. Improvement for drought tolerance can be achieved by the introduction of drought-grelated genes and QTLs to modern wheat cultivars. Therefore, identification of candidate molecules or loci involved in drought tolerance is necessary, which is undertaken by "omics" studies and QTL mapping. In this sense, wild counterparts of modern varieties, specifically wild emmer wheat (*T. dicoccoides*), which are highly tolerant to drought, hold a great potential. Prior to their introgression to modern wheat cultivars, drought related candidate genes are first characterized at the molecular level, and their function is confirmed via transgenic studies. After integration of the tolerance loci, specific environment targeted field trials are performed coupled with extensive analysis of morphological and physiological characteristics of developed cultivars, to assess their performance under drought conditions and their possible contributions to yield in certain regions. This paper focuses on recent advances on drought related gene/QTL identification, studies on drought related molecular pathways, and current efforts on improvement of wheat cultivars for drought tolerance.

## 1. Introduction

Current climate change is projected to have a significant impact on temperature and precipitation profiles, increasing the incidence and severity of drought. Drought is the single largest abiotic stress factor leading to reduced crop yields, so high-yielding crops even in environmentally stressful conditions are essential [1, 2]. This is not the first time we face this situation, in which increasing demands on existing resources are not feasible, and higher-yielding crops are required to balance crop production with increasing human food consumption. A similar scenario occurred 50 years ago due to the high rate of population growth, and it was overcome by selective breeding of high grain yielding semidwarf mutants of wheat, a process coined Green Revolution [3]. In relation to current development of cultivars, which are higher yielding even in water-limited environments, one of the major targets is *Triticum* species, being one of the leading human food source, accounting for more than half of total human consumption [2, 4].

The increasing incidence and importance of drought in relation to crop production has rendered it as a major focus of research for several decades. However, studying drought response is challenged by the complex and quantitative nature of the trait. Drought tolerance is complicated with environmental interactions. In the analysis of a plant's drought response, the mode, timing, and severity of the dehydration stress and its occurrence with other abiotic and biotic stress factors are significant [5]. Furthermore different species, subspecies, and cultivars of crops show variation in their drought tolerance under same conditions, emphasizing the importance of genetic diversity as an underlying factor of drought and its significance in drought-related research. Plants exhibiting high drought tolerance are the most suitable targets of drought-related research and are the most promising sources of drought-related gene and gene regions

to be used in the improvement of modern crop varieties. These include the natural progenitors of cultivated crops, and for wheat improvement, *Ae. tauschii,* which is more drought tolerant than *Triticum* and wild emmer wheat (*T. dicoccoides*), which harbors drought tolerance characteristics, lost during cultivation of modern lines, is of great importance [6].

Although development of higher-yielding crops under water-limited environments is the most viable solution to stabilizing and increasing wheat production under current climatic conditions, it is challenged by the nature of drought response as a trait and the complex genomic constitution of wheat [16]. However, recently, the utilization of drought tolerant wild species and the rapid advances in molecular biological, functional genomics, and transgenics technologies have facilitated drought-related studies, resulting in significant progress in the identification of related genes and gene regions and dissection of some of its molecular aspects. This paper summarizes the current state of drought-related research in *Triticum* species, focusing on the identification and functional characterization of drought-related molecules, analysis of their interactions in the complex network of drought response, and applications of these data to improve wheat cultivars utilizing molecular based-technologies.

## 2. *T. dicoccoides* and Drought Tolerance

Wild emmer wheat (*T. turgidum* ssp. *dicoccoides* (körn.) Thell) is the tetraploid ($2n = 4x = 28$; genome BBAA) progenitor of both domesticated tetraploid durum wheat (*T. turgidum* ssp. *durum* (Desf.) MacKey) and hexaploid ($2n = 6x = 42$; BBAADD) bread wheat (*T. aestivum* L.). It is thought to have originated and diversified in the Near East Fertile Crescent region through adaptation to a spectrum of ecological conditions. It is genetically compatible with durum wheat (*T. turgidum* ssp. *durum*) and can be crossed with bread wheat (*T. aestivum* L.) [17]. Wild emmer germplasm harbors a rich allelic pool, exhibiting a high level of genetic diversity, showing correlation with environmental factors, reported by population-wide analysis of allozyme and DNA marker variations [18–24].

Wild emmer wheat is important for its high drought tolerance, and some of *T. dicoccoides* genotypes are fully fertile in arid desert environments. Wild emmer wheat accessions were shown to thrive better under water-limited conditions in terms of their productivity and stability, compared to durum wheat. The wild emmer gene pool was shown to offer a rich allelic repertoire of agronomically important traits including drought tolerance [23, 25–28]. Hence, *T. dicoccoides* is an important source of drought-related genes and highly suitable as a donor for improving drought tolerance in cultivated wheat species.

Wild emmer wheat, being a potential reservoir of drought-related research, has been the source of several identified candidate drought-related genes with the development of "omics" approaches in the recent decades. In recent years, transcript profiling of leaf and root tissues from two *T. dicoccoides* genotypes, originating from Turkey, TR39477 (tolerant variety), TTD-22 (sensitive variety), was performed by our group, in two separate studies, utilizing different methodologies. In one report, subtractive cDNA libraries were constructed from slow dehydration stressed plants, and over 13,000 ESTs were sequenced. In another study, Affymetrix GeneChip Wheat Genome Array was used to profile expression in response to shock drought stress [1, 29]. Wild emmer wheat was shown to be capable of engaging in known drought responsive mechanisms, harboring elements present in modern wheat varieties and also in other crop species. Additionally several genes or expression patterns, unique to tolerant wild emmer wheat, indicative of its distinctive ability to tolerate water deficiency, were also revealed. Transcript and metabolite profiling studies were also undertaken for two *T. dicoccoides* genotypes, originating from Israel, Y12-3 (tolerant variety) and A24-39 (sensitive variety), under drought stress and nonstress conditions. Leaf transcript profiling indicated differential multilevel regulation among cultivars and conditions [30]. Integration of root transcript and metabolite profiling data emphasized drought adaptation through regulation of energy related processes involving carbon metabolism and cell homeostasis (Table 1) [14]. Recently, in wild emmer wheat, our group also profiled drought induced expression of microRNA (miRNAs), small regulatory molecules known to be involved in several cellular processes including stress responses. In this study, leaf and root tissues of resistant wild emmer wheat varieties, TR39477 and TR38828, were screened via a microarray platform, and 13 differentially expressed miRNAs were found to be differentially expressed in response to drought (Table 1) [15].

Following the identification of *T. dicoccoides* drought-related gene candidates, as discussed previously, a number of these potential drought resistant genes were cloned and further characterized. In one of the recent reports, TdicTMPIT1 (integral transmembrane protein inducible by Tumor Necrosis Factor-$\alpha$, TNF-$\alpha$) was cloned from wild emmer root tissue and shown to be a membrane protein, associated with the drought stress response, exhibiting increased levels of expression, specifically in wild emmer wheat upon osmotic stress [31]. In a different study, TdicDRF1 (DRE binding factor 1), conserved between crop species, was cloned for the first time from wild emmer wheat. Its DNA binding domain, AP2/ERF (APETALA2/ethylene-responsive element binding factor), was shown to bind to drought responsive element (DRE), using an electrophoretic mobility shift assay (EMSA). It was revealed to exhibit cultivar and tissue specific regulation of its expression, through mechanisms involving alternative splicing [32]. Moreover, the relations between autophagy and drought response were analyzed in another line of research by the cloning of TdATG8 (autophagy related protein 8) and its further functional investigation with yeast complementation assay and virus induced gene silencing (VIGS) of plants. In this study, autophagy was shown to be induced in drought-stressed plants in an organ-specific mode, and silencing of ATG8 was shown to decrease drought tolerance of plants, revealing it as a positive regulator of drought stress [33] (Tables 2 and 3).

Table 1: Transcript, protein, metabolite profiling studies conducted in the last three years.

| Species | Cultivars | Tissue | Drought stress application | Method | Reference |
|---|---|---|---|---|---|
| *T. aestivum* | Drought tolerance: Plainsman V: tolerant; Kobomugi: sensitive | Root | Moderate drought stress applied on tillering stage | cDNA microarray | [7] |
| *T. aestivum* | Drought tolerance: information can not be accessed | Grain | Short water shortage in early grain development | cDNA microarray | [8] |
| *T. aestivum* | Efficiency of stem reserve mobilization in peduncles: N49: tolerant; N14: sensitive | Stem | Progressive drought stress after anthesis | 2D gel and MS | [9] |
| *T. aestivum* | Cultivar Vinjett | Grain | Drought applied at terminal spiklet or at anthesis | 2D gel and MS | [10] |
| *T. aestivum* | Yield under drought: Excalibur: tolerant; RAC875: tolerant; Kukri: sensitive | Leaf | Cyclic drought applied after first flag leaf formation mimicking field conditions | SCX column HPLC and MS | [11] |
| *T. durum* | Able to acquire drought tolerance: Ofanto: tolerant | Leaf | Drought applied at booting stage (controls SWC: irrigated when it decreases %50 of field capacity; drought SWC: irrigated when it decreases %12.5 of field capacity) | cDNA-AFLP | [12] |
| *T. durum* | Drought tolerance: Om Rabia3: tolerant; Mahmoudi: sensitive | Embryo | Drought applied at final development stage of seed maturity | 2D gel and HPRP column and MS | [13] |
| *T. dicoccoides* | Yield under drought conditions: Y12-3: tolerant; A24-39: sensitive | Leaf | Terminal drought applied at inflorescence emergence stage | Transcript profiling | [14] |
| *T. dicoccoides* | Yield under drought conditions: Y12-3: tolerant; A24-39: sensitive | Leaf | Drought applied after germination at five/six leaf stage | Transcript and metabolite profiling | [14] |
| *T. dicoccoides* | Drought tolerance: TR39477: tolerant; TR38828: tolerant | Leaf/root | Shock drought stress | miRNA profiling | [15] |

*T*: *Triticum*; SWC: soil water content; 2D: 2-dimensional; SCX: strong cation exchange; HPLC: high performance liquid chromatography; MS: mass spectrometry; cDNA: complementary DNA; AFLP: amplified fragment length polymorphisms; HPRP: human prion protein.

## 3. Phenotyping for Drought Tolerance in Wheat with Physiological Traits

For screening out transgenic wheat lines with desirable drought tolerance, the physiological traits and processes which can be genetically manipulated to improve wheat adaptation to drought have to be taken into account. The genetic basis of drought tolerance in wheat is still elusive. At present the physiological traits (PTs) linked to heat tolerance appear to be a superlative accessible tool since they exhibit the favorable allele combination for drought tolerance. Such alleles interact with the environment and genetic background which includes variation in gene expression and hence are still poorly understood through the QTL approach [50]. Hybridization of heat tolerance PTs may not always have a predictable outcome related to net crop yield particularly in varying environmental conditions, but breeding such varieties with complementary PTs could augment the cumulative gene effect [51]. Thus the physiological phenotyping along with gene discovery can be valuable to pin down desired alleles and understand their genetic mechanism [50]. Cossani and Reynolds have proposed a model based on this concept of genetically characterized PT for improved drought tolerance of wheat [52]. The model focuses on 3 major genetic parameters of yield when water and nutrients are not limiting factors. The genetic parameters are discussed in the following.

### 3.1. Light Interception (LI) Traits

*3.1.1. Canopy Architecture.* Since increase in temperature is linked with a decrease in green area duration and leaf area index, light interception or LI traits can be manipulated by studying the variation in the rapid ground cover (RGC) and leaf senescence of wheat. RGC shows genotypic variability in relatively heritable and simple breeding targets such as embryo and grain size, specific leaf area, or seedling emergence rate [53]. Optimized distribution of light may improve radiation use efficiency (RUE) and LI traits since wheat displays a vast diversity in canopy structure. Furthermore leaves are more erect and smaller in size in many modern cultivars thereby facilitating RUE and allowing more light penetration to lower leaves.

*3.1.2. Hindrance of Leaf Senescence.* Leaf senescence during drought can be hindered by delayed expression of senescence related green thereby giving stay-green (SG) genotypes with normal photosynthesis [54]. Stay green is thus identified as an important adaptive PT for drought stress conditions,

Table 2: List of identified and characterized drought related genes in the last three years.

| Gene | Function | Related mechanism/stress | Reference |
|---|---|---|---|
| TaPIMP1 | Transcription factor: R2R3 type MYB TF | Drought | [34] |
| TaSRG | Transcription factor: *Triticum aestivum* salt response gene | Drought | [35] |
| TaMYB3R1 | Transcription factor: MYB3R type MYB TF | drought | [36] |
| TaNAC (NAM/ATAF/CUC) | Transcription factor: plant specific NAC (NAM/ATAF/CUC) TF | Drought | [37] |
| TaMYB33 | Transcription factor: R2R3 type MYB TF | Drought | [38] |
| TaWRKY2, TaWRKY19 | Transcription factor: WRKY type TF | Drought | [39] |
| TdicDRF1 | Transcription factor: DRE binding protein | Drought | [32] |
| TaABC1 | Kinase: protein kinase ABC1 (activity of bc(1) complex) | Drought | [40] |
| TaSnRK2.4 | Kinase: SNF1 type serine/threonine protein kinase | Drought | [41] |
| TaSnRK2.7 | Kinase: SNF1 type serine/threonine protein kinase | drought | [42] |
| TdTMKP1 | Phosphatase: MAP kinase phosphatase | Drought | [43] |
| TaCHP | CHP rich zinc finger protein with unknown function | ABA-dependent and -independent pathways | [44] |
| TaCP | Protein degradation: cysteine protease | Drought | [45] |
| TaEXPR23 | Cell wall expansion: expansin | Water retention ability and osmotic potential | [46] |
| TaL5 | Nucleocytoplasmic transport of 5S ribosomal RNA: ribosomal L5 gene | Drought | [47] |
| TdPIP1;1, TdPIP1;2 | Protective protein: aquaporin | Drought | [48] |
| TdicATG8 | Autophagy: autophagy related gene 8 | Drought | [33] |
| TdicTMPIT1 | Autophagy: integral transmembrane protein inducible by TNF-$\alpha$ | Drought | [31] |
| *Era1*, *Sal1* | Enhanced response to ABA, inositol polyphosphate 1-phosphatase | Drought | [49] |

Ta: *Triticum aestivum*; Td: *Triticum durum*; Tdic: *Triticum dicoccoides*; DRE: drought related element; SNF: Sucrose nonfermenting; MAP: mitogen activated protein; ABA: abscisic acid; CHP: cysteine histidine proline; TNF-$\alpha$: tumor necrosis factor $\alpha$; PIMP: pathogen induced membrane protein; CP: cysteine protease; EXPR: expansin; PIP: plasma membrane intrinsic proteins.

but its role in improving grain yield in drought is still a matter of extensive research. However, some correlations were shown between SG and yield and identified QTLs in mapping populations [55]. Since chlorosis in plants is not expressed homogenously in plant organs aboveground, many approaches have been developed to estimate SG including spectral reflectance, but these also need to be more specific to functional SG.

### 3.2. Radiation Use Efficiency Traits

*3.2.1. Photosynthesis and Photorespiration.* According to Cossani and Reynolds**,** once the LI traits are optimized the focus on increased crop biomass will depend on RUE traits which include dark respiration, photorespiration, and other photosynthetic strategies. A central player of the photosynthetic pathway, Rubisco was observed to show lower affinity for $CO_2$ over $O_2$ in higher temperatures [52]. Thus, increasing the affinity of Rubisco is especially significant for adaptation to warm conditions. The importance of $CO_2$ fixation by Rubisco for high temperature adaptation is also emphasised by the observation that C4 plants adapt to warm conditions by concentrating $CO_2$. Present transgenic attempts to convert C3 plants into C4 plants are still in progress and require more knowledge of the maintenance of the C4 pathway. Studies of the Rubisco kinetic properties of *Limonium gibertii* may be used in transgenics in wheat even though wheat Rubisco has an excellent $CO_2$ affinity. One model shows 12% increase in net assimilation when substrate specificity factor of wheat Rubisco was replaced from *L. gibertii* [56]. Rubisco activase active sites become inactive progressively under drought, thus associating the activase with heat shock chaperone cpn60$\beta$ could provide Rubisco protection [57]. This has great potential since thermotolerant types of Rubisco in tropical species and diverse optimum temperature of Rubisco have been found in nature [58]. By exploiting this fact a chimeric enzyme was created thus increasing the heat resistance in Arabidopsis by combining the Arabidopsis Rubisco recognition domain and tobacco activase [55].

## 4. Identification of Drought-Related Genes and QTLs

Prior to focusing on individual drought-related components, drought response, due to its complex nature, must be viewed as a whole system, for which large scale identification of probable dehydration stress-related genes or QTLs is necessary. Potential markers for stress tolerance can be identified either through "omics" studies or QTL mapping of yield related traits under drought prone environments. In the long run, these markers can aid in screening cultivars for drought tolerance/sensitivity and/or improvement of drought tolerance in wheat.

*4.1. Drought-Related Gene Identification by "Omics".* "Omics" techniques examine all or a representative subset of an organism's genes, transcripts, proteins, or metabolites. As well as accumulating genomic sequence knowledge, data from profiling studies is also crucial in understanding the drought response, which is largely mediated by differential accumulations of drought-related components.

In the recent decades, high-throughput profiling techniques have been utilized for the identification of potential drought tolerance markers from different wheat species (Table 1). Some of the large scale profiling studies undertaken in wild emmer wheat were mentioned in Section 2 [14, 15, 59]. "Omics" studies were also performed to monitor dehydration induced transcripts and proteins of bread and durum wheat cultivars with differing sensitivities to drought, both in stress and nonstress conditions. Methodologies used in transcript profiling studies range from cDNA microarrays to cDNA-AFLP (amplified fragment length polymorphism). For differential protein identification, the common procedures used include 2D (2-Dimensional) gels, various chromatography techniques, and mass spectrometry. In these recent high-throughput studies, molecular mechanisms behind various drought induced physiological or morphological events were targeted, using related tissues and appropriate mode/timing/severity of stress treatments for each profiling experiment. In two of these studies, underlying molecular mechanisms of early grain development upon shock dehydration response and root functional responses upon moderate drought at tillering were investigated in bread wheat by transcript profiling [8, 60]. Proteome profiling was established in several bread wheat tissues: grain upon drought at terminal spikelet or at anthesis; leaf under field like cyclic drought conditions after first flag leaf formation; stem upon progressive drought stress after anthesis were established. The latter research was conducted to understand the underlying molecular mechanisms of mobilization of stem carbohydrate reserves to grains, a process that contributes to yield under terminal drought conditions and its findings pointed out to the involvement of senescence and protection against oxidative stress in effectiveness of the mobilization process [9]. In recent years, transcript profiling in durum wheat flag leaf upon field like drought at booting was performed [12]. In a different line of research, proteomic profiles of *T. durum* mature embryos were established, which is especially important since embryos are good model systems for drought studies, sustaining germination in extreme conditions of desiccation [13]. An overview of recently established profiling studies is provided in Table 1.

*4.2. QTL Mapping.* Dissection of drought tolerance, a complex quantitative phenotype, affected by multiple loci requires the identification of related quantitative trait loci (QTLs). QTL cloning is a large effort in terms of the technology, resources, and time required, but determination of QTLs is proceeded by great advantages in applications of marker-assisted selection (MAS) and better yielding cultivar development. Identification of QTLs takes advantage of molecular maps, developed by the use of DNA markers. The establishment of these molecular maps has been enabled by the recent advances in functional genomics, which have supplied bacterial artificial chromosomes (BACs), gene sequence data, molecular marker technology, and bioinformatic tools for comparative genomics. Mapping and fine mapping for the identification of candidate regions for a trait prior to positional cloning requires suitable mapping populations: recombinant inbred lines (RILs) and near isogenic lines (NILs), several of which have been established for wheat varieties. However, up to now, only a limited number of studies has succeeded in the positional cloning of wheat QTLs and none in the context of drought [2, 4].

In recent years, several yield QTLs were identified in wheat through linkage analysis and association mapping. Since yield is the most crucial trait to breeders, most QTLs for drought tolerance in wheat have been determined through yield and yield related measurements under water-limited conditions [64–68]. However, these studies are challenged by the factors that yield and drought are both complex traits, involving multiple loci and showing genotype and environment interactions. Yield is difficult to be described accurately with respect to water use, and its accurate phenotyping is a challenge since QTLs established in one environment may not be confirmed in other. For this reason, large scale phenotyping trials, carried out in multiple fields, taking into consideration the environmental varieties are crucial. Until now, a number of studies have identified QTLs associated with specific components of drought response using *T. durum, T. aestivum,* and *T.durum* X *T. dicoccoides* mapping populations; however the genomic regions associated with individual QTLs are still very large and unsuitable for screening in breeding programmes. However, in recent years, several yield related QTLs were mapped using (*T. aestivum* L.) RAC875/Kukri doubled haploid populations grown under a variety of environmental conditions including nonirrigated environments. In one study, inbred population was assessed under heat, drought, and high yield potential conditions to identify genetic loci for grain yield, yield components, and key morphophysiological traits [69]. In another study, regions associated with QTLs for grain yield and physical grain quality were assessed under 16 field locations and year combinations in three distinct seasonal conditions [70]. In a third study, QTLs were identified for days to ear emergence and flag leaf glaucousness

Table 3: List of genes confirmed to function in drought by transgenic studies in last three years.

| Type of transgenic study | Source of the gene | Gene | Function | Related mechanism/ stress | Reference |
|---|---|---|---|---|---|
| Overexpression in *A. thaliana* | From *T. Aestivum* | WRKY2, WRKY19 | Transcription factor: WRKY type TF | Drought | [39] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | MYB33 | transcription factor: R2R3 type MYB TF | Drought | [38] |
| Overexpression in *N. tabacum* | From *T. Aestivum* | PIMP1 | Transcription factor: R2R3 type MYB TF | Drought | [34] |
| Overexpression in *N. tabacum* | From *T. Aestivum* | NAC (NAM/ATAF /CUC) | Transcription factor: plant-specific NAC (NAM/ATAF/CUC) TF | Drought | [37] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | ABC1 | Kinase: protein kinase ABC1 (activity of bc(1) complex) | Drought | [40] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | SnRK2.4 | Kinase: SNF1-type serine/threonine protein kinase | Drought | [41] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | SnRK2.7 | Kinase: SNF1-type serine/threonine protein kinase | Drought | [42] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | CP | Protein degradation: cysteine protease | Drought | [45] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | CHP | CHP rich zinc finger protein with unknown function | ABA-dependent and -independent pathways | [44] |
| Overexpression in *N. tabacum* | From *T. Aestivum* | EXPR23 | Cell wall expansion: expansin | Water retention ability and osmotic potential | [46] |
| Overexpression in *A. thaliana* | From *T. Aestivum* | TaSIP | Salt induced protein with unknown function | Drought and salinity | [61] |
| Overexpression in *N. tabacum* | From *T. Durum* | PIP1;1, PIP1;2 | Protective protein: aquaporin | Drought | [48] |
| Overexpression in *T. aestivum* | From *H. Vulgare* | HVAI | Protective protein: LEA | Drought | [62] |
| Transgenic ubiquitin: TaCHP | — | CHP | CHP rich zinc finger protein with unknown function | ABA-dependent and -independent pathways | [44] |
| TaABA08′OF1 deletion line | — | ABA08 | ABA catabolism: ABA 8′-hydroxylase | Drought | [63] |
| VIGS silencing in *T. dicoccoides* | — | ATG8 | Autophagy: autophagy related gene 8 | Drought | [33] |
| VIGS silencing in *T. aestivum* | — | *Era1*, *Sal1* | Enhanced response to ABA, inositol polyphosphate 1-phosphatase | Drought | [49] |

ABA: abscisic acid; CHP: cysteine histidine proline; SNF: sucrose nonfermenting; PIMP: pathogen induced membrane protein; CP: cysteine protease; EXPR: expansin; PIP: plasma membrane intrinsic proteins; LEA: late embryogenesis abundant; HVA: *Hordeum vulgare* aleurone; TaSIP: *Triticum aestivum* salt induced protein; VIGS: virus induced gene silencing.

under southern Australian conditions [71]. Another multi-environmental analysis provided a basis for fine mapping and cloning the genes linked to a yield related QTL [72]. These recent studies are promising, and along with the recent advances in DNA sequencing technology and new approaches of coupling linkage analysis with "omics" studies, these data will find their way into practical wheat breeding programmes in relation to drought [2, 4]. Drought-related QTLs identified in these studies are listed in Supplementary Table 1 (see Supplementary Materials available online at http://dx.doi.org/10.1155/2013/548246).

## 5. Identification of Molecular Mechanisms Related to Drought

Probable drought-related genes and QTLs, identified in "omics" and "QTL mapping" studies, should be further characterized, prior to their use in the development of better yielding cultivars. Elucidation of these components includes analyzing their gene and protein structure and determining their roles and interactions in the complex network of stress response signaling. Their functional relevance to drought should be shown and eventually confirmed with transgenic

studies. This section summarizes the recent research regarding the characterization of drought-related genes, in detail, dissection of drought-related molecular pathways, and functional genomics studies.

*5.1. Characterization of Drought-Related Genes.* Prior to its utilization for drought tolerance improvement, for each putative drought-related gene region or molecule identified in "QTL mapping" or "omics" study, the immediate step is the cloning and in detail characterization of the gene and its protein. This process exploits a variety of *in silico* and basic molecular biology methods and involves several aspects that differ on the nature of the research, including analysis of gene and protein structure, phylogeny-based studies and determination of gene chromosomal localization, protein-protein and protein-DNA interactions, and transcript and protein subcellular localizations. Further characterization involves transcript and protein monitoring in response to stress conditions and functional analysis of the protein. Utilizing these strategies, in recent years several drought-related proteins were elucidated, the majority being stress-related transcription factors (TFs) and signal transducers.

Drought is known to be regulated at the transcriptional level, and TFs have been the focus of attention for the improvement of better yielding cultivars since targeting a single TF can affect several downstream-regulatory aspects of drought tolerance. Classically, two transcriptional regulatory circuits induced by drought have been studied: ABA-dependent and DREB-(dehydration-responsive element binding protein-) mediated (ABA-independent) pathways. These pathways are schematically depicted in Figure 1. One of the major classes of TFs involved in ABA-dependent stress responses is MYB TFs, and in the recent years, there has been a focus on the elucidation of bread wheat R2R3 and MYB3R type MYB TFs, known to be involved in ABA signaling of drought. In three different lines of research, drought responsive MYBs, TaPIMP1 (pathogen induced membrane protein), TaMYB33, and TaMYB3R1, were cloned and studied via the analysis of their domains, determination of their nuclear subcellular localizations, and assessment of transcriptional activation function to proteins [34, 36, 38]. Phylogenetic analysis of their protein sequences classified TaMYB3R1 as MYB3R type and the others as R2R3 type MYB TFs. The R2R3 type MYB TaPIMP1 was originally described as the first defense related MYB in wheat; however, detailed analyses indicated that TaPIMP1 is also induced by abiotic stresses, particularly drought. In addition, the induction of its expression by ABA and its inability to bind to the DRE-box element as indicated by EMSA suggest that TaPIMP1 acts in the ABA-dependent pathways of drought response [73]. Similarly, TaMYB33, another drought responsive R2R3 type MYB, was shown to be induced by ABA treatment, and the overexpression in *Arabidopsis* plants could not detect a significant increase in DREB2, suggesting that TaMYB33 is also involved in ABA-dependent mechanisms [38]. Both TaPIMP1 and TaMYB33 appear to enhance drought tolerance through ROS detoxification and reinforcement of osmotic balance. An elevated level of proline or proline synthesis common to both

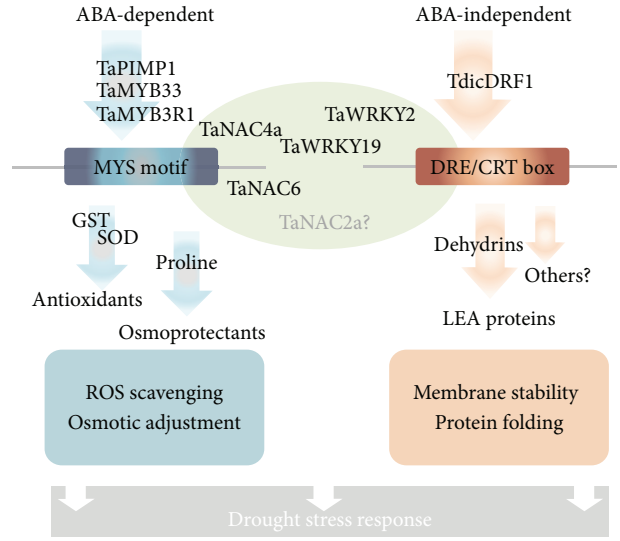


Figure 1: ABA-dependent and ABA-independent pathways of stress response. MYB and DREB TFs are given as examples to ABA-dependent and-independent routes. While ABA-dependent pathways appear to recruit antioxidant and osmoprotectant mechanisms, ABA-independent pathways generally involve protective proteins. NAC and WRKY TFs provide crosstalk between these pathways; where some members, such as TaNAC4 and TaNAC6, may predominantly act in an ABA-dependent fashion, some members may be closer to ABA-independent pathways. In several cases, such as TaWRKY19, both pathways are employed. It should be noted that both pathways are highly intermingled, and functions of several regulators, such as TaNAC2a, as well as entire pathways are yet to be elucidated.

TaPIMP1 and TaMYB33 mediated response is noteworthy [34, 38]. MYB3R type MYB TFs are less pronounced class of MYB proteins in stress response. TaMYB3R1, one of the few examples of MYB3R type MYBs in wheat, has been implicated in drought stress response and is also responsive to ABA, similar to TaPIMP1 and TaMYB33. However, the downstream events of TaMYB3R1-mediated drought stress response remains to be elucidated [36].

ABA-independent also called DREB-mediated pathways are largely governed by dehydration-responsive element-binding (DREB)/C-repeat-binding (CBF) proteins which recognize dehydration-responsive element (DRE)/C-repeat (CRT) motifs through a conserved AP2 domain. While DREB1 TFs are mainly responsive against cold stress, DREB2 TFs are more pronounced in drought stress response; although functional overlaps are possible where a certain DREB responds to multiple stresses [74]. It should be noted that DREB-mediated stress responses may also cooperate or overlap with ABA-dependent stress responses (Figure 1). A number of DREB homologs have deen identified in wheat and, although DREB2-mediated drought response is not fully elucidated yet, enhanced drought tolerance through DREB-mediated pathways is considered to involve LEA proteins [75]. As noted in Section 2, recently, a DREB2 homolog, TdicDRF1, was identified, cloned, and characterized for the

first time in wild emmer wheat. Comparison of drought-stressed resistant and sensitive genotypes revealing differential expressions of TdicDRF1 suggested not only a conserved role on drought stress response but also a promising mechanism that can be utilized in improvement of wheat cultivars for drought tolerance [32].

In recent years, evidence has accumulated that there is crosstalk between classical ABA-dependent and ABA-independent pathways (Figure 1). The best known example of such occurrence is NAC TFs, which regulate drought stress response through both ABA-dependent and ABA-independent pathways. In a recent study, *T. aestivum* NAC (NAM/ATAF/CUC) TFs were identified *in silico*, phylogenetically classified and characterized, and their expression profiles were monitored in response to ABA and drought stress. In response to these treatments, TaNAC4a and TaNAC6 exhibited similar expression trends, suggesting an ABA-dependent regulation of drought, while in the case of TaNTL5 and TaNAC2a, the changes in the expression were not parallel [37]. In another study, WRKY type transcription factors (TaWRKY2 and TaWRKY19), which are known to be involved in plant abiotic stress response and ABA signaling were identified computationally, localized to the nucleus and shown to bind specifically to cis-element, W box. This report revealed that WRKY19 as a component of both ABA and DREB pathways, showing WRKY19 expression level, was responsive to ABA application, and in transgenic WRKY19 deficient plants, the expression levels of DREB pathway components were altered [39].

In addition to these known players of ABA-dependent and DREB-mediated pathways, other novel TFs are also discovered, and one such TF is the recently discovered *T. aestivum* salt response gene TF (TaSRG) which was shown to be induced in response to drought and ABA [35].

Other major targets of recent research have been enzymes that aid in reversible phosphorylation of signaling molecules in drought-related network of protein interactions. Although the major classes of stress-related kinases and phosphatases taking part in these cascades are known, namely, mitogen activated protein kinases (MAPKs), SNF-1-like kinases (SnRKs), calcium-dependent protein kinases (CDPKs), and MAP kinase phosphatases (MKPs), information regarding to these components is far from complete. For this purpose, known components should be further investigated since their exact positions and interactions in the complex signaling network are currently unknown. This has been applied recently, in two separate studies, in which SNF-1-like kinases, namely, TaSnRK2.4, TaSnRK2.7 from bread wheat, were characterized further in detail [41, 42]. In a different research, a MAP kinase phosphatase, TdTMKP1 was cloned from durum wheat, and its specific interaction with two MAPKs, TdTMPK3, and TdTMPK6, was verified, in accordance of its role as a negative regulator of MAPKs [43]. In an independent line of research, a priorly poorly characterized kinase TaABC1 (*T. aestivum* L. protein kinase) was investigated and shown to be involved in drought [40].

Although transcription factors and signal transducers have been the major focus of research in terms of characterization, in recent years other putative drought-related molecules were also isolated from wheat varieties, investigated, and supporting evidence for their roles in drought stress was obtained [31–33, 44–48]. Information regarding these studies is listed in Table 2. Most of the drought-related genes identified were confirmed either by using overexpressor plants or wheat deletion lines or silencing the gene of interest via virus induced gene silencing revealing its function [33, 39, 63] (Table 3).

*5.2. Studies of Drought-Related Pathways.* Plants in environments prone to drought stress have developed several tolerance strategies, resistance and avoidance mechanisms, which enable them to survive and reproduce under conditions of water scarcity.

The fundamental plant drought responses include growth limitation, changes in gene expression, altered hormonal levels, induced and suppressed signaling pathways, accumulation of compatible solutes and osmoprotectant proteins, suppression of metabolism, increased lipid peroxidation with higher levels of ROS, and counter-acting increased levels of antioxidant activity. Drought is regulated both at the transcriptional level and posttranscriptional level, the latter including the action of miRNAs and posttranslational modifications for proteosomal degradation [76] (Supplementary Table 2).

*5.2.1. Compatible Solutes.* Compatible solutes are nontoxic molecules that accumulate in the cytoplasm upon drought stress. Common compatible solutes are sugars, sugar alcohols, glycine betaine, amino acids, and proline. They are known to be involved in osmotic adjustment, function as ROS scavengers, protect proteins and cell structures, and exhibit adaptive value in metabolic pathways. In a recent study, compatible solutes in *T. aestivum* leaves were screened in response to water deficit at the reproduction stage. Major contributors to osmotic adjustment were revealed to be $K^+$ in the early stages of stress and molecules including glycinebetaine, proline, and glucose, in the late stress [77]. Recently, compatible solutes were also assessed in *T. aestivum* cultivars under different irrigation regimes. Drought application decreased the levels of inorganic solutes but increased the levels of organic solutes [78].

*5.2.2. Protective Proteins.* Protective proteins known to be involved in drought stress response include LEA proteins, aquaporins, heat shock proteins, and ion channels. Recently, in *T. aestivum* cultivars, changes in the transcript and protein levels of dehydrins and LEA proteins, in response to progressive drought applied at early vegetation and during its recovery, were monitored [79]. In a different study, two aquaporins were overexpressed providing direct evidence of their drought-related functions [48]. Additionally in another research, SNP and InDEL (insertion/deletion) repertoire of a $Na^+/K^+$ transporter, HKT-1, was assessed and observed to be compromised of mostly missense mutations, predominantly present in the tolerant wheat variety [80].

*5.2.3. Signaling.* Drought signaling includes signal perception and transduction. Known drought-related signal transducers and some recent reports on their characterization and functional assessment are summarized in Sections 5.1–5.3 [40–42]. Some of these studies were performed on calcium dependent protein kinases (CDPK), which sense and respond to $Ca^{2+}$ an important secondary messenger of signal transduction cascades. In a recent study, two bread wheat CDPKs, CPK7 and CPK12, were revealed to be molecularly evolved through gene duplication followed by functional diversification. In this study, they were shown to contain different putative cis-element combinations in their promoters, and two CDPKs were shown to respond differently to drought, PEG, salt (NaCl), cold, hydrogen peroxide ($H_2O_2$), and ABA applications [81]. Other suggested signaling molecules of drought stress network are SA (salicylic acid) and NO (nitric oxide). In recent studies, NO was shown to be present in higher levels in drought tolerant wheat variety and may have a possible role in the drought induced limitation in root growth [82]. Recently, SA was shown to aid drought tolerance through increasing accumulation of solutes [78].

*5.2.4. Photosynthesis and Respiration.* Upon dehydration conditions, basal metabolic activities, including photosynthesis and respiration, are known to be altered in plants. Recently, *T. aestivum* and *T. durum* genotypes with differing sensitivities to osmotic stress were evaluated in terms of their gas exchange in response dehydration. Photosynthesis was analyzed in depth, in relation to ROS levels and physiological parameters, for elucidation of the mechanisms by which the tolerant cultivar sustains a high performance of water-use efficiency, maintaining respiration rate and photosynthesis even under stress conditions [83]. PS II (Photosystem II) is a key protein pigment complex of photosynthesis, which aids in light harvesting. Upon dehydration, PS II repair cycle is impaired affecting oxygen evolving process of PS II reaction center, leading to photooxidative damage. PS II has a biphasic primary phytochemistry kinetics, which is referred to as PS II heterogeneity. In recent studies, photosynthetic efficiency of PSII complex was measured in different wheat varieties under different environmental conditions and the extent and nature of this heterogeneity was assessed in detail, in relation to osmotic stress [84].

*5.2.5. Growth.* Stress response of plants in relation to growth differentiates based both on the tissue and the severity, timing, and mode of stress applied. The degree of osmotic stress induced limitation of plant organ growth also differs from cultivar to cultivar and does not have a correlation with drought tolerance [82, 85, 86]. Recently, dehydration induced retardation of root and leaf growth was studied in detail, in relation to several components of the drought response pathway. In some of these studies, cell wall-bound peroxidases, ROS and NO, were shown to be unfavorable for root expansion and suggested to have possible roles in retarding root cell wall extension [85]. Another report analyzed the underlying mechanisms of *T. aestivum* root elongation in detail, showing that even during plasmolysis upon stress, although root elongation is retarded, new root hair cell formation is sustained [87]. In a different study, monitoring of *T. durum* leaves for ABA, water status, and leaf elongation rates upon drought stress and recovery revealed a retardation in leaf elongation even after stress recovery, suggesting that a rapid accumulation of ABA during stress may have caused the loss of cell wall extensibility [86].

Additionally, recently, expansin, known to be involved in cell wall loosening, was overexpressed in tobacco, confirming its role in plant water retention ability and osmotic potential [83].

*5.2.6. Transcriptional Regulation and Posttranscriptional/Translational Modifications.* The expression of several drought-related gene products is regulated at the transcriptional level. Known drought-related TFs and some recent reports on their characterization and functional assessment are summarized in Sections 5.1–5.3 [32, 34–39]. Additional studies undertaken in the recent years include preliminary research, drought induced expression profiling of MYB transcripts, revealing TaMYBsdu1 as a potential drought-related TF and characterization of SNP and INDEL repertoire of *T. durum* DREB1 and WRKY1 [80, 88].

As well as on the genomic level, drought stress is also regulated at the posttranscriptional and posttranslational levels. The major players of posttranscriptional regulation are miRNAs, which have been identified in a variety of crops, including *Triticeae* species, using both computational and experimental approaches, and their expression profiling was performed in wild emmer wheat and crop model species including *Brachypodium* [15, 76, 89–92]. Posttranslational modifications include protein degradation, mostly via ubiquitination. In a recent study, the leaves of resistant *T. aestivum* cultivar were shown to exhibit a relatively small increase in cysteine protease function upon dehydration, limiting protein loss of this cultivar. In this study, a cysteine protease present only under drought conditions was detected [93]. In a different report, the functional role of a cysteine protease protein was confirmed by its overexpression in *Arabidopsis* [45].

*5.2.7. ROS and Antioxidants.* Upon drought stress, ROS generation occurs mainly in the chloroplast and mitochondria, which results in oxidative damage and lipid peroxidation. Nonenzymatic and enzymatic antioxidants are produced by the plant to detoxify ROS. In a recent report, drought induced ROS generation and antioxidant activity was screened comparatively in the root whole cells and mitochondria of drought acclimated and nonacclimated *T. aestivum* seedlings. A special role of mitochondrial scavenging mechanisms was highlighted by the finding that in specifically nonacclimated seedlings, mitochondrial antioxidants were found to be predominantly active. In the same study, a quick increase in antioxidant mechanisms was observed with stress recovery, thus it was proposed that accumulation of high levels of $H_2O_2$ upon stress can inhibit antioxidant mechanisms [94]. Another study was undertaken in two *T. aestivum* cultivars,

differing in their drought tolerance, focusing especially on the level and activity of ascorbate glutathione cycle related enzymes in stem and leaf tissues. It was shown that ascorbate processing and oxidation were differentially changed in the two cultivars [7]. Recently several other studies of ROS were performed to determine its role in the drought response network in relation to photosynthesis, NO, root growth, ABA, and BABA ($\beta$-aminobutyric acid) [82, 95, 96].

*5.2.8. Abscisic Acid.* ABA is a plant hormone that is known to be involved in plant developmental processes and was also shown to be an inducer of stress-related pathways. Recently the role of ABA catabolism in drought was supported by direct evidence from a wheat deletion line [63]. ABA is a major hot topic of drought research, and recently several studies are performed to determine its role in drought in relation to several drought-related molecules: protective proteins, NO, ROS, leaf and root growth, osmotic adjustment, BABA, ROS, and antioxidants [79, 82, 86, 95]. Additionally, in a recent study alginate oligosaccharides (AOS) prepared from degradation of alginate were shown to play a role in enhancing drought resistance in *T. aestivum* growth period by upregulating the drought tolerance related genes involved in ABA signal pathway, such as LEA1, SnRK2, and pyrroline-5-carboxylate synthetase gene (P5CS) [97].

*5.3. Transgenic Studies for Identification of Gene Function.* Transgenic plants provide the most straightforward way to demonstrate the functional relevance of the potential drought-related gene. Using functional genomics methods, modification of a single gene can be achieved in an identical genetic background. Analyzing the functional role of a protein of interest is achieved by the creation of overexpressor plants or loss-of-function mutants. These studies are most often carried out in model species, *Arabidopsis thaliana* or *Nicotiana benthamiana.* The advantages of these species are basically their rapid reproduction time and the ease of genetic transformation. However, other transgenic model systems, which hold the advantage of being phylogenetically more similar to monocot crop species, are also being developed, most importantly *Brachypodium distachyon*. The use of even a phylogenetically very close model plant in function verification of a gene of interest does not exclude the possibility that its role can differ in the crop of interest. Therefore transgenic studies applied directly on wheat are being developed but currently still more time and labor consuming. Other systems, which hold the advantage of straightforward analysis of gene function in the target crops are deletion lines and virus induced gene silencing (VIGS), which aids in functional characterization through silencing of targeted transcripts.

*5.3.1. Overexpression Studies.* Direct evidence for the functional role of several drought response candidates was established via their overexpression in *A. thaliana* or tobacco by *Agrobacterium* mediated transformation. To gain insight into the mechanisms the molecule of concern exerts a role in drought, and these studies are often coupled by the profiling of stress-related genes and characterization of the overexpressor plants in terms of their altered morphological and physiological properties. In recent years, drought-related molecular function of several transcription factors, signal transducers, and some other proteins were confirmed via their overexpression in *Arabidopsis* or *N. tabacum*. Information regarding these studies is summarized in Table 3.

Recently, in four independent studies, overexpression of transcription factors (TaWRKY2, TaWRKY19, TaMYB33, TaPIMP1, and TaNAC) was shown to confer elevated drought tolerance in target model organisms [34, 37–39]. With the use of these overexpressor plants, WRKY proteins were shown to be involved in the DREB pathway [39], and MYB proteins were revealed to function in ROS detoxification [34, 38]. MYB TaPIMP1 overexpressors were also shown to exhibit increased levels of ABA synthesis and its restricted signaling [38]. Additionally, recently introgression of three kinases (TaABC1, TaSnRK2.7, and TaSnRK2.4) into *Arabidopsis* in separate studies was shown to improve drought tolerance evident by the water content and related measurements of overexpressor plants. All three kinases were observed to improve photosynthetic efficiency. TaABC1 was shown to be involved in DREB and ABA pathways [40]. TaSnRK2.7 was revealed to be involved in carbohydrate metabolism and mechanisms involving root growth [42]. TaSnRK2.4 overexpressors displayed differences in development and showed strengthened cell membrane stability [41]. In addition to transcription factors and kinases, overexpression studies of other proteins were also performed in model organisms, in relation to drought and drought-related capabilities. Cysteine protease, TaCP transgenics were observed to have higher survival rates under drought [45]. CHP rich zinc finger protein TaCHP was revealed to be involved in ABA-dependent and -independent signaling pathways [44]. Expansin, TaEXPR23, was shown to increase water retention ability and decrease osmotic potential [46]. Durum wheat aquaporins (TdPIP1; 1 and TdPIP2; 1) were revealed to regulate root and leaf growth [48]. Besides, in a recent study, *T. aestivum* salt induced protein (TaSIP) was shown to have a role in drought and salt tolerance via overexpression of the gene in *A. thaliana* resulted superior physiological properties [61] (Table 3).

*5.3.2. Wheat Deletion Lines and Virus Induced Gene Silencing.* In recent years, a number of other transgenic studies were also performed for function determination. In such a study, *T. aestivum* deletion lines which lack ABA 8′-hydroxylase gene, involved in ABA catabolism, were used to study the role of ABA metabolism in the reproductive stage drought tolerance of cultivars. This study revealed a parallel between sensitivity to osmotic stress and higher spike ABA levels [63]. In a different study, ubiquitin:TaCHP transgenic wheat lines were used in studies in relation to the role of CHP rich zinc finger protein in drought stress [44]. Additionally, VIGS via barley stripe mosaic virus (BSMV) derived vectors was undertaken to silence ATG8 in wild emmer wheat, revealing it as a positive regulator of drought stress [33] (Table 3). VIGS was also used in another research in order to investigate the roles of *Era1* (enhanced response to abscisic acid), *Sal1*

(inositol polyphosphate 1-phosphatase), and *Cyp707a* (ABA 8′-hydroxylase) in response to limiting water conditions in wheat. When subjected to limiting water conditions, VIGS-treated plants for *Era1* and *Sal1* resulted in increased relative water content (RWC), improved water use efficiency (WUE). Compared to other tested genes *Era1* was found to be the most promising as a potential target for drought tolerance breeding in wheat [49].

## 6. Improvement of Modern Wheat

Recent advances in molecular biological, functional, and comparative tools open up new opportunities for the molecular improvement of modern wheat. Recently developed techniques enable faster identification and characterization of drought-related gene(s) and gene region(s). Natural variants of modern species harbor a large repertoire of potential drought-related genes and hold a tremendous potential for wheat improvement. Introduction of drought-related components of wheat can be performed either with breeding through marker-assisted selection or transgenic methods. Recent increase in sequence availability due to recently developed high-throughput sequencing strategies has provided several high quality genetic markers for breeding. Transgenic strategies with enhanced transformation and selection methods are currently being developed.

*6.1. Marker-Assisted Selection.* Molecular breeding approaches based on specific traits utilize molecular markers for the screening of drought tolerance in cultivars. Loci that are targeted in marker-assisted selection (MAS) are most often derived from QTL mapping studies of quantitative traits [98]. MAS is most often performed based on physiomorphological characteristics related to yield under drought conditions. Markers that are utilized in such a context include SSR (simple sequence repeat) markers, *Xgwm136,* and NW3106, which are linked to genes that effect tillering capacity and coleoptile length, respectively [99]. Other selection markers are linked to Rht (reduced height) genes, which are known to be associated with harvest index. Additionally, transcription factor-derived markers, especially DREB proteins hold a great potential as PCR-based selection markers that can be useful in MAS [100]. However, the isolation of transcription factors is a challenge since they belong to large gene families containing members with high sequence similarities. Identification and successful isolation of a single drought-related loci is compelling also in general due to the complex genomic structure of wheat. However, in the near future, completion of wheat genome sequencing will pace identification of specific loci and the development of markers to be used in selection during breeding processes [98, 101].

*6.2. Use of Transgenics.* An alternative to ongoing breeding programmes is transgenic methods, which enable the transfer of only the desired loci from a source organism to elite wheat cultivars, avoiding possible decrease in yield due to the cotransfer of unwanted adjacent gene segments. Until now, transcription factors have been the most appealing targets for transgenic wheat improvement, due to their role in multiple stress-related pathways. In two different lines of research, overexpression of cotton and *A. thaliana* DREB was performed in wheat, resulting in transgenic lines with improved drought tolerance [102–104]. In another study, a barley LEA protein, *HVA1*, was also overexpressed in wheat, and overexpressors were observed to have better drought tolerance [105]. Transgenic wheat obtained with *Arabidopsis* DREB and *HVA1* protein overexpression was also shown to produce higher yield in the field under drought conditions, but further studies are required to confirm their performance under different environments [105].

It is not unreasonable to predict in the following decades: GM (genetically modified) wheat will be transferred to the fields as a common commercial crop. However, to pace this process, new transgenics methodologies should be developed since the current methods are laborious and time consuming. In a recent study, drought enhancement of bread wheat was established with the overexpression of barley *HVA1,* using a novel technique, which combines doubled haploid technology and *Agrobacterium* mediated genetic transformation [62] (Table 3).

*6.3. Use of Proteomics.* Despite the impressive technological breakthroughs in the genomics of drought resistant cultivars the overall scenario is not so promising, and new dimensions have to be explored for the exact elucidation of the wheat drought response process. Hence new studies are focusing to study wheat tolerance at the proteomic level to target different proteins and understand their role in stress. One particular study during grain development used comparative proteomic analysis and used 2 varieties of wheat resistant (CIMMYT wheat variety Kauz) and sensitive (Janz to drought). They applied linear and nonlinear 2-DE and MALDI-TOF mass spectrometry and elucidated that non-linear 2-DE showed a high resolution and identifies 153 spots of proteins that were differentially expressed, 122 of which were detected by MALDI-TOF. The characterized proteins were primarily metabolism proteins (26% carbohydrate metabolism), proteins involved in defense and detoxification (23%), and the rest of 17% were storage proteins. The study successfully showed the differential expression of various proteins in drought resistant and tolerant varieties. Kauz wheat variety showed high expression of LEA and alpha-amylase inhibitors and catalase isozyme 1, WD40 repeat protein, whereas these proteins were either unchanged or downregulated in Janz variety. Vice versa ascorbate peroxidase G beta-like protein and ADP glucose pyrophosphorylase remained unchanged in Kauz but were all downregulated in Janz. Proteins such as triticin precursor and sucrose synthase showed a considerably higher expression in Kauz water deficit variety compared to Janz water deficit plants. Thus the differential expression shows that biochemical and protein level expression could be a simpler approach to understanding and manipulating drought stress in plants [106].

A parallel approach to understanding the protein expression and posttranslational modification in wheat was carried out by Budak et al. in which 2 wild varieties of emmer wheat

*Triticum turgidum* ssp. *dicoccoides* TR39477 and TTD22 were used along with one modern wheat cultivar *Triticum turgidum* ssp. *durum* cv. Kızıltan. The complete leaf proteome profiles of all three genotypes were compared by 2-DE gel electrophoresis and nanoscale liquid chromatographic electrospray ionization tandem mass spectrometry. Instead of using only drought tolerant and drought resistant varieties another third intermediate variety (modern) was also used. Although many proteins were common in all 3 cultivars both modern and durum but 75 differentially expressed proteins were detected [107]. Consequently comparative proteomics may provide a clearer picture and alternate way to evaluate and characterize drought resistant genes and proteins in wheat varieties.

## 7. Conclusion and Future Perspectives

Drought stress is one of the major limitations to crop production. To develop improved cultivars with enhanced tolerance to drought stress, identification of osmotic stress-related molecules and determination of their roles and locations in several physiological, biochemical, and gene regulatory networks is necessary. Several QTLs for key morphopysiological characteristics and yield were identified under water-limited conditions through creation of linkage maps using parentals with different drought coping abilities. In recent decades, application of high-throughput screening, "omics" strategies on *Triticum* species with differential drought tolerance coping abilities, has revealed several stress-related candidate gene(s) or gene block(s). Furthermore, using a variety of bioinformatics, molecular biology, and functional genomics tools, drought-related candidates were characterized, and their roles in drought tolerance were studied. Major drought-related molecules were revealed to be signal transduction pathway components and transcription factors. Several osmoprotectants, compatible solutes, ROS, and antioxidants were shown to accumulate in response to dehydration. Drought stress was found to alter various ongoing metabolic processes, such as growth, photosynthesis, and respiration.

Analysis of drought response has been complicated in the absence of wheat genomic sequence data. However, with the recent advances in sequencing technologies, genome sequence of bread wheat is almost complete by the efforts of ITMI (The International *Triticeae* Mapping Initiative) and IWGSC (International Wheat Genome Sequencing Consortium). Availability of whole wheat genome sequence will contribute to the ongoing studies of exploring the extensive reservoir of alleles in drought tolerant wild germplasm, and this also enables better marker development, genome analysis and large scale profiling experiments. "Omics" strategies have especially contributed to drought research since osmotic stress response is not only genomic based but also regulated at the posttranscriptional and posttranslational levels. Advances in transformation/selection strategies have paced molecular transformation of wheat, which has an advantage to conventional and marker-assisted breeding for targeted introduction of only the desired loci.

It is reasonable to predict that in the following years higher yielding wheat under drought conditions will be developed through breeding or molecular transformation of novel genes obtained from screening of wheat germplasms and will be commercially grown to balance the production with the consumption of the increasing human population. Research exploiting recent advances in genomics technologies has made it possible to dissect and resynthesize molecular regulation of drought and manipulate crop genomes for drought tolerance. The future efforts will be to integrate and translate these resources into practical higher yielding field products.

## References

[1] N. Z. Ergen and H. Budak, "Sequencing over 13 000 expressed sequence tags from six subtractive cDNA libraries of wild and modern wheats following slow drought stress," *Plant, Cell & Environment*, vol. 32, no. 3, pp. 220–236, 2009.

[2] D. Fleury, S. Jefferies, H. Kuchel, and P. Langridge, "Genetic and genomic tools to improve drought tolerance in wheat," *Journal of Experimental Botany*, vol. 61, no. 12, pp. 3211–3222, 2010.

[3] M. Tester and P. Langridge, "Breeding technologies to increase crop production in a changing world," *Science*, vol. 327, no. 5967, pp. 818–822, 2010.

[4] D. Z. Habash, Z. Kehel, and M. Nachit, "Genomic approaches for designing durum wheat ready for climate change with a focus on drought," *Journal of Experimental Botany*, vol. 60, no. 10, pp. 2805–2815, 2009.

[5] M. P. Reynolds, "Drought adaptation in wheat," in *Drought Tolerance in Cereals*, J. M. Ribaut, Ed., chapter 11, pp. 402–436, Haworth's Food Products Press, New York, NY, USA, 2006.

[6] M. Ashraf, M. Ozturk, and H. R. Athar, *Salinity and Water Stress: Improving Crop Efficiency*, Berlin, Germany, Springer, 2009.

[7] M. Sečenji, E. Hideg, A. Bebes, and J. Györgyey, "Transcriptional differences in gene families of the ascorbate-glutathione cycle in wheat during mild water deficit," *Plant Cell Reports*, vol. 29, no. 1, pp. 37–50, 2010.

[8] A. Szucs, K. Jäger, M. E. Jurca et al., "Histological and microarray analysis of the direct effect of water shortage alone or combined with heat on early grain development in wheat (*Triticum aestivum*)," *Physiologia Plantarum*, vol. 140, no. 2, pp. 174–188, 2010.

[9] M. M. Bazargani, E. Sarhadi, A. A. Bushehri et al., "A proteomics view on the role of drought-induced senescence and oxidative stress defense in enhanced stem reserves remobilization in wheat," *Journal of Proteomics*, vol. 74, no. 10, pp. 1959–1973, 2011.

[10] F. Yang, A. D. Jørgensen, H. Li et al., "Implications of high-temperature events and water deficits on protein profiles in wheat (*Triticum aestivum* L. cv. Vinjett) grain," *Proteomics*, vol. 11, no. 9, pp. 1684–1695, 2011.

[11] K. L. Ford, A. Cassin, and A. Bacic, "Quantitative proteomic analysis of wheat cultivars with differing drought stress tolerance," *Frontiers in Plant Science*, vol. 2, article 44, 2011.

[12] P. Rampino, G. Mita, P. Fasano et al., "Novel durum wheat genes up-regulated in response to a combination of heat and drought stress," *Plant Physiology and Biochemistry*, vol. 56, pp. 72–78, 2012.

[13] S. Irar, F. Brini, A. Goday, K. Masmoudi, and M. Pagès, "Proteomic analysis of wheat embryos with 2-DE and liquid-phase chromatography (ProteomeLab PF-2D)—a wider perspective

of the proteome," *Journal of Proteomics*, vol. 73, no. 9, pp. 1707–1721, 2010.

[14] T. Krugman, Z. Peleg, L. Quansah et al., "Alteration in expression of hormone-related genes in wild emmer wheat roots associated with drought adaptation mechanisms," *Functional & Integrative Genomics*, vol. 11, no. 4, pp. 565–583, 2011.

[15] M. Kantar, S. J. Lucas, and H. Budak, "miRNA expression patterns of *Triticum dicoccoides* in response to shock drought stress," *Planta*, vol. 233, no. 3, pp. 471–484, 2011.

[16] S. Farooq, "Triticeae: the ultimate source of abiotic stress tolerance improvement in wheat," in *SalInity and Water Stress*, M. Ashraf, Ed., chapter 7, pp. 65–71, Springer, Berlin, Germany, 2009.

[17] M. Feldman and E. R. Sears, "The wild genetic resources of wheat," *Scientific American*, vol. 244, pp. 102–112, 1981.

[18] P. Dong, Y. M. Wei, G. Y. Chen et al., "EST-SSR diversity correlated with ecological and genetic factors of wild emmer wheat in Israel," *Hereditas*, vol. 146, no. 1, pp. 1–10, 2009.

[19] T. Fahima, M. S. Röder, K. Wendehake, V. M. Kirzhner, and E. Nevo, "Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides*, in Israel," *Theoretical and Applied Genetics*, vol. 104, no. 1, pp. 17–29, 2002.

[20] T. Fahima, G. L. Sun, A. Beharav, T. Krugman, A. Beiles, and E. Nevo, "RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel," *Theoretical and Applied Genetics*, vol. 98, no. 3-4, pp. 434–447, 1999.

[21] E. Nevo and A. Beiles, "Genetic diversity of wild emmer wheat in Israel and Turkey—structure, evolution, and application in breeding," *Theoretical and Applied Genetics*, vol. 77, no. 3, pp. 421–455, 1989.

[22] E. Nevo, E. Golenberg, A. Beiles, A. H. D. Brown, and D. Zohary, "Genetic diversity and environmental associations of wild wheat, *Triticum dicoccoides*, in Israel," *Theoretical and Applied Genetics*, vol. 62, no. 3, pp. 241–254, 1982.

[23] Z. Peleg, Y. Saranga, T. Krugman, S. Abbo, E. Nevo, and T. Fahima, "Allelic diversity associated with aridity gradient in wild emmer wheat populations," *Plant, Cell & Environment*, vol. 31, no. 1, pp. 39–49, 2008.

[24] J. R. Wang, Y. M. Wei, X. Y. Long et al., "Molecular evolution of dimeric $\alpha$-amylase inhibitor genes in wild emmer wheat and its ecological association," *BMC Evolutionary Biology*, vol. 8, no. 1, article 91, 2008.

[25] Z. Peleg, T. Fahima, S. Abbo et al., "Genetic diversity for drought resistance in wild emmer wheat and its ecogeographical associations," *Plant, Cell & Environment*, vol. 28, no. 2, pp. 176–191, 2005.

[26] J. Peng, D. Sun, and E. Nevo, "Domestication evolution, genetics and genomics in wheat," *Molecular Breeding*, vol. 28, no. 3, pp. 281–301, 2011.

[27] J. Peng, D. Sun, and E. Nevo, "Wild emmer wheat, *Triticum dicoccoides*, occupies a pivotal position in wheat domestication process," *Australian Journal of Crop Science*, vol. 5, no. 9, pp. 1127–1143, 2011.

[28] J. H. Peng, D. F. Sun, Y. L. Peng, and E. Nevo, "Gene discovery in *Triticum dicoccoides*, the direct progenitor of cultivated wheats," *Cereal Research Communications*, vol. 41, no. 1, pp. 1–22, 2013.

[29] N. Z. Ergen, J. Thimmapuram, H. J. Bohnert, and H. Budak, "Transcriptome pathways unique to dehydration tolerant relatives of modern wheat," *Functional and Integrative Genomics*, vol. 9, no. 3, pp. 377–396, 2009.

[30] T. Krugman, V. Chagué, Z. Peleg et al., "Multilevel regulation and signalling processes associated with adaptation to terminal drought in wild emmer wheat," *Functional and Integrative Genomics*, vol. 10, no. 2, pp. 167–186, 2010.

[31] S. Lucas, E. Dogan, and H. Budak, "TMPIT1 from wild emmer wheat: first characterisation of a stress-inducible integral membrane protein," *Gene*, vol. 483, no. 1-2, pp. 22–28, 2011.

[32] S. Lucas, E. Durmaz, B. A. Akpnar, and H. Budak, "The drought response displayed by a DRE-binding protein from *Triticum dicoccoides*," *Plant Physiology and Biochemistry*, vol. 49, no. 3, pp. 346–351, 2011.

[33] D. Kuzuoglu-Ozturk, O. Cebeci Yalcinkaya, B. A. Akpinar et al., "Autophagy-related gene, TdAtg8, in wild emmer wheat plays a role in drought and osmotic stress response," *Planta*, vol. 236, no. 4, pp. 1081–1092, 2012.

[34] H. Liu, X. Zhou, N. Dong, X. Liu, H. Zhang, and Z. Zhang, "Expression of a wheat MYB gene in transgenic tobacco enhances resistance to *Ralstonia solanacearum*, and to drought and salt stresses," *Functional & Integrative Genomics*, vol. 11, no. 3, pp. 431–443, 2011.

[35] X. He, X. Hou, Y. Shen, and Z. Huang, "TaSRG, a wheat transcription factor, significantly affects salt tolerance in transgenic rice and *Arabidopsis*," *FEBS Letters*, vol. 585, no. 8, pp. 1231–1237, 2011.

[36] H. Cai, S. Tian, C. Liu, and H. Dong, "Identification of a MYB3R gene involved in drought, salt and cold stress in wheat (*Triticum aestivum* L.)," *Gene*, vol. 485, no. 2, pp. 146–152, 2011.

[37] Y. Tang, M. Liu, S. Gao et al., "Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco," *Plant Physiology*, vol. 144, no. 3, pp. 210–224, 2012.

[38] Y. Qin, M. Wang, Y. Tian, W. He, L. Han, and G. Xia, "Over-expression of TaMYB33 encoding a novel wheat MYB transcription factor increases salt and drought tolerance in *Arabidopsis*," *Molecular Biology Reports*, vol. 39, no. 6, pp. 7183–7192, 2012.

[39] C. F. Niu, W. Wei, Q. Y. Zhou et al., "Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic *Arabidopsis* plants," *Plant, Cell & Environment*, vol. 35, no. 6, pp. 1156–1170, 2012.

[40] C. Wang, R. Jing, X. Mao, X. Chang, and A. Li, "TaABC1, a member of the activity of bc1 complex protein kinase family from common wheat, confers enhanced tolerance to abiotic stresses in *Arabidopsis*," *Journal of Experimental Botany*, vol. 62, no. 3, pp. 1299–1311, 2011.

[41] X. Mao, H. Zhang, S. Tian, X. Chang, and R. Jing, "TaSnRK2.4, an SNF1-type serine/threonine protein kinase of wheat (*Triticum aestivum* L.), confers enhanced multistress tolerance in *Arabidopsis*," *Journal of Experimental Botany*, vol. 61, no. 3, pp. 683–696, 2010.

[42] H. Zhang, X. Mao, R. Jing, X. Chang, and H. Xie, "Characterization of a common wheat (*Triticum aestivum* L.) TaSnRK2.7 gene involved in abiotic stress responses," *Journal of Experimental Botany*, vol. 62, no. 3, pp. 975–988, 2011.

[43] I. Zaïdi, C. Ebel, M. Touzri et al., "TMKP1 is a novel wheat stress responsive MAP kinase phosphatase localized in the nucleus," *Plant Molecular Biology*, vol. 73, no. 3, pp. 325–338, 2010.

[44] C. Li, J. Lv, X. Zhao et al., "TaCHP: a wheat zinc finger protein gene down-regulated by abscisic acid and salinity stress plays a positive role in stress tolerance," *Plant Physiology*, vol. 154, no. 1, pp. 211–221, 2010.

[45] Q. W. Zang, C. X. Wang, X. Y. Li et al., "Isolation and characterization of a gene encoding a polyethylene glycol-induced cysteine protease in common wheat," *Journal of Biosciences*, vol. 35, no. 3, pp. 379–388, 2010.

[46] Y. Y. Han, A. X. Li, F. Li, M. R. Zhao, and W. Wang, "Characterization of a wheat (*Triticum aestivum* L.) expansin gene, TaEXPB23, involved in the abiotic stress response and phytohormone regulation," *Plant Physiology and Biochemistry*, vol. 54, pp. 49–58, 2012.

[47] G. Z. Kang, H. F. Peng, Q. X. Han, Y. H. Wang, and T. C. Guo, "Identification and expression pattern of ribosomal L5 gene in common wheat (*Triticum aestivum* L.)," *Gene*, vol. 493, no. 1, pp. 62–68, 2012.

[48] M. Ayadi, D. Cavez, N. Miled, F. Chaumont, and K. Masmoudi, "Identification and characterization of two plasma membrane aquaporins in durum wheat (*Triticum turgidum* L. subsp. durum) and their role in abiotic stress tolerance," *Plant Physiology and Biochemistry*, vol. 49, no. 9, pp. 1029–1039, 2011.

[49] H. Manmathan, D. Shaner, J. Snelling, N. Tisserat, and N. Lapitan, "Virus-induced gene silencing of Arabidopsis thaliana gene homologues in wheat identifies genes conferring improved drought tolerance," *Journal of Experimental Botany*, vol. 64, no. 5, pp. 1381–1392, 2013.

[50] M. Reynolds and R. Tuberosa, "Translational research impacting on crop productivity in drought-prone environments," *Current Opinion in Plant Biology*, vol. 11, no. 2, pp. 171–179, 2008.

[51] M. Reynolds and G. Rebetzke, "Application of plant physiology in wheat breeding," in *The World Wheat Book: A History of Wheat Breeding*, A. P. Bonjean, W. J. Angus, and M. van Ginkel, Eds., vol. 2, Paris, France, 2011.

[52] C. M. Cossani and M. P. Reynolds, "Physiological traits for improving heat tolerance in wheat," *Plant Physiology*, vol. 160, no. 4, pp. 1710–1718, 2012.

[53] R. A. Richards and Z. Lukacs, "Seedling vigour in wheat—sources of variation for genetic and agronomic improvement," *Australian Journal of Agricultural Research*, vol. 53, no. 1, pp. 41–50, 2002.

[54] P. O. Lim, H. J. Kim, and H. G. Nam, "Leaf senescence," *Annual Review of Plant Biology*, vol. 58, pp. 115–136, 2007.

[55] U. Kumar, A. K. Joshi, M. Kumari, R. Paliwal, S. Kumar, and M. S. Röder, "Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the "Chirya 3" × "Sonalika" population," *Euphytica*, vol. 174, no. 3, pp. 437–445, 2010.

[56] M. A. J. Parry, M. Reynolds, M. E. Salvucci et al., "Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency," *Journal of Experimental Botany*, vol. 62, no. 2, pp. 453–467, 2011.

[57] M. E. Salvucci and S. J. Crafts-Brandner, "Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis," *Physiologia Plantarum*, vol. 120, no. 2, pp. 179–186, 2004.

[58] I. Kurek, K. C. Thom, S. M. Bertain et al., "Enhanced thermostability of *Arabidopsis* rubisco activase improves photosynthesis and growth rates under moderate heat stress," *The Plant Cell*, vol. 19, no. 10, pp. 3230–3241, 2007.

[59] T. Krugman, V. Chagué, Z. Peleg et al., "Multilevel regulation and signalling processes associated with adaptation to terminal drought in wild emmer wheat," *Functional and Integrative Genomics*, vol. 10, no. 2, pp. 167–186, 2010.

[60] M. Sečenji, Á. Lendvai, P. Miskolczi et al., "Differences in root functions during long-term drought adaptation: comparison of active gene sets of two wheat genotypes," *Plant Biology*, vol. 12, no. 6, pp. 871–882, 2010.

[61] H.-Y. Du, Y.-Z. Shen, and Z.-J. Huang, "Function of the wheat TaSIP gene in enhancing drought and salt tolerance in transgenic *Arabidopsis* and rice," *Plant Molecular Biology*, vol. 81, no. 4-5, pp. 417–429, 2013.

[62] H. Chauhan and P. Khurana, "Use of doubled haploid technology for development of stable drought tolerant bread wheat (*Triticum aestivum* L.) transgenics," *Plant Biotechnology Journal*, vol. 9, no. 3, pp. 408–417, 2011.

[63] X. Ji, B. Dong, B. Shiran et al., "Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals," *Plant Physiology*, vol. 156, no. 2, pp. 647–662, 2011.

[64] A. A. Diab, R. V. Kantety, N. Z. Ozturk, D. Benscher, M. M. Nachit, and M. E. Sorrells, "Drought—inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat," *Scientific Research and Essays*, vol. 3, no. 1, pp. 9–27, 2008.

[65] M. Maccaferri, M. C. Sanguineti, S. Corneti et al., "Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability," *Genetics*, vol. 178, no. 1, pp. 489–511, 2008.

[66] K. L. Mathews, M. Malosetti, S. Chapman et al., "Multi-environment QTL mixed models for drought stress adaptation in wheat," *Theoretical and Applied Genetics*, vol. 117, no. 7, pp. 1077–1091, 2008.

[67] C. L. McIntyre, K. L. Mathews, A. Rattey et al., "Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions," *Theoretical and Applied Genetics*, vol. 120, no. 3, pp. 527–541, 2010.

[68] Z. Peleg, T. Fahima, T. Krugman et al., "Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population," *Plant, Cell & Environment*, vol. 32, no. 7, pp. 758–779, 2009.

[69] D. Bennett, M. Reynolds, D. Mullan et al., "Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments," *Theoretical and Applied Genetics*, vol. 125, no. 7, pp. 1473–1485, 2012.

[70] D. Bennett, A. Izanloo, M. Reynolds, H. Kuchel, P. Langridge, and T. Schnurbusch, "Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments," *Theoretical and Applied Genetics*, vol. 125, no. 2, pp. 255–271, 2012.

[71] D. Bennett, A. Izanloo, J. Edwards et al., "Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions," *Theoretical and Applied Genetics*, vol. 124, no. 4, pp. 697–711, 2012.

[72] J. Bonneau, J. Taylor, B. Parent et al., "Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat," *Theoretical and Applied Genetics*, vol. 126, no. 3, pp. 747–761, 2013.

[73] Z. Zhang, X. Liu, X. Wang et al., "An R2R3 MYB transcription factor in wheat, TaPIMP1, mediates host resistance to Bipolaris sorokiniana and drought stresses through regulation of defense- and stress-related genes," *New Phytologist*, vol. 196, no. 4, pp. 1155–1170, 2012.

[74] P. K. Agarwal, P. Agarwal, M. K. Reddy, and S. K. Sopory, "Role of DREB transcription factors in abiotic and biotic stress tolerance in plants," *Plant Cell Reports*, vol. 25, no. 12, pp. 1263–1274, 2006.

[75] C. Egawa, F. Kobayashi, M. Ishibashi, T. Nakamura, C. Nakamura, and S. Takumi, "Differential regulation of transcript accumulation and alternative splicing of a DREB2 homolog under abiotic stress conditions in common wheat," *Genes & Genetic Systems*, vol. 81, no. 2, pp. 77–91, 2006.

[76] M. Kantar, S. J. Lucas, and H. Budak, "Drought stress: molecular genetics and genomics approaches," *Advances in Botanical Research*, vol. 57, pp. 445–493, 2011.

[77] S. A. Nio, G. R. Cawthray, L. J. Wade, and T. D. Colmer, "Pattern of solutes accumulated during leaf osmotic adjustment as related to duration of water deficit for wheat at the reproductive stage," *Plant Physiology and Biochemistry*, vol. 49, no. 10, pp. 1126–1137, 2011.

[78] N. Loutfy, M. A. El-Tayeb, A. M. Hassanen, M. F. Moustafa, Y. Sakuma, and M. Inouhe, "Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum*)," *Journal of Plant Research*, vol. 125, no. 1, pp. 173–184, 2012.

[79] I. I. Vaseva, B. S. Grigorova, L. P. Simova-Stoilova, K. N. Demirevska, and U. Feller, "Abscisic acid and late embryogenesis abundant protein profile changes in winter wheat under progressive drought stress," *Plant Biology*, vol. 12, no. 5, pp. 698–707, 2010.

[80] L. Mondini, M. Nachit, E. Porceddu, and M. A. Pagnotta, "Identification of SNP mutations in DREB1, HKT1, and WRKY1 genes involved in drought and salt stress tolerance in durum wheat (*Triticum turgidum* L. var durum)," *OMICS*, vol. 16, no. 4, pp. 178–187, 2012.

[81] S. Geng, Y. Zhao, L. Tang et al., "Molecular evolution of two duplicated CDPK genes CPK7 and CPK12 in grass species: a case study in wheat (*Triticum aestivum* L.)," *Gene*, vol. 475, no. 2, pp. 94–103, 2011.

[82] I. Tari, A. Guóth, D. Benyó, J. Kovács, P. Poór, and B. Wodala, "The roles of ABA, reactive oxygen species and Nitric Oxide in root growth during osmotic stress in wheat: comparison of a tolerant and a sensitive variety," *Acta Biologica Hungarica*, vol. 61, no. 1, pp. 189–196, 2010.

[83] V. Vassileva, C. Signarbieux, I. Anders, and U. Feller, "Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.)," *Journal of Plant Research*, vol. 124, no. 1, pp. 147–154, 2011.

[84] R. Singh-Tomar, S. Mathur, S. I. Allakhverdiev, and A. Jajoo, "Changes in PS II heterogeneity in response to osmotic and ionic stress in wheat leaves (*Triticum aestivum*)," *Journal of Bioenergetics and Biomembranes*, vol. 44, no. 4, pp. 411–419, 2012.

[85] J. Csiszar, A. Gallé, E. Horváth et al., "Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress," *Plant Physiology and Biochemistry*, vol. 52, pp. 119–129, 2012.

[86] M. Mahdid, A. Kameli, C. Ehlert, and T. Simonneau, "Rapid changes in leaf elongation, ABA and water status during the recovery phase following application of water stress in two durum wheat varieties differing in drought tolerance," *Plant Physiology and Biochemistry*, vol. 49, no. 10, pp. 1077–1083, 2011.

[87] M. Volgger, I. Lang, M. Ovečka, and I. Lichtscheidl, "Plasmolysis and cell wall deposition in wheat root hairs under osmotic stress," *Protoplasma*, vol. 243, no. 1–4, pp. 51–62, 2010.

[88] M. Rahaie, G. P. Xue, M. R. Naghavi, H. Alizadeh, and P. M. Schenk, "A MYB gene from wheat (*Triticum aestivum* L.) is up-regulated during salt and drought stresses and differentially regulated between salt-tolerant and sensitive genotypes," *Plant Cell Reports*, vol. 29, no. 8, pp. 835–844, 2010.

[89] T. Unver and H. Budak, "Conserved microrna and their targets in model grass species brachypodium distachyon," *Planta*, vol. 230, no. 4, pp. 659–669, 2009.

[90] H. Budak and A. Akpinar, "Dehydration stress-responsive miRNA in Brachypodium distachyon: evident by genome-wide screening of microRNAs expression," *OMICS*, vol. 15, no. 11, pp. 791–799, 2011.

[91] S. J. Lucas and H. Budak, "Sorting the wheat from the chaff: identifying miRNAs in genomic survey sequences of *Triticum aestivum* chromosome 1AL," *PLoS One*, vol. 7, no. 7, Article ID e40859, 2012.

[92] M. Kantar, B. A. Akpınar, M. Valárik et al., "Subgenomic analysis of microRNAs in polyploid wheat," *Functional & Integrative Genomics*, vol. 12, no. 3, pp. 465–479, 2012.

[93] L. Simova-Stoilova, I. Vaseva, B. Grigorova, K. Demirevska, and U. Feller, "Proteolytic activity and cysteine protease expression in wheat leaves under severe soil drought and recovery," *Plant Physiology and Biochemistry*, vol. 48, no. 2-3, pp. 200–206, 2010.

[94] D. S. Selote and R. Khanna-Chopra, "Antioxidant response of wheat roots to drought acclimation," *Protoplasma*, vol. 245, no. 1–4, pp. 153–163, 2010.

[95] Y.-L. Du, Z.-Y. Wang, J.-W. Fan, N. C. Turner, T. Wang, and F.-M. Li, "Beta-Aminobutyric acid increases abscisic acid accumulation and desiccation tolerance and decreases water use but fails to improve grain yield in two spring wheat cultivars under soil drying," *Journal of Experimental Botany*, vol. 63, no. 13, pp. 4849–4860, 2012.

[96] I. M. Huseynova, "Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought," *Biochimica et Biophysica Acta*, vol. 1817, no. 8, pp. 1516–1523, 2012.

[97] H. Liu, Y. H. Zhang, H. Yin, W. X. Wang, X. M. Zhao, and Y. G. Du, "Alginate oligosaccharides enhanced *Triticum aestivum* L. tolerance to drought stress," *Plant Physiology and Biochemistry*, vol. 62, pp. 33–40, 2013.

[98] J. R. Witcombe, P. A. Hollington, C. J. Howarth, S. Reader, and K. A. Steele, "Breeding for abiotic stresses for sustainable agriculture," *Philosophical Transactions of the Royal Society B*, vol. 363, no. 1492, pp. 703–716, 2008.

[99] S. Gulnaz, M. Sajjad, I. Khaliq, A. S. Khan, and S. H. Khan, "Relationship among coleoptile length, plant height and tillering capacity for developing improved wheat varieties," *International Journal of Agriculture and Biology*, vol. 13, no. 1, pp. 130–133, 2011.

[100] B. Wei, R. Jing, C. Wang et al., "Dreb1 genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs," *Molecular Breeding*, vol. 23, no. 1, pp. 13–22, 2009.

[101] E. Nevo and G. Chen, "Drought and salt tolerances in wild relatives for wheat and barley improvement," *Plant, Cell & Environment*, vol. 33, no. 4, pp. 670–685, 2010.

[102] P. Guo, M. Baum, S. Grando et al., "Differentially expressed genes between drought-tolerant and drought-sensitive barley

genotypes in response to drought stress during the reproductive stage," *Journal of Experimental Botany*, vol. 60, no. 12, pp. 3531–3544, 2009.

[103] A. Pellegrineschi, M. Reynolds, M. Pacheco et al., "Stress-induced expression in wheat of the *Arabidopsis* thaliana DREB1A gene delays water stress symptoms under greenhouse conditions," *Genome*, vol. 47, no. 3, pp. 493–500, 2004.

[104] D. Hoisington and R. Ortiz, "Research and field monitoring on transgenic crops by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)," *Euphytica*, vol. 164, no. 3, pp. 893–902, 2008.

[105] A. Bahieldin, H. T. Mahfouz, H. F. Eissa et al., "Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance," *Physiologia Plantarum*, vol. 123, no. 4, pp. 421–427, 2005.

[106] S.-S. Jiang, X. N. Liang, X. Li et al., "Wheat drought-responsive grain proteome analysis by linear and nonlinear 2-DE and MALDI-TOF mass spectrometry," *International Journal of Molecular Sciences*, vol. 13, no. 12, pp. 16065–16083, 2012.

[107] H. Budak, B. A. Akpinar, T. Unver, and M. Turktas, "Proteome changes in wild and modern wheat leaves upon drought stress by two-dimensional electrophoresis and nanoLC-ESI-MS/MS," *Plant Molecular Biology*, 2013.

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*Research Article*

# Establishing an Efficient Way to Utilize the Drought Resistance Germplasm Population in Wheat

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Drought resistance breeding provides a hopeful way to improve yield and quality of wheat in arid and semiarid regions. Constructing core collection is an efficient way to evaluate and utilize drought-resistant germplasm resources in wheat. In the present research, 1,683 wheat varieties were divided into five germplasm groups (high resistant, HR; resistant, *R*; moderate resistant, MR; susceptible, *S*; and high susceptible, HS). The least distance stepwise sampling (LDSS) method was adopted to select core accessions. Six commonly used genetic distances (Euclidean distance, Euclid; Standardized Euclidean distance, Seuclid; Mahalanobis distance, Mahal; Manhattan distance, Manhat; Cosine distance, Cosine; and Correlation distance, Correlation) were used to assess genetic distances among accessions. Unweighted pair-group average (UPGMA) method was used to perform hierarchical cluster analysis. Coincidence rate of range (CR) and variable rate of coefficient of variation (VR) were adopted to evaluate the representativeness of the core collection. A method for selecting the ideal constructing strategy was suggested in the present research. A wheat core collection for the drought resistance breeding programs was constructed by the strategy selected in the present research. The principal component analysis showed that the genetic diversity was well preserved in that core collection.

## 1. Introduction

Drought is probably the most important abiotic stress that limits plant growth [1]. Drought stress is one of the most serious environmental factors that can severely limit the yield and quality of agricultural crops [2]. With global climate change, the lack of water for agronomic purposes will become the major problem for crop production [3]. In agronomical point-of-view, drought stress is a situation in which lack of water exceeds the capacity of plants which leads to the growth prevention. Thus, improving the drought tolerance is a major adaptation strategy for plant production in arid and semiarid regions [4]. In drought prone environments, crop drought resistance is a major factor in the stabilization of crop performance. Drought resistance is now considered by both breeders and molecular biologists as a valid breeding target.

Wheat (*Triticum aestivum* L.) is one of the most important cereals in the world. Drought stress may reduce all yield components in wheat [5]. Drought is the major factor limiting wheat growth and productivity in many regions of the world, and the changing global climate is making the situation more serious [6, 7]. Developing high-yielding wheat cultivars under drought conditions in arid and semiarid regions is an important objective of breeding programs [5]. Although great efforts have been made in wheat drought resistance breeding, the decrease in agricultural productivity induced by drought stress still remains unsolved [8]. One reason is that the numerous germplasm resources were not effectively utilized in wheat breeding programs. However, with continuous collection of germplasm resources, the size of populations has been becoming bigger and bigger, which hindered the evaluation and utilization of the wheat germplasm resources.

Core collections provide an efficient way to evaluate and utilize germplasm resources. A core collection is a representative sample of the whole collection which has minimum repetitiveness and maximum genetic diversity of a plant species [9]. The core collection serves as a working

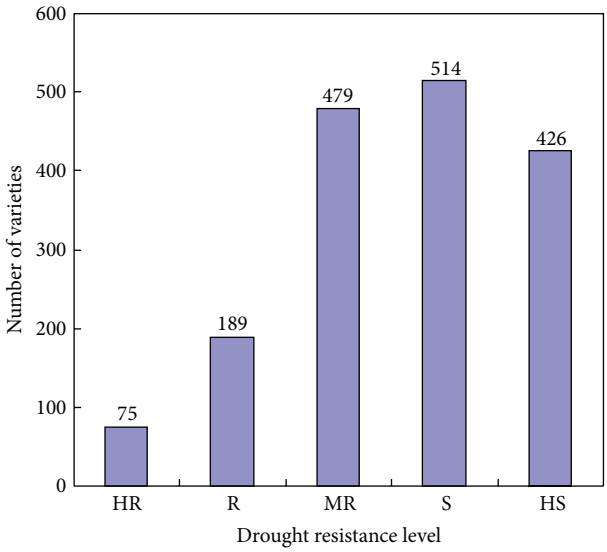


Figure 1: The distribution of 1,683 wheat varieties: HR: high resistant; *R*: resistant; MR: moderate resistant; *S*: susceptible; HS: high susceptible.

collection to be evaluated and utilized preferentially [10–13]. In this way, it is possible to preserve most of the genes in large germplasm populations using a small sample. Thus, the objectives of this research were (1) to investigate the ideal constructing strategy on wheat core collection based on data of agronomic traits combining drought resistance information and (2) to construct such a wheat core collection for the drought resistance breeding programs.

## 2. Materials and Methods

*2.1. Materials.* Wheat varieties were introduced from abroad. The drought resistance level combining four yield traits (plant height, spike length, grain numbers per spike, and 1000-grain weight) and four quality traits (crude protein content, lysine content, sedimentation, and hardness) of 1,683 varieties have been investigated. All data were downloaded from "Chinese Crop Germplasm Resources Information System" (http://icgr.caas.net.cn/).

*2.2. Core Collection Construction.* According to the drought resistance level, all 1,683 wheat varieties were divided into five germplasm groups (high resistant, HR; resistant, *R*; moderate resistant, MR; susceptible, *S*; and high susceptible, HS). The distribution of varieties was shown in Figure 1. The procedure for core collection construction was conducted by two steps. First, subcore collections were selected from each germplasm groups. Second, all the sub-core collections were combined together to construct a core collection.

The least distance stepwise sampling (LDSS) method [14] was adopted to construct sub-core collections from germplasm groups. The procedure was as follows. (1) The genetic distances among accessions were calculated, and accessions were classified by hierarchical cluster analysis based on their genetic distance. (2) One accession from a subgroup with the least distance was randomly removed, and another accession of the subgroup was sampled. (3) The genetic distances among the remaining accessions were calculated, and the sampling was repeated in the same way. The stepwise samplings were performed until the percentage of the remaining accessions reached the desired one. This method performs sampling based on the subgroup with the least genetic distance, which can efficiently eliminate redundant accessions and ignore the effect of the cluster methods [15].

*2.3. Genetic Distances and Evaluating Parameters.* Six commonly used genetic distances (Euclidean distance, Euclid; Standardized Euclidean distance, Seuclid; Mahalanobis distance, Mahal; Manhattan distance, Manhat; Cosine distance, Cosine; and Correlation distance, Correlation) were used to assess genetic distances among accessions. Unweighted pair-group average (UPGMA) method was used for performing hierarchical cluster analysis [15].

Coincidence rate of range (CR) and variable rate of coefficient of variation (VR) [16, 17] were adopted to evaluate the representativeness of core collection. Those four parameters were formulated as follows: $\mathrm{CR} = (1/n)\sum_{i=1}^{n}(R_{C(i)}/R_{I(i)}) \times 100$, where $R_{C(i)}$ is the range of the $i$th trait in the core collection; $R_{I(i)}$ is the range of the corresponding trait in the initial collection; $n$ and is total number of traits, $\mathrm{VR} = (1/n)\sum_{i=1}^{n}(\mathrm{CV}_{C(i)}/\mathrm{CV}_{I(i)}) \times 100$, where $\mathrm{CV}_{C(i)}$ is the coefficient of variation of the $i$th trait in the core collection; $\mathrm{CV}_{I(i)}$ is the coefficient of variation of the corresponding trait in the initial collection; $n$ is total number of traits.

*2.4. Data Analysis.* The genetic distances calculation, the LDSS procedures, and the evaluating parameters calculation were performed using computer code programmed by the authors based on MATLAB software (version 6.5) [18].

## 3. Results

*3.1. The Assessment of Subcore Collections Constructed by Different Genetic Distances.* Subcore collections were constructed by different genetic distances at the sampling percentage of 10%, 20%, and 30% (Table 1). In any germplasm group, CR and VR of sub-core collections constructed by the genetic distance of Cosine and Correl were much lower than of those constructed by the other four genetic distances at the three sampling percentages (Table 1). In HR group, subcore collections constructed by Manhat had larger CR and VR than those constructed by Euclid, Seuclid, and Mahal at the three sampling percentages (Table 1). In *R* group, sub-core collections constructed by Euclid had the largest CR at the sampling percentage of 10% and 30%, and those constructed by Manhat had the largest VR at the sampling percentage of 10% and 20% (Table 1). In MR group, sub-core collections constructed by Mahal had the largest CR at the sampling percentage of 20% and 30%, and those constructed by Seuclid had the largest VR at the sampling percentage

Table 1: The values of CR and VR of different subcore collection constructed by six genetic distances at the sampling percentage of 10%, 20%, and 30%.

| Parameter | DRL[a] | SP[b] | Genetic distance | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Euclid | Seuclid | Mahal | Manhat | Cosine | Correl |
| CR (%) | HR | 10% | 76.17 | 87.16 | 80.48 | 82.36 | 51.34 | 52.54 |
| | | 20% | 89.55 | 91.96 | 88.77 | 91.98 | 71.08 | 70.14 |
| | | 30% | 95.78 | 94.17 | 93.76 | 97.59 | 77.57 | 80.69 |
| | *R* | 10% | 91.51 | 81.60 | 84.16 | 89.24 | 60.62 | 59.34 |
| | | 20% | 95.98 | 86.40 | 92.55 | 96.09 | 73.60 | 65.00 |
| | | 30% | 98.93 | 96.11 | 93.64 | 96.88 | 81.25 | 72.20 |
| | MR | 10% | 94.20 | 92.61 | 94.58 | 95.37 | 58.36 | 70.18 |
| | | 20% | 97.41 | 96.27 | 98.00 | 97.10 | 66.23 | 78.06 |
| | | 30% | 98.37 | 97.35 | 99.31 | 97.11 | 75.63 | 86.26 |
| | *S* | 10% | 96.46 | 96.91 | 96.05 | 95.24 | 69.75 | 65.32 |
| | | 20% | 97.81 | 99.58 | 98.92 | 99.46 | 75.42 | 72.74 |
| | | 30% | 98.04 | 99.58 | 99.35 | 99.49 | 82.47 | 77.19 |
| | HS | 10% | 95.33 | 93.92 | 95.88 | 93.88 | 61.97 | 63.68 |
| | | 20% | 98.65 | 96.43 | 97.30 | 96.81 | 80.81 | 73.24 |
| | | 30% | 99.20 | 97.82 | 97.82 | 99.20 | 82.20 | 82.80 |
| VR (%) | HR | 10% | 122.67 | 127.61 | 124.51 | 131.10 | 85.27 | 79.12 |
| | | 20% | 122.60 | 120.78 | 119.30 | 125.19 | 92.14 | 90.12 |
| | | 30% | 117.73 | 119.07 | 113.57 | 122.80 | 91.74 | 91.82 |
| | *R* | 10% | 129.66 | 118.85 | 118.24 | 132.61 | 90.21 | 82.94 |
| | | 20% | 119.06 | 115.52 | 117.51 | 120.73 | 88.99 | 82.92 |
| | | 30% | 112.89 | 114.32 | 113.78 | 113.18 | 90.82 | 88.82 |
| | MR | 10% | 128.26 | 128.60 | 126.81 | 128.51 | 80.95 | 90.70 |
| | | 20% | 119.56 | 122.15 | 120.58 | 120.39 | 85.01 | 92.93 |
| | | 30% | 116.79 | 116.78 | 113.45 | 115.29 | 89.06 | 92.51 |
| | *S* | 10% | 130.14 | 129.53 | 126.03 | 127.10 | 90.40 | 85.16 |
| | | 20% | 120.64 | 120.67 | 119.72 | 121.22 | 88.94 | 88.29 |
| | | 30% | 115.44 | 115.40 | 115.53 | 114.36 | 92.36 | 90.24 |
| | HS | 10% | 125.25 | 128.97 | 133.16 | 124.54 | 89.94 | 90.06 |
| | | 20% | 122.00 | 122.77 | 122.13 | 120.35 | 92.00 | 91.91 |
| | | 30% | 117.09 | 116.11 | 117.42 | 115.94 | 87.68 | 93.25 |

[a]DRL: drought resistance level (HR: high resistant; *R*: resistant; MR: moderate resistant; *S*: susceptible; and HS: high susceptible).
[b]SP: sampling percentage.

of 10% and 20%, but similar VR than that constructed by Euclid at the sampling percentage of 30% (Table 1). In S group, sub-core collections constructed by Seuclid had the largest CR at the three sampling percentage, while there was no significant pattern in VR (Table 1). In HS group, sub-core collections constructed by Euclid had the largest CR at the sampling percentage of 20% and 30%, and those constructed by Mahal had the largest VR at the sampling percentage of 10% and 30% (Table 1). Synthesizing the results above, five ideal combinations for sub-core collection were selected: HR-Manhat, *R*-Euclid, MR-Mahal, *S*-Seuclid, and HS-Euclid.

*3.2. Selection of the Optimal Sampling Percentage.* In each germplasm group, sub-core collections were constructed based on the selected genetic distance with the sampling percentage increasing from 5% to 30%. The value of CR of each sub-core collection was calculated. Thus, 26 CRs were calculated in each group. The constructing results of the five groups were summarized in Figure 2. In each group, the CR showed logarithmic changing. The CR increased drastically when the sampling percentage was small. With the sampling percentage increasing, CR increased steady (Figure 2). The rangeability in the group of HR and *R* was larger than that in the groups of MR, *S*, and HS (Figure 2).

Each curve of in Figure 2 was treated by curve fitting analysis, and the results were summarized in Table 2. The equations showed logarithmic form, and the coefficient of determination of fitted equations ($R^2$) of each equation was larger than 0.9 (Table 2). Based on the equations, the optimal sampling percentage was calculated by setting the value of CR (%) to 95.00 (Table 2).

*3.3. Validation of the Ideal Constructing Strategy.* The principal component analysis was adopted to validate sub-core

Table 2: The logarithmic equations on the CR's value responded to the sampling percentage in five combinations of subcore collection construction. The optimal sampling percentage was calculated by the equation when the value of CR (%) was set to 95.00.

| Combination[a] | Equation[b] | $R^{2}$[c] | Optimal sampling percentage (%) |
|---|---|---|---|
| HR-Manhat | $y = 12.19\ln(x) + 58.41$ | 0.9732 | 20.12 |
| *R*-Euclid | $y = 6.52\ln(x) + 78.17$ | 0.9616 | 13.21 |
| MR-Mahal | $y = 2.67\ln(x) + 90.16$ | 0.9451 | 6.13 |
| *S*-Seuclid | $y = 2.80\ln(x) + 91.16$ | 0.9392 | 3.94 |
| HS-Euclid | $y = 2.47\ln(x) + 91.14$ | 0.9498 | 4.77 |

[a]HR-Manhat: high resistant group combining Manhattan distance; *R*-Euclid: resistant group combining Euclidean distance; MR-Mahal: moderate resistant group combining Mahalanobis distance; *S*-Seuclid: susceptible group combining Standardized Euclidean distance; and HS-Euclid: high susceptible group combining Euclidean distance.

[b]$x$: the sampling percentage (%); $y$: the value of CR (%).

[c]$R^2$: coefficient of determination of fitted equations.

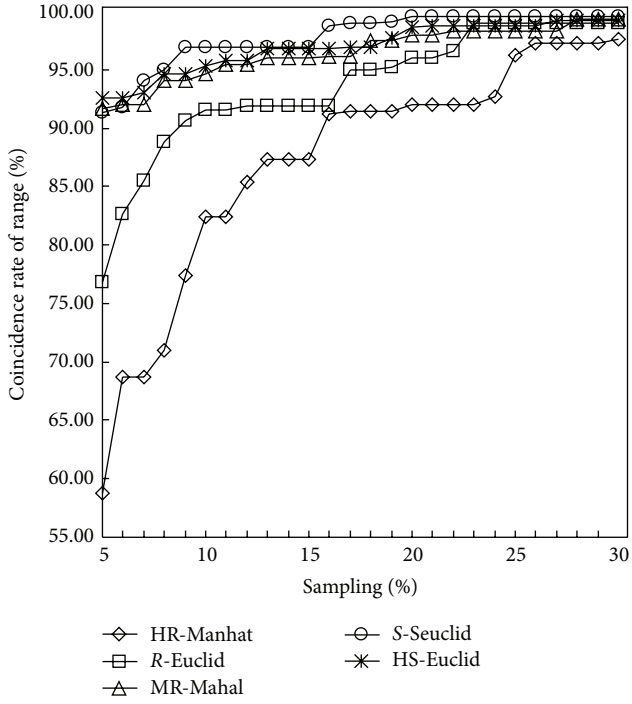


Figure 2: The coincidence rate of the range (CR) of subcore collections constructed by five combinations with the sampling percentage increasing from 5% to 30%. HR-Manhat, high resistant group combining Manhattan distance; *R*-Euclid, resistant group combining Euclidean distance; MR-Mahal, moderate resistant group combining Mahalanobis distance; *S*-Seuclid, susceptible group combining Standardized Euclidean distance; the HS-Euclid, high susceptible group combining Euclidean distance.

collections constructed by the ideal strategy selected by the present research. Principal component plots of core accessions and reserve accessions in each germplasm group were drown in Figure 3. The total genetic variation percentage of the first two principal components was 71.51% in HR group, 67.67% in *R* group, 66.90% in MR group, 68.45% in *S* group, and 71.83% in HS group. At the same sampling percentage, compared to the sub-core collections constructed by complete random selection, the core accessions selected by the present strategy showed more symmetrical distribution in the whole germplasm group, and most extreme accessions were selected (Figure 3).

## 4. Discussion

Core collection has been studied for about twenty years [19, 20]. A valid core collection provides a high-efficient way to assess genetic diversity or to find beneficial genes [21–24]. Most core collection researches focused on finding efficient ways in sub-core collection selection [25–27]. However, there is not a widely accepted strategy for constructing sub-core collection up to now. One common approach for constructing a core collection is splitting the whole germplasm population into several groups, then, selecting representative core accessions from each group to form sub-core collections, and combining all sub-core collections to form the final core collection [16, 28]. The present research divided the whole wheat germplasm population into five groups based on drought resistance level. The results showed that the distribution pattern of accessions was various in different germplasm group, which might lead to different suitable strategy for sub-core collection construction. Therefore, different germplasm group required different constructing strategy, and it is needlessly to try to find a widely accepted constructing strategy.

The representativeness is the most important character for a core collection. The VR represents the difference of variance between core collection and the initial collection. The value of VR is affected greatly by the number of accessions in the core collection. In core collection construction based on a valid strategy, with the sampling percentage increasing, the variance decreased and the mean almost keeps unchanging, which led to the decrease of VR. However, at the same sampling percentage, bigger VR means more variation preserved in core collection. The CR shows the extent of preservation of the trait scope in a core collection. The value of CR is not affected greatly by the number of accessions. In the present research, the CR showed sensitivity to the representativeness of a sub-core collection. The CR has been reported to be an important parameter for the evaluation of the representativeness of the core collections [9, 29, 30]. Based on the above analysis, the ideal genetic distance for different

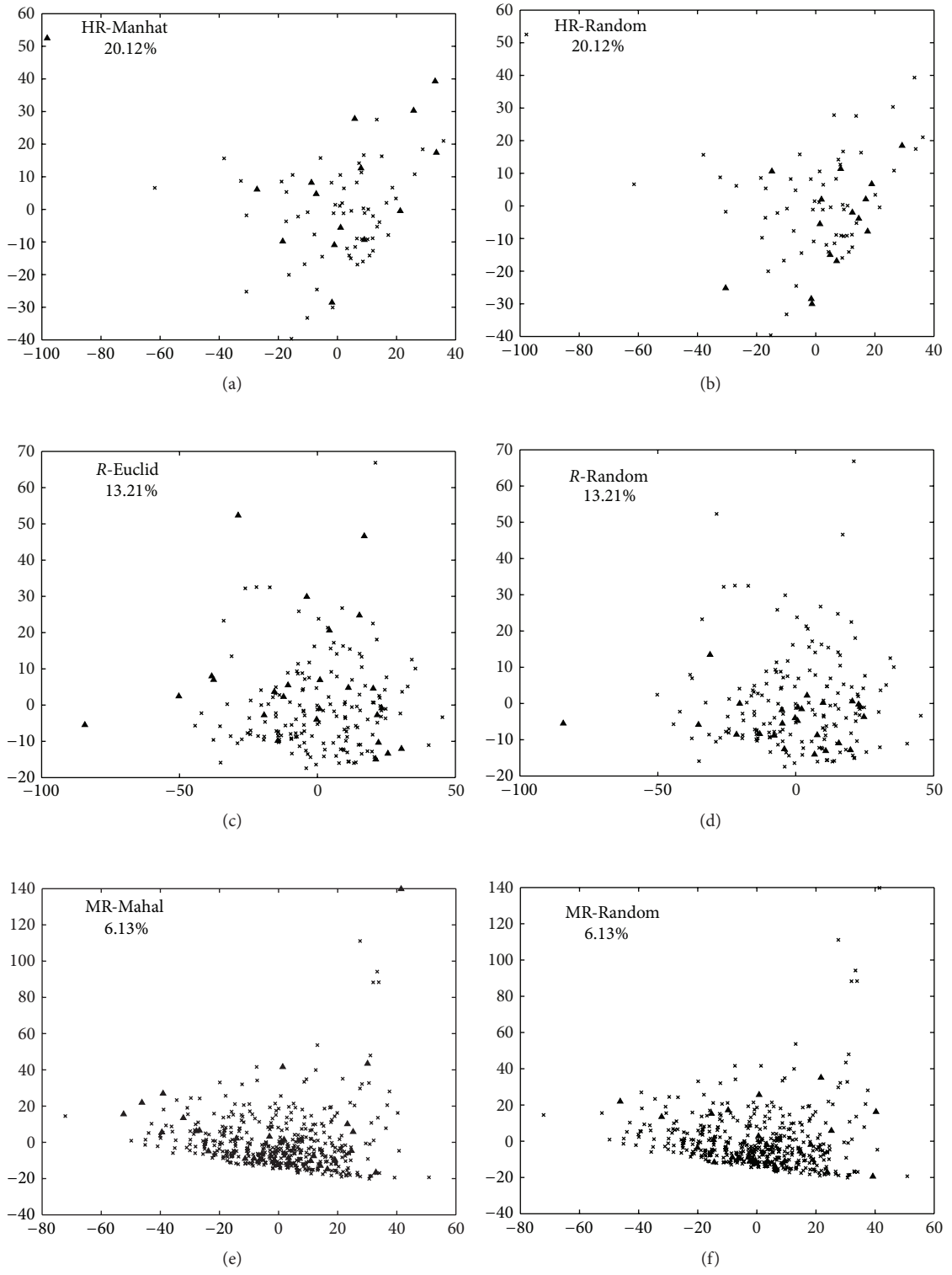


Figure 3: Continued.

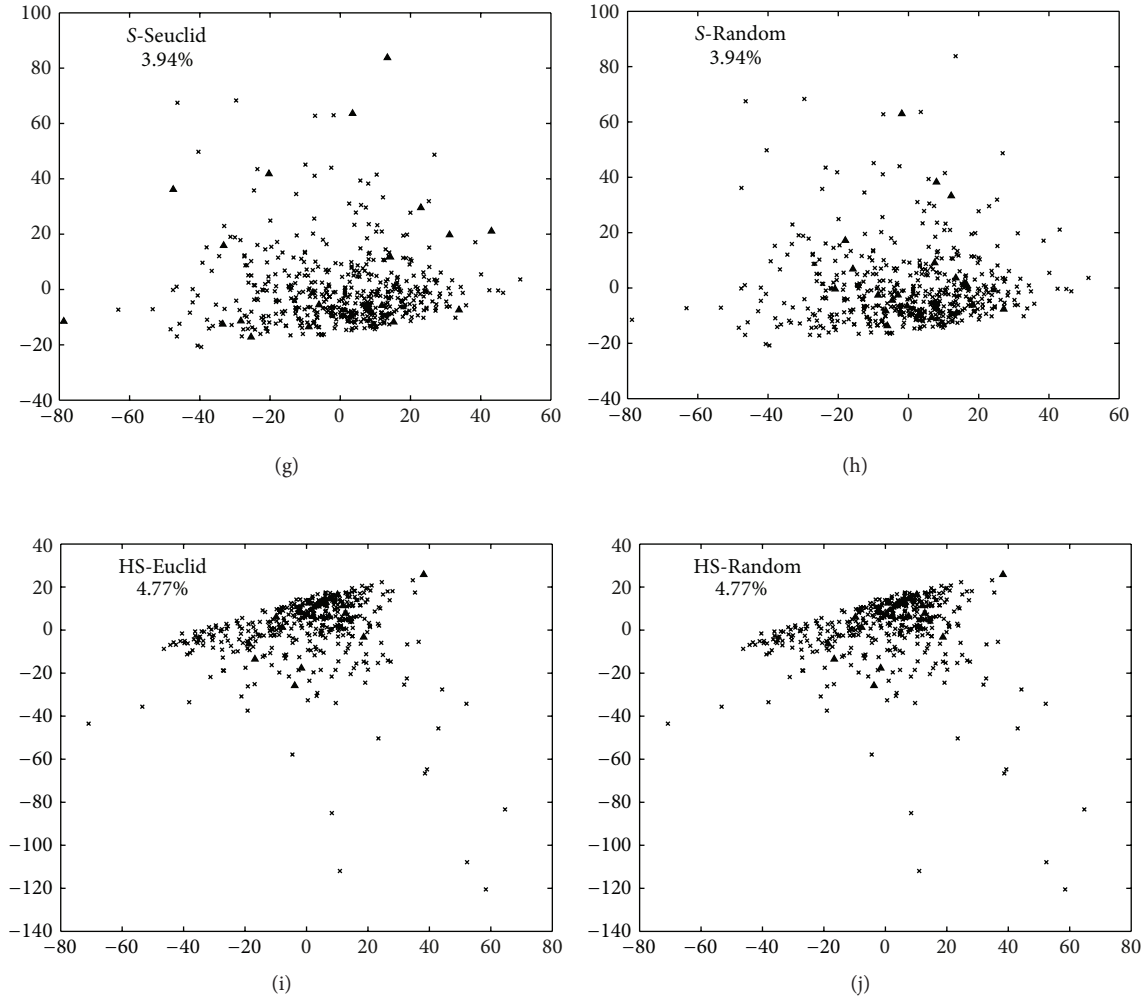


Figure 3: Principal component plots of core accessions and reserve accessions in the sampling percentage. The upward pointing triangles represented the core accessions; the crosses represented the reserved accessions. The left column showed plots for subcore collection constructed by LDSS method based on the selected genetic distance and sampling percentage; the right column showed plots for subcore collection constructed by complete random selection based on the same sampling percentage. HR-Manhat, high resistant group combining Manhattan distance; *R*-Euclid, resistant group combining Euclidean distance; MR-Mahal, moderate resistant group combining Mahalanobis distance; *S*-Seuclid, susceptible group combining Standardized Euclidean distance; and HS-Euclid, high susceptible group combining Euclidean distance.

group was determined first by CR, then by VR. Moreover, a genetic distance that could make higher CR at low sampling percentage might be more valid than others.

In the present research, data of eight agronomic traits in 1,683 wheat varieties were downloaded from public database of "Chinese Crop Germplasm Resources Information System." Such a big number of wheat germplasm might not be planted within one area or one year. Therefore, the upper data might not be collected based on the same cultivating standards, which might affect the precision of the final core collection. However, there were more than one agronomic trait used in the present research. Data of eight agronomic traits were used to calculate CR and VR. The two evaluating parameters reflected the mean representativeness of the eight agronomic traits in the core collection, which reduced the error mentioned above. A wheat core collection for the drought resistance breeding programs was constructed by the strategy selected in the present research based on the upper dataset. Table 2 showed the optimal genetic distance and the relative optimal sampling percentage for sub-core collection in each germplasm group. Therefore, the whole core collection was constructed by combining all sub-core collections. The principal component analysis showed that the genetic diversity was well preserved in that core collection. The method for the ideal constructing strategy selection suggested in the present research is also valuable in other crop's core collection construction.

## Authors' Contribution

Jiancheng Wang and Yajing Guan contributed equally to this work.

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## References

[1] C. Saint Pierre, J. L. Crossa, D. Bonnett, K. Yamaguchi-Shinozaki, and M. P. Reynolds, "Phenotyping transgenic wheat for drought resistance," *Journal of Experimental Botany*, vol. 63, no. 5, pp. 1799–1808, 2012.

[2] L. Simova-Stoilova, I. Vaseva, B. Grigorova, K. Demirevska, and U. Feller, "Proteolytic activity and cysteine protease expression in wheat leaves under severe soil drought and recovery," *Plant Physiology and Biochemistry*, vol. 48, no. 2-3, pp. 200–206, 2010.

[3] K. Bürling, Z. G. Cerovic, G. Cornic, J. M. Ducruet, G. Noga, and M. Hunsche, "Fluorescence-based sensing of drought-induced stress in the vegetative phase of four contrasting wheat genotypes," *Environmental and Experimental Botany*, vol. 89, no. 3, pp. 51–59, 2013.

[4] I. C. Dodd, W. R. Whalley, E. S. Ober, and M. A. J. Parry, "Genetic and management approaches to boost UK wheat yields by ameliorating water deficits," *Journal of Experimental Botany*, vol. 62, no. 15, pp. 5241–5248, 2011.

[5] A. Nouri, A. Etminan, J. A. T. da Silva, and R. Mohammadi, "Assessment of yield, yield-related traits and drought tolerance of durum wheat genotypes (*Triticum turjidum* var. durum Desf.)," *Australian Journal of Crop Science*, vol. 5, no. 1, pp. 8–6, 2011.

[6] M. Farooq, M. Irfan, T. Aziz, I. Ahmad, and S. A. Cheema, "Seed priming with ascorbic acid improves drought resistance of wheat," *Journal of Agronomy and Crop Science*, vol. 199, no. 1, pp. 12–22, 2013.

[7] C. Wendelboe-Nelson and P. C. Morris, "Proteins linked to drought tolerance revealed by DIGE analysis of drought resistant and susceptible barley varieties," *Proteomics*, vol. 12, no. 22, pp. 3374–3385, 2012.

[8] C. He, W. Zhang, Q. Gao, A. Yang, X. Hu, and J. Zhang, "Enhancement of drought resistance and biomass by increasing the amount of glycine betaine in wheat seedlings," *Euphytica*, vol. 177, no. 2, pp. 151–167, 2011.

[9] O. H. Frankel and A. H. D. Brown, "Plant genetics resources today: a critical appraisal," in *Crop Gentic Resources: Conservation and Evaluation*, J. H. W. Holden and J. T. Williams, Eds., pp. 249–257, George Allen and Unwin, London, UK,, 1984.

[10] C. Silvar, A. M. Casas, D. Kopahnke et al., "Screening the Spanish Barley Core Collection for disease resistance," *Plant Breeding*, vol. 129, no. 1, pp. 45–52, 2010.

[11] A. Biabani, L. Carpenter-Boggs, C. J. Coyne, L. Taylor, J. L. Smith, and S. Higgins, "Nitrogen fixation potential in global chickpea mini-core collection," *Biology and Fertility of Soils*, vol. 47, no. 6, pp. 679–685, 2011.

[12] D. Pino Del Carpio, R. K. Basnet, R. C. H. de Vos, C. Maliepaard, R. Visser, and G. Bonnema, "The patterns of population differentiation in a *Brassica rapa* core collection," *Theoretical and Applied Genetics*, vol. 122, no. 6, pp. 1105–1118, 2011.

[13] C. Wang, S. Chen, and S. Yu, "Functional markers developed from multiple loci in GS3 for fine marker-assisted selection of grain length in rice," *Theoretical and Applied Genetics*, vol. 122, no. 5, pp. 905–913, 2011.

[14] J. C. Wang, J. Hu, H. M. Xu, and S. Zhang, "A strategy on constructing core collections by least distance stepwise sampling," *Theoretical and Applied Genetics*, vol. 115, no. 1, pp. 1–8, 2007.

[15] J. C. Wang, J. Hu, Y. J. Guan, and Y. F. Zhu, "Effect of the scale of quantitative trait data on the representativeness of a cotton germplasm sub-core collection," *Journal of Zhejiang University-SCIENCE B*, vol. 14, no. 2, pp. 162–170, 2012.

[16] J. C. Wang, J. Hu, X. X. Huang, and S. C. Xu, "Assessment of different genetic distances in constructing cotton core subset by genotypic values.," *Journal of Zhejiang University-SCIENCE B*, vol. 9, no. 5, pp. 356–362, 2008.

[17] Y. J. Mei, J. P. Zhou, H. M. Xu, and S. J. Zhu, "Development of Sea Island cotton (*Gossypium barbadense* L.) core collection using genotypic values," *Australian Journal of Crop Science*, vol. 6, no. 4, pp. 673–680, 2012.

[18] The MathWorks Inc., *MATLAB Software*, The MathWorks Inc., Natick, Mass, USA, 2002.

[19] J. Bordes, C. Ravel, J. P. Jaubertie et al., "Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection," *Theoretical and Applied Genetics*, vol. 126, no. 3, pp. 805–822, 2013.

[20] A. Diederichsen, P. M. Kusters, D. Kessler, Z. Bainas, and R. Gugel, "Assembling a core collection from the flax world collection maintained by Plant Gene Resources of Canada," *Genetic Resources and Crop Evolution*, no. 1, pp. 1–7, 2012.

[21] Y. X. Zhang, X. R. Zhang, Z. Che, L. H. Wang, W. L. Wei, and D. H. Li, "Genetic diversity assessment of sesame core collection in China by phenotype and molecular markers and extraction of a mini-core collection," *BMC Genetics*, vol. 13, no. 1, p. 102, 2012.

[22] M. Sharma, A. Rathore, U. N. Mangala et al., "New sources of resistance to Fusarium wilt and sterility mosaic disease in a mini-core collection of pigeonpea germplasm," *European Journal of Plant Pathology*, vol. 133, no. 3, pp. 707–714, 2012.

[23] G. Saeidi, M. Rickauer, and L. Gentzbittel, "Tolerance for cadmium pollution in a core-collection of the model legume, Medicago truncatula L. at seedling stage," *Australian Journal of Crop Science*, vol. 6, no. 4, pp. 641–648, 2012.

[24] P. E. McClean, J. Terpstra, M. McConnell, C. White, R. Lee, and S. Mamidi, "Population structure and genetic differentiation among the USDA common bean (*Phaseolus vulgaris* L.) core collection," *Genetic Resources and Crop Evolution*, pp. 1–17, 2011.

[25] R. R. Coimbra, G. V. Miranda, C. D. Cruz, D. J. H. Silva, and R. A. Vilela, "Development of a Brazilian maize core collection," *Genetics and Molecular Biology*, vol. 32, no. 3, pp. 538–545, 2009.

[26] E. S. Rao, P. Kadirvel, R. C. Symonds, S. Geethanjali, and A. W. Ebert, "Using SSR markers to map genetic diversity and population structure of Solanum pimpinellifolium for development of a core collection," *Plant Genetic Resources*, vol. 1, no. 1, pp. 1–11, 2011.

[27] P. A. Reeves, L. W. Panella, and C. M. Richards, "Retention of agronomically important variation in germplasm core collections: implications for allele mining," *Theoretical and Applied Genetics*, vol. 124, no. 6, pp. 1155–1171, 2012.

[28] C. M. Díez, A. Imperato, L. Rallo, D. Barranco, and I. Trujillo, "Worldwide core collection of olive cultivars based on simple sequence repeat and morphological markers," *Crop Science*, vol. 52, no. 1, pp. 211–221, 2012.

[29] J. Hu, J. Zhu, and H. M. Xu, "Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops," *Theoretical and Applied Genetics*, vol. 101, no. 1-2, pp. 264–268, 2000.

[30] M. F. Oliveira, R. L. Nelson, I. O. Geraldi, C. D. Cruz, and J. F. F. de Toledo, "Establishing a soybean germplasm core collection," *Field Crops Research*, vol. 119, no. 2-3, pp. 277–289, 2010.

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*Research Article*

# Physiological and Biochemical Responses of *Ulva prolifera* and *Ulva linza* to Cadmium Stress

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Responses of *Ulva prolifera* and *Ulva linza* to $Cd^{2+}$ stress were studied. We found that the relative growth rate (RGR), Fv/Fm, and actual photochemical efficiency of PSII (Yield) of two *Ulva* species were decreased under $Cd^{2+}$ treatments, and these reductions were greater in *U. prolifera* than in *U. linza*. *U. prolifera* accumulated more cadmium than *U. linza* under $Cd^{2+}$ stress. While *U. linza* showed positive osmotic adjustment ability (OAA) at a wider $Cd^{2+}$ range than *U. prolifera*. *U. linza* had greater contents of N, P, $Na^+$, $K^+$, and amino acids than *U. prolifera*. A range of parameters (concentrations of cadmium, $Ca^{2+}$, N, P, $K^+$, $Cl^-$, free amino acids (FAAs), proline, organic acids and soluble protein, Fv/Fm, Yield, OAA, and $K^+/Na^+$) could be used to evaluate cadmium resistance in *Ulva* by correlation analysis. In accordance with the order of the absolute values of correlation coefficient, contents of $Cd^{2+}$ and $K^+$, Yield, proline content, Fv/Fm, FAA content, and OAA value of *Ulva* were more highly related to their adaptation to $Cd^{2+}$ than the other eight indices. Thus, *U. linza* has a better adaptation to $Cd^{2+}$ than *U. prolifera*, which was due mainly to higher nutrient content and stronger OAA and photosynthesis in *U. linza*.

## 1. Introduction

Heavy metal contamination is an environmental problem in the margin sea [1]. As the economy in Asian countries continues to grow, the release of heavy metals and other contaminants has increased noticeably [2, 3]. Due to their acute toxicity, cadmium (Cd), lead, and mercury are among the most hazardous metals to the environment and living things [4].

Cd, an oxophilic and sulfophilic element, forms complexes with various organic particles and thereby triggers a wide range of reactions that collectively put the aquatic ecosystems at risk. Cadmium also poses a serious threat to human health due to its accumulation in the food chain [5, 6]. It has been classified as group (I) a human carcinogen by the International Agency for Research on Cancer (IARC) [7]. Cadmium toxicity may be characterized by a variety of syndromes and effects, including renal dysfunction, hypertension, hepatic injury, lung damage, and teratogenic effects [8]. To remove Cd pollutants, various treatment technologies, such as precipitation, ion exchange, adsorption, and biosorption, have been employed [9]. Biosorption is one of the promising techniques for removal of heavy metals. Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physicochemical pathways of uptake [10]. Among the biological materials investigated for heavy metal removal, marine macroalgae have high uptake capacities for a number of heavy metal ions [11, 12].

Green algae species of Ulvaceae, especially the members of the green algal genus *Ulva*, have been considered as monitors of heavy metals in estuaries [13–15]. Numerous studies have shown that green macroalgae such as *Ulva lactuca* are able to absorb Cd. These studies mainly focused

on metabolism-independent Cd accumulation [6], synthetic surfactants exerting impact on uptake of Cd [12], effect of pH, contact time, biomass dosage and temperature on the Cd uptake kinetics [2], and induced oxidative stress by Cd [7]. However, little information is available regarding physiological responses of different *Ulva* species to increased $Cd^{2+}$ concentrations.

In this study, *Ulva prolifera* and *Ulva linza* were studied for their responses to different $Cd^{2+}$ concentrations. Their growth, chlorophyll fluorescence parameters, osmotic adjustment ability, and accumulation of inorganic ions and organic solutes were investigated in indoor seawater culture systems. The specific objective of this study was to determine if there was species variation in $Cd^{2+}$ adaptation, and what were the major physiological parameters involved in the adaptation.

## 2. Materials and Methods

*2.1. The Seaweed Collection, Cultivation, and $Cd^{2+}$ Treatment.* Green algae were collected from the sea in Dafeng (*Ulva prolifera*) and Lianyungang (*Ulva linza*), Jiangsu province, China. Upon arrival in the laboratory, the seaweeds were washed with distilled water and then cultured in 250 mL flasks containing 200 mL of sterilized artificial seawater (33.33 psu, pH 8.0) enriched with VSE medium [16] for 5 d. The composition of artificial seawater was (g $L^{-1}$) $HCO_3^-$ 0.25, $SO_4^{2-}$ 3.84, $Cl^-$ 17.45, $Ca^{2+}$ 0.76, $Mg^{2+}$ 1.00, $K^+$ 0.57, and $Na^+$ 9.46. The composition of VSE nutrient solution was (mg $L^{-1}$) $NaNO_3$ 42.50, $Na_2HPO_4 \cdot 12H_2O$ 10.75, $FeSO_4 \cdot 7H_2O$ 0.28, $MnCl_2 \cdot 4H_2O$ 0.02, $Na_2EDTA \cdot 2H_2O$ 3.72, vitamin $B_1$ 0.20, Biotin 0.001, and vitamin $B_{12}$ 0.001. After 5 d acclimation, healthy samples (0.5 g fresh weight) were cultured in 250 mL flasks with 200 mL medium as described earlier. $CdCl_2$ was added to each flask at the following concentrations: 0, 5, 10, 20, 40, 80, or 120 $\mu$mol $L^{-1}$. After 7 d treatment, *U. prolifera* and *U. linza* were harvested and analyzed for selected parameters as described later. All experiments were performed in three replicates. During the preculture and the treatment, seaweeds were grown in a GXZ intelligent light incubator at temperature of 20 ± 1°C, light intensity of 50 $\mu$mol $m^{-2}$ $s^{-1}$, and photoperiod of 12/12 h. The culture medium was altered every other day.

*2.2. Measurement of Relative Growth Rate (RGR).* Fresh weight was determined by weighing the algae after blotting by absorbent paper. RGR was calculated according to the formula RGR (% $d^{-1}$) = $[\ln(M_t/M_0)/t] \times 100\%$, where $M_0$ and $M_t$ are the fresh weights (g) at days 0 and 7, respectively [17].

*2.3. Measurement of Osmotic Adjustment Ability (OAA).* Saturated osmotic potential was measured by the freezing-point depression principle. Seaweeds were placed in double-distilled water for 8 h and then rinsed 5 times with double-distilled water. After blotting dry with absorbent paper, seaweeds were dipped into liquid nitrogen for 20 min. The frozen seaweeds were thawed in a syringe for 50 min, and the seaweed sap was then collected by pressing the seaweed in the syringe [18]. The $\pi_{100}$ was measured by using a fully automatic freezing-point osmometer (8P, Shanghai, China). OAA was calculated by the following equation:

$$\Delta\pi_{100} = \pi_{100}^{\mu} - \pi_{100}^{s}, \quad (1)$$

whereby $\pi_{100}^{\mu}$ was the $\pi_{100}$ of control seaweeds, and $\pi_{100}^{s}$ was the $\pi_{100}$ of $Cd^{2+}$-stressed seaweeds.

*2.4. Measurements of Chlorophyll (Chl) and Carotenoid (Car) Contents.* Determination of Chl and Car was carried out by the method of Häder et al. [19]. Weighed 0.1 g fresh seaweeds were cut with scissors and extracted with 95% (v/v) ethanol (10 mL) in the dark for 24 h. The absorbance of pigment extract was measured at wavelengths of 470, 649, and 665 nm with a spectrophotometer. From the measured absorbance, concentrations of Chl a, Chl b, and Car were calculated on a weight basis.

*2.5. Determination of Chlorophyll Fluorescence Parameters.* A PHYTO-PAM Phytoplankton Analyzer (PAM 2003, Walz, Effeltrich, Germany) was used to determine *in vivo* chlorophyll fluorescence from chlorophyll in photosystem II (PSII) using different experimental protocols [19]. Before determination, samples were adapted for 15 min in the total darkness to complete reoxidation of PSII electron acceptor molecules. The maximal photochemical efficiency of PSII (Fv/Fm) and the actual photochemical efficiency of PSII in the light (Yield) were then determined.

*2.6. Measurement of Nitrogen (N) and Phosphorous (P) Concentrations.* Dried samples were ground in a mortar and pestle. Total N in seaweed tissue was analyzed by an N gas analyzer using an induction furnace and thermal conductivity. Total P in seaweed tissue was quantitatively determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Optima 2100 DV, PerkinElmer, USA) following nitric acid/hydrogen peroxide microwave digestion. The total amounts of N and P in the seaweed tissue were calculated by multiplying N and P contents in tissue as a proportion of dry weight by the total dry weight of the sample [20].

*2.7. Measurement of Inorganic Elements.* After 7 d, seaweeds were harvested, washed, and oven-dried at 65°C for 3 d. A 50 mg sample was ashed in a muffle furnace. The ash was dissolved in 8 mL of $HNO_3 : HClO_4$ (3 : 1, v : v) and diluted to 50 mL with distilled water. The contents of Cd, Na, K, Ca, and Mg were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Optima 2100 DV, PerkinElmer, USA) [21]. To determine Cl content, the ash was dissolved in 100 mL distilled water and analyzed by potentiometric titration with silver nitrate ($AgNO_3$) [18]. Total nitrate was measured as described previously [22] with nitrate extracted from the tissue by boiling fresh seaweeds (20 mg) in distilled water (400 $\mu$L) for 20 min. The nitrate concentrations in the samples were measured spectrophotometrically at 540 nm.

*2.8. Measurement of Organic Solutes.* Soluble sugars (SS) determination was carried out by the anthrone method [23]. Water extract of fresh seaweeds was added to 0.5 mL of 0.1 mol $L^{-1}$ anthrone-ethyl acetate and 5 mL $H_2SO_4$. The mixture was heated at 100°C for 1 min, and its absorbance at 620 nm was read after cooling to room temperature. A calibration curve with sucrose was used as a standard. Soluble proteins (SPs) were measured by Coomassie Brilliant Blue G-250 staining [24]. Fresh seaweeds (0.5 g) were homogenized in 1 mL phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5,000 g for 10 min. An aliquot of 0.5 mL of freshly prepared trichloroacetic acid (TCA) was added and mixture centrifuged at 8.000 g for 15 min. The pellets were dissolved in 1 mL of 0.1 mol $L^{-1}$ NaOH, and 5 mL of Bradford reagent was added. Absorbance was recorded at 595 nm using bovine serum albumin as a standard. Free amino acids (FAAs) were extracted and determined following the method of Zhou and Yu [23]. A total of 0.5 g fresh tissue was homogenized in 5 mL 10% (w/v) acetic acid, extracts were supplemented with 1 mL distilled water and 3 mL ninhydrin reagent, then boiled for 15 min and fast cooled, and the volume was made up to 5 mL with 60% (v/v) ethanol. Absorbance was read at 570 nm. The content of total free amino acids was calculated from a standard curve prepared using leucine. Proline (PRO) concentration was determined spectrophotometrically by adopting the ninhydrin method of Irigoyen et al. [25]. We first homogenized 300 mg fresh leaf samples in sulphosalicylic acid. To the extract, 2 mL each of ninhydrin and glacial acetic acid were added. The samples were heated at 100°C. The mixture was extracted with toluene, and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard. Organic acids (OAs) were extracted with boiling distilled water. The concentration of total OA was determined by 0.01 mmol $L^{-1}$ NaOH titration method, with phenolphthalein as indicator [26].

*2.9. Statistical Analyses.* All experiments were performed in three replicates. The data are presented as the mean ± SD. Data were analyzed using SPSS statistical software. Significant differences between means were determined by Duncan's multiple range test. Unless otherwise stated, differences were considered statistically significant when $P \leq 0.05$. Statistical analysis on two-way variance analysis (ANOVA), and correlation coefficient was performed using Microsoft Excel.

## 3. Results

*3.1. Effect of Cadmium Stress on RGR and OAA of U. prolifera and U. linza.* Compared to the control, treatments with 5 $\mu$mol $L^{-1}$ $Cd^{2+}$ for 7 d did not change RGR of *U. linza,* but significantly decreased RGR of *U. prolifera*. The RGR of both *Ulva* species was significantly decreased as $Cd^{2+}$ concentration increased. After 7 d exposure to 10, 20, 40, 80; or 120 $\mu$mol $L^{-1}$ $Cd^{2+}$, RGR of *U. linza* decreased by 53, 75, 116, 177, and 277%, respectively; *U. prolifera* decreased by 93, 139, 271, and 357%, respectively. *U. prolifera* died at 120 $\mu$mol $L^{-1}$ $Cd^{2+}$ on day 7 (Figure 1).

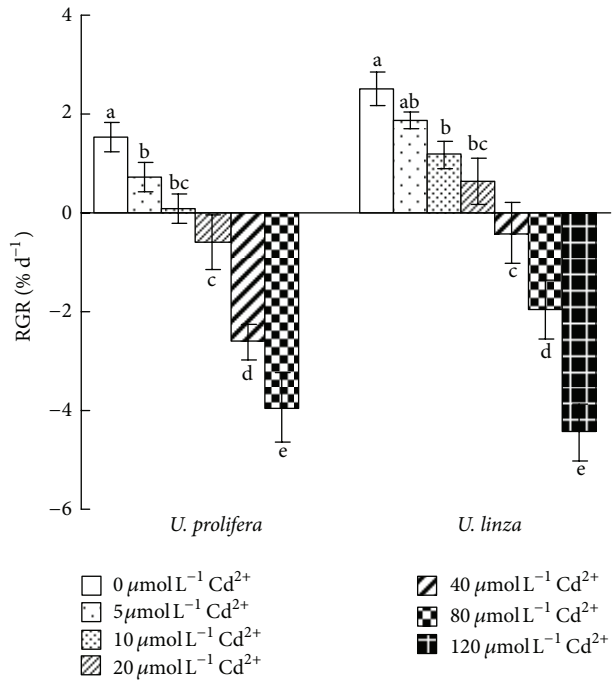


Figure 1: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 20, 40, 80, and 120 $\mu$mol $L^{-1}$) on relative growth rate (RGR) in *U. prolifera* and *U. linza.*

The OAA of both species was enhanced by low $Cd^{2+}$ concentration treatments. The enhancement occurred at 5 and 10 $\mu$mol $L^{-1}$ for *U. prolifera* and 5, 10 and 20 $\mu$mol $L^{-1}$ for *U. linza* (Figure 2). However, OAA was negative when *U. prolifera* was treated by 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, and *U. linza* treated by 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ (Figure 2).

*3.2. Effect of Cadmium Stress on Cadmium Content in U. prolifera and U. linza.* Cadmium contents in *U. prolifera* and *U. linza* increased as $Cd^{2+}$ concentrations increased (Figure 3). At 5, 10, 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, Cd contents in *U. prolifera* was 32, 78, 114, 140, and 165 times of the $Cd^{2+}$ = 0 treatment, respectively, and 10, 26, 44, 65, and 79 times of its control treatment in *U. linza*, respectively.

*3.3. Effect of Cadmium Stress on Chl and Car Contents in U. prolifera and U. linza.* Both Chl and Car contents decreased with the increased $Cd^{2+}$ concentration. There was no significant change in Chl and Car when both species were treated by 5 and 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ for 7 d. However, significant declines in Chl and Car contents were observed when they were exposed to 20, 40, or 80 $\mu$mol $L^{-1}$ $Cd^{2+}$. Compared to the control treatment, Chl contents decreased by 18, 25, and 45% at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ in *U. prolifera,* respectively; and the decreases were 16, 20, and 39% in *U. linza*, respectively (Figure 4(a)). The Car content declined by 16, 29 and 54% at 20, 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ in *U. prolifera*, respectively; and by 13, 16, and 44% in *U. linza*, respectively (Figure 4(b)).

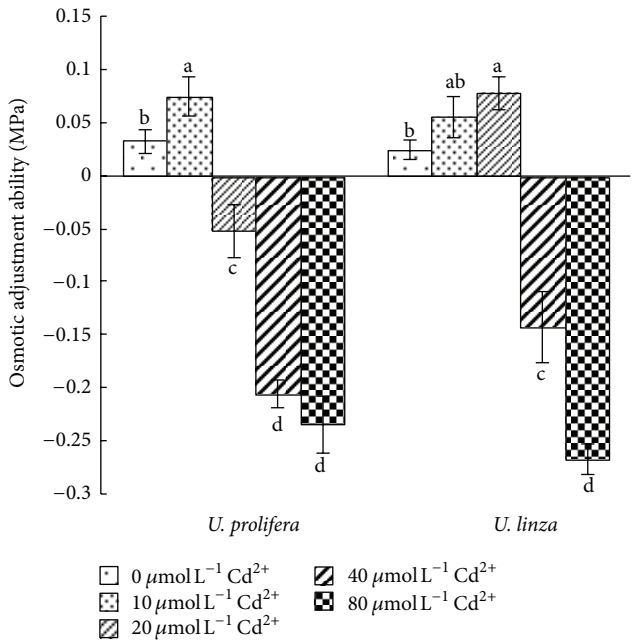


FIGURE 2: Effects of different concentrations of $Cd^{2+}$ (5, 10, 20, 40, and 80 $\mu$mol $L^{-1}$) on osmotic adjustment ability (OAA) of *U. prolifera* and *U. linza*.

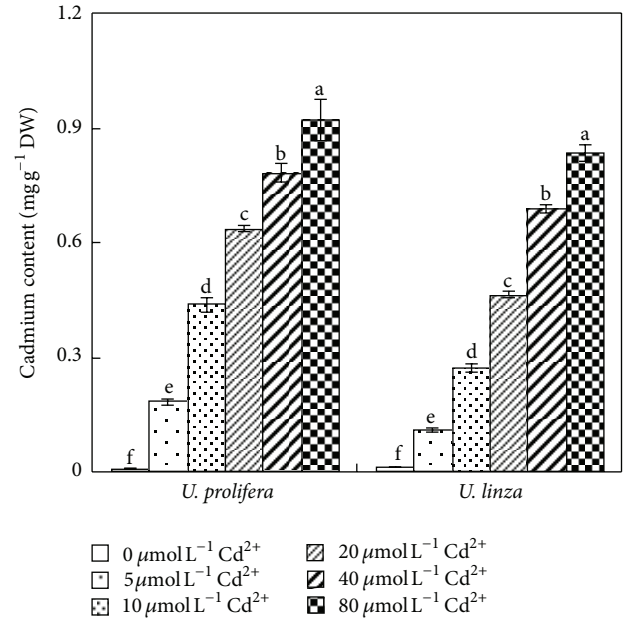


FIGURE 3: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 20, 40, and 80 $\mu$mol $L^{-1}$) on cadmium concentration of *U. prolifera* and *U. linza*.

*3.4. Effect of Cadmium Stress on Chlorophyll Fluorescence Parameters of U. prolifera and U. linza.* Compared to the control treatment, Fv/Fm of *U. prolifera* and *U. linza* were not significantly affected by the treatments of 5 or 10 $\mu$mol $L^{-1}$ $Cd^{2+}$. However, Fv/Fm of both *Ulva* species fell significantly when $Cd^{2+}$ concentrations reached 20 $\mu$mol $L^{-1}$. In comparison with the control, Fv/Fm of *U. prolifera* decreased 17, 22, and 31% at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$; whereas Fv/Fm of *U. linza* decreased 9, 10, and 15% after exposure to 20, 40, or 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, respectively (Figure 5(a)). For actual photochemical efficiency of PSII (Yield) of *U. prolifera*, there was an obvious decrease when $Cd^{2+}$ concentrations rose from 20 to 80 $\mu$mol $L^{-1}$; whereas Yield of *U. linza* showed no significant decline until $Cd^{2+}$ concentration was 80 $\mu$mol $L^{-1}$ (Figure 5(b)).

*3.5. Effect of Cadmium Stress on Contents of N and P in U. prolifera and U. linza.* Contents of N and P in both *Ulva* species showed a declining trend after an initial increase. The highest N content was recorded at 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ in *U. prolifera* and at 20 $\mu$mol $L^{-1}$ $Cd^{2+}$ in *U. linza*. N contents in *U. linza* in all $Cd^{2+}$ treatments were higher than those of control; however, in *U. prolifera*, N contents at 20, 40, or 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ were significantly decreased compared to the control (Figure 6(a)).

*U. prolifera* had the highest P concentration at 5 $\mu$mol $L^{-1}$ $Cd^{2+}$; but the highest P concentration was observed when *U. linza* was treated by 10 $\mu$mol $L^{-1}$ $Cd^{2+}$. The P contents decreased 31, 40, and 54% at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ in *U. prolifera,* respectively. Compared to the control, the P concentration of *U. linza* at 20 $\mu$mol $L^{-1}$ $Cd^{2+}$ increased significantly, and then decreased by 11 and 27% under 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, respectively (Figure 6(b)).

*3.6. Effect of Cadmium Stress on Inorganic Elements of U. prolifera and U. linza.* The $Na^+$ content of *U. prolifera* grown at 5 or 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ was not significantly different from the control, and it increased by 42, 67, and 83% at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, respectively. However, in *U. linza*, 5, 10, 20, and 40 $\mu$mol $L^{-1}$ $Cd^{2+}$ had no significant influence on $Na^+$ content, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ increased $Na^+$ content by 36% (Table 1). The $K^+$ content of *U. prolifera* grown at 5 or 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ remained unaffected compared to the control; it decreased significantly by 41, 45, and 62% at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, respectively. In *U. linza*, 5, 10, and 20 $\mu$mol $L^{-1}$ $Cd^{2+}$ had no significant influence on $K^+$ content, whereas 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ decreased $K^+$ content by 34 and 50%, respectively (Table 1). The $Ca^{2+}$ content of *U. prolifera* grown at 5, 10, 20, or 40 $\mu$mol $L^{-1}$ $Cd^{2+}$ remained unaffected, but increased significantly (24%) at 80 $\mu$mol $L^{-1}$ $Cd^{2+}$. However, in *U. linza*, 5 and 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ had no significant influence on $Ca^{2+}$ contents, whereas 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ increased $Ca^{2+}$ content by 22, 39, and 50%, respectively (Table 1). The $Mg^{2+}$ content of *U. prolifera* grown at 5, 10, 20, 40 or 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ remained unaffected. With increasing $Cd^{2+}$ concentrations, $Mg^{2+}$ contents of *U. linza* showed an increasing trend after an initial decline (Table 1). The $Cl^-$ contents appeared to have a declining trend with increasing $Cd^{2+}$ concentration similarly to Mg concentrations. However, no obvious difference in $Cl^-$

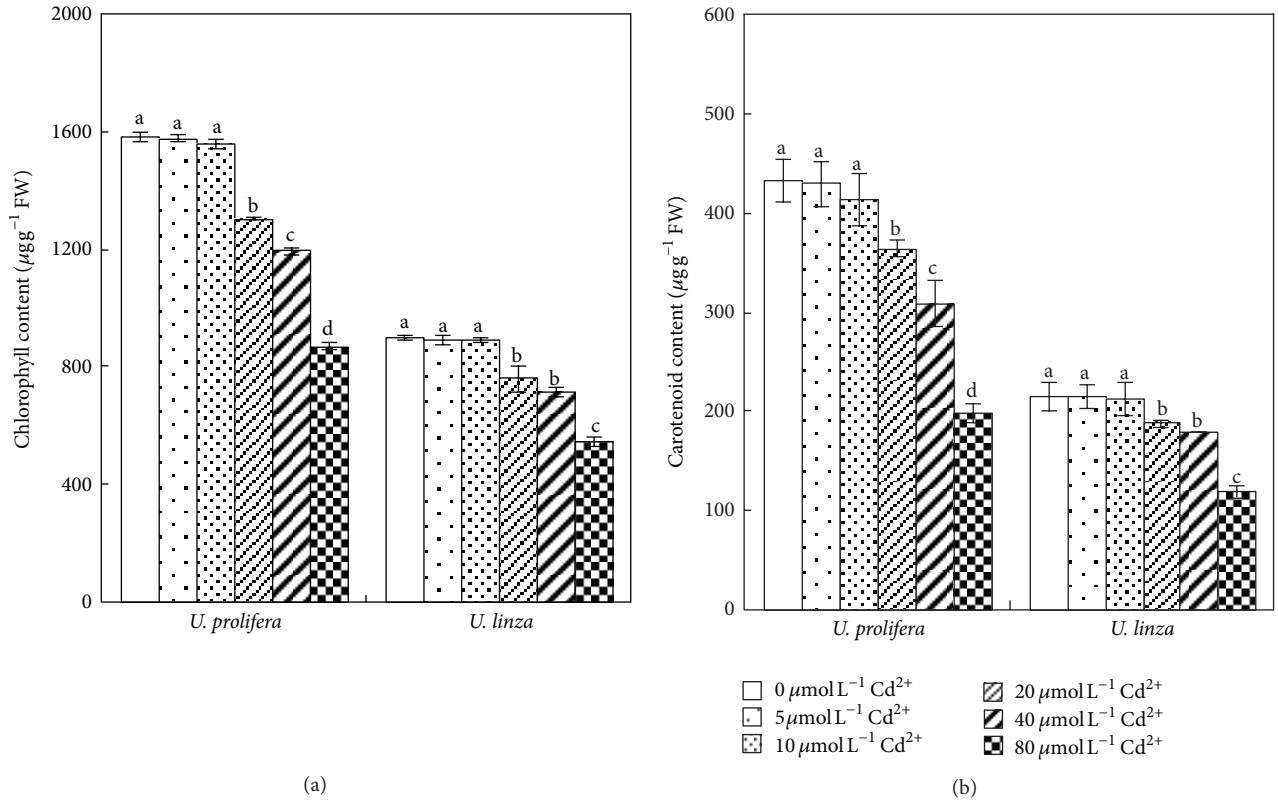


FIGURE 4: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 20, 40, and 80 $\mu$mol $L^{-1}$) on chlorophyll content (a) and carotenoid content (b) in *U. prolifera and U. linza.*

contents among all $Cd^{2+}$ treatments was noted in the two *Ulva* species (Table 1). Nitrate content in *U. prolifera* showed an uptrend with increasing $Cd^{2+}$ concentration; however, with increasing $Cd^{2+}$ concentrations, nitrate content of *U. linza* showed a decline trend after an initial increase. We also found that nitrate contents of *U. linza* were much more than those of *U. prolifera* under all treatments except for 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ treatment (Table 1).

The $K^+/Na^+$ and $Ca^{2+}/Na^+$ ratios in *U. prolifera* were not influenced by 5 and 10 $\mu$mol $L^{-1}$ $Cd^{2+}$, but they showed declining trends at 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ (Table 1). In *U. linza*, 5 and 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ had no significant influence on the $K^+/Na^+$ ratio, whereas 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ decreased that ratio by 6, 45, and 64%, respectively. However, in *U. prolifera*, 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ decreased the $K^+/Na^+$ ratio by 55, 65, and 78%. No $Cd^{2+}$ treatment significantly changed the $Ca^{2+}/Na^+$ ratio in *U. linza*.

*3.7. Effect of Cadmium Stress on Organic Solutes in U. prolifera and U. linza.* With increasing $Cd^{2+}$ concentration, soluble sugar (SS) content appeared to have an ascending trend after an initial decline in both *Ulva* species. In *U. prolifera*, 40 $\mu$mol $L^{-1}$ $Cd^{2+}$ did not change the SS content, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ increased SS concentration by 27% compared to the control. However, in *U. linza*, 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ increased SS content by 40 and 90%, respectively (Table 2). In *U. prolifera* and *U. linza*, 5 $\mu$mol $L^{-1}$ $Cd^{2+}$ significantly increased free amino acid (FAA) content by 25 and 16%, respectively. However, 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ had no obvious change on FAA contents of the two *Ulva* species. Treatments with 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ significantly decreased FAA content by 52, 79, and 87% in *U. prolifera* and by 2, 25, and 43% in *U. linza* (Table 2). Proline (PRO) content was greatly enhanced by $Cd^{2+}$ treatments in both *Ulva* species. At 5, 10, 20, 40, and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$, PRO content was increased 154, 431, 715, 1031, and 1069%, respectively, in *U. prolifera*; and increased 147, 420, 726, 1040, and 1147%, respectively, in *U. linza* (Table 2). Organic acid (OA) content in *U. prolifera* was not affected at 5, 10 and 20 $\mu$mol $L^{-1}$ $Cd^{2+}$, and OA concentration in *U. linza* was not affected at 5, 10, 20, and 40 $\mu$mol $L^{-1}$ $Cd^{2+}$. Treatments with 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ decreased OA content by 29 and 47%, respectively, in *U. prolifera*, whereas in *U. linza* only 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ decreased OA content by 27% (Table 2). The soluble protein (SP) content in the two *Ulva* species was not affected at 5, 10 and 20 $\mu$mol $L^{-1}$ $Cd^{2+}$ and was decreased at 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$. Treatments with 40 and 80 $\mu$mol $L^{-1}$ $Cd^{2+}$ significantly decreased SP content by, respectively, 16 and 42% in *U. prolifera* and by 8 and 25% in *U. linza* (Table 2).

*3.8. Correlation Analysis between RGR and Other Physiological and Biochemical Indexes under Cadmium Stress.* Correlation

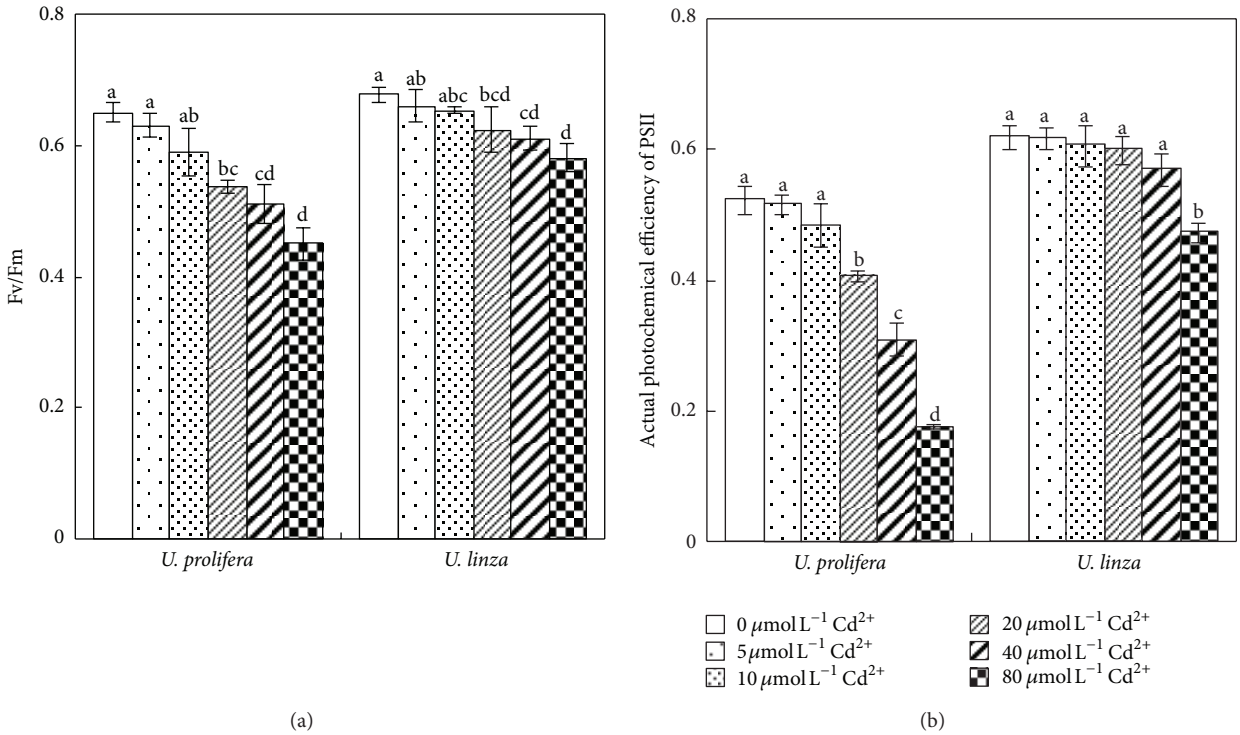


Figure 5: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 30, 40, and 80 $\mu$mol $L^{-1}$) on Fv/Fm (a) and Yield (actual photochemical efficiency of PSII) (b) of *U. prolifera* and *U. linza*.

analysis indicated that RGR of both *Ulva* species was insignificantly related to contents of Chl, Car, $Na^+$ and $Mg^{2+}$, and the $Ca^{2+}/Na^+$ ratio. In contrast, RGR was highly negative correlated with the contents of $Cd^{2+}$, $Ca^{2+}$, SS, and PRO, and highly positive correlated with the contents of N, P, K, Cl, FAA, OA and SP, $K^+/Na^+$ ratio, OAA, Fv/Fm, and Yield (Table 3).

## 4. Discussion

Plant growth can be suppressed by Cd [7, 17]. It was reported that *Ulva lactuca* was sensitive to cadmium, as obviously shown by growth reduction and lethal effects at 40 $\mu$mol $L^{-1}$ $Cd^{2+}$ within 6 days [27]. In the study presented here, *U. prolifera* and *U. linza*, the dominant free-floating *Ulva* species of green tide bloom in the Yellow Sea of China [28], showed sensitivity to $Cd^{2+}$ (reduction in RGR, Fv/Fm, and Yield). Furthermore, this reduction was found to be more pronounced in *U. prolifera* than *U. linza*. After 7 d, *U. prolifera* died at 120 $\mu$mol $L^{-1}$ $Cd^{2+}$, whereas *U. linza* was still alive (Figures 1 and 4). This result indicated that *U. linza* had better adaptation to $Cd^{2+}$ toxicity than *U. prolifera*.

It is known that marine macroalgae can concentrate heavy metals to a large extent [2, 29]. In this study, Cd accumulation in *U. prolifera* and *U. linza* increased significantly in response to increased $Cd^{2+}$ concentrations. However, *U. prolifera* accumulated more Cd than *U. linza* (Figure 3). In general, plant accumulation of a given metal is a function of uptake capacity and intracellular binding sites [30]. The cell walls of plant cells contain proteins and different carbohydrates that can bind metal ions. After the binding sites in the cell wall become saturated, intracellular Cd accumulation mediated by metabolic processes may lead to cell toxicity [31].

*Ulva* species are widely distributed in the coastal intertidal zones where had full change on salinity level. Thus, many *Ulva* species have strong OAA to cope with variable and heterogeneous environments. Similarly to a number of other stresses, heavy metal toxicity can decrease cell water content and lower the cell water potential ($\psi_w$) through increased net concentrations of solutes (osmotic adjustment), which is a common response to water stress and an important mechanism for maintaining cell water content and, thus, turgor [18, 32]. In our experiments, OAA of *U. linza* had positive values in the treatments with 5, 10, or 20 $\mu$mol $L^{-1}$ $Cd^{2+}$, whereas *U. prolifera* had positive OAA only at 5 and 10 $\mu$mol $L^{-1}$ $Cd^{2+}$ (Figure 2). When OAA values in *Ulva* were positive, that is, OAA contributed to maintaining turgor, *Ulva* could continue growing, and RGR was positive. However, when OAA in *Ulva* was negative resulting in turgor loss, the growth was stopped, and RGR was negative. Correlation analysis also showed that RGR was positively related to OAA, suggesting that OAA played an important role in maintaining algal growth. Also, good osmotic adjustment enabled plants to maintain high photosynthetic activity (Figure 5).

Cadmium is a nonessential element for plant growth, and it inhibits uptake and transport of many macro- and

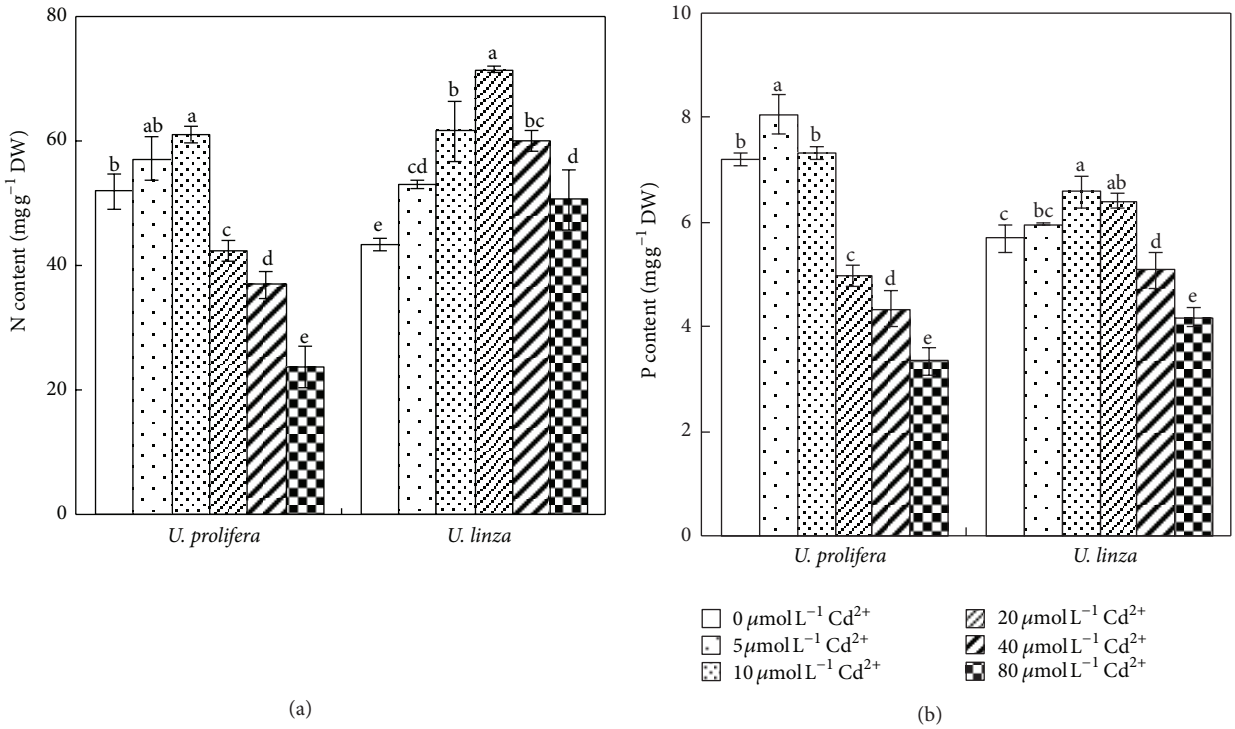


Figure 6: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 20, 40, 80 $\mu$mol $L^{-1}$) on contents of N (a) and P (b) of *U. prolifera* and *U. linza*.

micronutrients, inducing nutrient deficiency [7, 17]. Contradictory data can be found in the literature on the effects exerted by $Cd^{2+}$ on terrestrial plant. Cadmium was reported to reduce uptake of N, P, K, Ca, Mg, Fe, Zn, Cu, Mn, Ni, and Na in many crop plants [33], whereas other authors found reduced K uptake but unchanged P uptake or even an increase in K content of several crop varieties under $Cd^{2+}$ stress [34, 35]. Obata and Umebayashi [36] reported that $Cd^{2+}$ treatment increased Cu content in the roots of pea, rice, and maize, but unchanged Cu content in cucumber and pumpkin plants. With $Cd^{2+}$ stress, Maksimović et al. [37] observed a reduction in the maize root influx and root-shoot transport of Cu, Zn, and Mn, a reduction in the root-shoot transport of Fe, but an increase in Fe influx and Ca and Mg transport. In this study, the response of total N and P concentrations in tissues of the two *Ulva* species to $Cd^{2+}$ treatments was positively related to their Cd resistance. We found that the treatment with low concentration of $Cd^{2+}$ enhanced N and P contents, but high concentrations of $Cd^{2+}$ ($\geq$20 $\mu$mol $L^{-1}$) decreased N and P contents in both *Ulva* species. The maintenance of total N and total P was more pronounced in less Cd-sensitive *U. linza* than Cd-sensitive *U. prolifera* (Figure 6). This suggests that the maintenance of a normal level of total N content upon challenge with Cd is likely to be a feature in relative Cd-resistant marine macroalga, similarly to terrestrial plants [38]. In *Ulva*, we found that the contents of $K^+$, $Ca^{2+}$, and $Cl^-$ were related to RGR, especially $K^+$ reduction caused *Ulva* growth reduction significantly (Table 1). Thus, the $K^+/Na^+$ ratio in both *Ulva* species decreased significantly with increasing $Cd^{2+}$ treatment concentrations, and $Cd^{2+}$-sensitive *U. prolifera* showed a greater $K^+/Na^+$ decline than $Cd^{2+}$-sensitive *U. linza* (Table 1).

We measured a decline in soluble sugar (SS) concentration at low $Cd^{2+}$ treatment concentrations and an increase at high $Cd^{2+}$ concentrations in both *Ulva* species. Moreover, the SS increase of *U. linza* is more marked than that of *U. prolifera*. In other studies, the decline in SS concentration corresponded with the photosynthetic inhibition or stimulation of respiration rate, affecting carbon metabolism and leading to production of other osmotica [39]. The accumulating soluble sugars in plants growing in presence of $Cd^{2+}$ could provide an adaptive mechanism via maintaining a favorable osmotic potential under adverse conditions of $Cd^{2+}$ toxicity [40].

Soluble protein (SP) content in organisms is an important indicator of metabolic changes and responds to a wide variety of stresses [41]. In this work, SP contents in *U. prolifera* and *U. linza* declined with increasing $Cd^{2+}$ treatment concentrations. Free amino acid (FAA) contents in both *Ulva* species first increased and then declined, with such a decline more pronounced in *U. prolifera* than in *U. linza*. The decreased protein content together with the increased free amino acid content suggest that the protein synthesizing machinery was impaired due to the $Cd^{2+}$ effect [42].

PRO accumulation in plant tissues in response to a number of stresses, including drought, salinity, extreme temperatures, ultraviolet radiation, or heavy metals, is well documented [43]. In this study, even though PRO content was increased in $Cd^{2+}$-treated *Ulva*, its absolute amount was relatively low. Under assumed localization of inorganic ions

Table 1: Effects of different concentrations of $Cd^{2+}$ (0, 5, 10, 30, 40, and 80 $\mu$mol $L^{-1}$) on inorganic ion content (mmol $g^{-1}$ DW), $K^+/Na^+$ and $Ca^{2+}/Na^+$ of *U. prolifera* and *U. linza*.

| | $Cd^{2+}$ treatment $\mu$mol $L^{-1}$ | $Na^+$ mmol $g^{-1}$ DW | $K^+$ mmol $g^{-1}$ DW | $Ca^{2+}$ mmol $g^{-1}$ DW | $Mg^{2+}$ mmol $g^{-1}$ DW | $Cl^-$ mmol $g^{-1}$ DW | $NO_3^-$ mmol $g^{-1}$ DW | $K^+/Na^+$ | $Ca^{2+}/Na^+$ |
|---|---|---|---|---|---|---|---|---|---|
| *U. prolifera* | 0 | 0.12 ± 0.01 c | 0.64 ± 0.04 a | 0.20 ± 0.02 b | 0.82 ± 0.04 a | 0.15 ± 0.01 a | $0.34 \times 10^{-3} \pm 0.03 \times 10^{-3}$ c | 5.01 ± 0.12 a | 1.70 ± 0.08 a |
| | 5 | 0.13 ± 0.02 c | 0.62 ± 0.05 a | 0.23 ± 0.01 ab | 0.78 ± 0.05 a | 0.11 ± 0.01 b | $0.49 \times 10^{-3} \pm 0.06 \times 10^{-3}$ c | 4.86 ± 0.21 a | 1.66 ± 0.07 a |
| | 10 | 0.12 ± 0.01 c | 0.63 ± 0.04 a | 0.23 ± 0.02 ab | 0.76 ± 0.05 a | 0.10 ± 0.01 b | $0.78 \times 10^{-3} \pm 0.06 \times 10^{-3}$ b | 5.10 ± 0.14 a | 1.84 ± 0.12 a |
| | 20 | 0.17 ± 0.02 b | 0.38 ± 0.03 b | 0.23 ± 0.02 ab | 0.75 ± 0.04 a | 0.09 ± 0.01 b | $1.41 \times 10^{-3} \pm 0.08 \times 10^{-3}$ a | 2.26 ± 0.15 b | 1.39 ± 0.10 b |
| | 40 | 0.20 ± 0.02 ab | 0.35 ± 0.03 b | 0.25 ± 0.02 ab | 0.79 ± 0.04 a | 0.10 ± 0.01 b | $1.40 \times 10^{-3} \pm 0.11 \times 10^{-3}$ a | 1.74 ± 0.11 c | 1.24 ± 0.08 bc |
| | 80 | 0.22 ± 0.01 a | 0.24 ± 0.02 c | 0.26 ± 0.02 a | 0.73 ± 0.05 a | 0.10 ± 0.01 b | $1.43 \times 10^{-3} \pm 0.04 \times 10^{-3}$ a | 1.12 ± 0.08 d | 1.14 ± 0.07 c |
| *U. linza* | 0 | 0.25 ± 0.02 b | 0.74 ± 0.04 a | 0.18 ± 0.02 c | 0.78 ± 0.04 a | 0.16 ± 0.01 a | $0.86 \times 10^{-3} \pm 0.08 \times 10^{-3}$ d | 3.02 ± 0.15 a | 0.72 ± 0.09 a |
| | 5 | 0.24 ± 0.03 b | 0.74 ± 0.04 a | 0.17 ± 0.02 c | 0.75 ± 0.03 ab | 0.12 ± 0.01 b | $1.21 \times 10^{-3} \pm 0.10 \times 10^{-3}$ c | 3.10 ± 0.23 a | 0.73 ± 0.08 a |
| | 10 | 0.24 ± 0.02 b | 0.73 ± 0.04 a | 0.18 ± 0.01 c | 0.72 ± 0.04 ab | 0.10 ± 0.02 b | $1.89 \times 10^{-3} \pm 0.07 \times 10^{-3}$ a | 3.04 ± 0.12 a | 0.75 ± 0.06 a |
| | 20 | 0.24 ± 0.01 b | 0.68 ± 0.03 a | 0.22 ± 0.02 b | 0.64 ± 0.03 b | 0.10 ± 0.01 b | $2.07 \times 10^{-3} \pm 0.12 \times 10^{-3}$ a | 2.85 ± 0.11 b | 0.83 ± 0.07 a |
| | 40 | 0.26 ± 0.02 b | 0.49 ± 0.03 c | 0.25 ± 0.02 ab | 0.68 ± 0.03 b | 0.11 ± 0.01 b | $1.65 \times 10^{-3} \pm 0.05 \times 10^{-3}$ b | 1.67 ± 0.07 c | 0.88 ± 0.08 a |
| | 80 | 0.34 ± 0.02 a | 0.37 ± 0.02 d | 0.27 ± 0.02 a | 0.72 ± 0.04 ab | 0.12 ± 0.02 b | $1.12 \times 10^{-3} \pm 0.11 \times 10^{-3}$ c | 1.10 ± 0.05 d | 0.75 ± 0.06 a |

The data in the same column are statistically different if labeled with different letters according to Duncan's multiple range test ($P \leq 0.05$).

Table 2: Effects of different concentration of $Cd^{2+}$ (0, 5, 10, 30, 40, and 80 $\mu$mol $L^{-1}$) on organic solute content of *U. prolifera* and *U. linza*.

| | $Cd^{2+}$ treatment $\mu$mol $L^{-1}$ | SS mmol $g^{-1}$ DW | FAA mmol $g^{-1}$ DW | PRO mmol $g^{-1}$ DW | OA mmol $g^{-1}$ DW | SP mg $g^{-1}$ DW |
|---|---|---|---|---|---|---|
| *U. prolifera* | 0 | 0.15 ± 0.02 b | 1.03 ± 0.05 b | $0.13 \times 10^{-3} \pm 0.02 \times 10^{-3}$ e | 0.17 ± 0.01 a | 42.15 ± 2.33 a |
| | 5 | 0.15 ± 0.02 b | 1.29 ± 0.12 a | $0.33 \times 10^{-3} \pm 0.02 \times 10^{-3}$ d | 0.17 ± 0.01 a | 41.38 ± 2.76 a |
| | 10 | 0.12 ± 0.01 bc | 1.10 ± 0.04 ab | $0.69 \times 10^{-3} \pm 0.04 \times 10^{-3}$ c | 0.19 ± 0.02 a | 40.45 ± 1.86 a |
| | 20 | 0.10 ± 0.01 c | 0.49 ± 0.11 c | $1.06 \times 10^{-3} \pm 0.07 \times 10^{-3}$ b | 0.16 ± 0.02 a | 38.39 ± 2.75 ab |
| | 40 | 0.14 ± 0.01 b | 0.22 ± 0.05 d | $1.47 \times 10^{-3} \pm 0.09 \times 10^{-3}$ a | 0.12 ±0.01 b | 35.53 ± 2.63 b |
| | 80 | 0.19 ± 0.01 a | 0.13 ± 0.06 d | $1.52 \times 10^{-3} \pm 0.12 \times 10^{-3}$ a | 0.09 ± 0.01 c | 24.35 ± 1.88 c |
| *U. linza* | 0 | 0.10 ± 0.01 cd | 1.23 ± 0.03 b | $0.15 \times 10^{-3} \pm 0.05 \times 10^{-3}$ f | 0.11 ± 0.02 a | 39.27 ± 1.22 a |
| | 5 | 0.10 ± 0.01 cd | 1.43 ± 0.09 a | $0.37 \times 10^{-3} \pm 0.02 \times 10^{-3}$ e | 0.12 ± 0.01 a | 38.89 ± 2.37 ab |
| | 10 | 0.07 ± 0.01 d | 1.21 ± 0.10 b | $0.78 \times 10^{-3} \pm 0.03 \times 10^{-3}$ d | 0.13 ± 0.01 a | 38.52 ± 2.67 ab |
| | 20 | 0.10 ± 0.01 c | 1.20 ± 0.06 b | $1.24 \times 10^{-3} \pm 0.08 \times 10^{-3}$ c | 0.14 ± 0.02 a | 37.13 ± 1.89 ab |
| | 40 | 0.14 ± 0.02 b | 0.97 ± 0.06 c | $1.71 \times 10^{-3} \pm 0.07 \times 10^{-3}$ b | 0.12 ± 0.01 a | 35.95 ± 2.41 b |
| | 80 | 0.19 ± 0.01 a | 0.76 ± 0.08 d | $1.87 \times 10^{-3} \pm 0.15 \times 10^{-3}$ a | 0.08 ± 0.01 b | 29.34 ± 1.87 c |

Different letters in the same column indicate statistical difference according to Duncan's multiple range test ($P \leq 0.05$). "SS, FAA, PRO, OA, and SP" in the table indicate the content of soluble sugar, free amino acid, proline, organic acid, and soluble protein, respectively.

Table 3: Correlation coefficients between RGR and other indices for *U. prolifera* and *U. linza*.

| Index | Correlation coefficient |
|---|---|
| Chl content | 0.072 |
| Car content | 0.198 |
| Fv/Fm | 0.830** |
| Yield | 0.858** |
| $Cd^{2+}$ content | −0.899** |
| N content | 0.561** |
| P content | 0.687** |
| OAA | 0.766** |
| $Na^{+}$ content | −0.138 |
| $K^{+}$ content | 0.881** |
| $Ca^{2+}$ content | −0.677** |
| $Mg^{2+}$ content | 0.060 |
| $Cl^{-}$ content | 0.444** |
| $K^{+}/Na^{+}$ | 0.627** |
| $Ca^{2+}/Na^{+}$ | −0.079 |
| SS content | −0.617** |
| FAA content | 0.828** |
| PRO content | −0.841** |
| OA content | 0.731** |
| SP content | 0.752** |

*Significant at 5% level, **significant at 1% level (two-tailed, $n = 18$).

in the vacuole and organic solutes in the cytoplasm, FAA and PRO may be mainly in the cytoplasm, accounting for about 5%–10% volume in mature cells [44]. A small amount of FAA and PRO accumulating in the cytoplasm can increase concentration significantly and play an important role in balancing vacuolar osmotic potential [44]. It has often been suggested that PRO accumulation may contribute to osmotic adjustment at the cellular level [39]. In addition, PRO as a compatible solute may protect enzymes from dehydration and inactivation [18].

In conclusion, exposing *U. prolifera* and *U. linza* to different concentrations of $Cd^{2+}$ resulted in the changes in growth, pigment content, chlorophyll fluorescence parameters, Cd accumulation, OAA, and concentration of N, P, main inorganic ions, and organic solutes. These changes make *U. linza* better adapted to withstanding $Cd^{2+}$ stress in comparison with *U. prolifera*. Our results highlight the role of osmotic adjustment in *Ulva* during $Cd^{2+}$ stress as an important mechanism enabling *Ulva* to maintain photosynthetic activity and, thus, growth under $Cd^{2+}$ stress.

## Authors' Contribution

H. Jiang and B. Gao both contributed equally to this paper.

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## References

[1] L. Zhu, J. Xu, F. Wang, and B. Lee, "An assessment of selected heavy metal contamination in the surface sediments from the South China Sea before 1998," *Journal of Geochemical Exploration*, vol. 108, no. 1, pp. 1–14, 2011.

[2] A. Sari and M. Tuzen, "Biosorption of Pb(II) and Cd(II) from aqueous solution using green alga (*Ulva lactuca*) biomass," *Journal of Hazardous Materials*, vol. 152, no. 1, pp. 302–308, 2008.

[3] B. Xu, X. Yang, Z. Gu, Y. Zhang, Y. Chen, and Y. Lv, "The trend and extent of heavy metal accumulation over last one hundred years in the Liaodong Bay, China," *Chemosphere*, vol. 75, no. 4, pp. 442–446, 2009.

[4] P. Lodeiro, B. Cordero, J. L. Barriada, R. Herrero, and M. E. Sastre De Vicente, "Biosorption of cadmium by biomass of brown marine macroalgae," *Bioresource Technology*, vol. 96, no. 16, pp. 1796–1803, 2005.

[5] B. Volesky and Z. R. Holan, "Biosorption of heavy metals," *Biotechnology Progress*, vol. 11, no. 3, pp. 235–250, 1995.

[6] E. A. Webster, A. J. Murphy, J. A. Chudek, and G. M. Gadd, "Metabolism-independent binding of toxic metals by *Ulva lactuca*: cadmium binds to oxygen-containing groups, as determined by NMR," *BioMetals*, vol. 10, no. 2, pp. 105–117, 1997.

[7] M. Kumar, P. Kumari, V. Gupta, P. A. Anisha, C. R. K. Reddy, and B. Jha, "Differential responses to cadmium induced oxidative stress in marine macroalga *Ulva lactuca* (Ulvales, Chlorophyta)," *BioMetals*, vol. 23, no. 2, pp. 315–325, 2010.

[8] S. Hajialigol, M. A. Taher, and A. Malekpour, "A new method for the selective removal of cadmium and zion ions from aqueous solution by modified clinoptilolite," *Adsorption Science and Technology*, vol. 24, no. 6, pp. 487–496, 2006.

[9] P. X. Sheng, Y. P. Ting, J. P. Chen, and L. Hong, "Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms," *Journal of Colloid and Interface Science*, vol. 275, no. 1, pp. 131–141, 2004.

[10] A. El-Sikaily, A. E. Nemr, A. Khaled, and O. Abdelwehab, "Removal of toxic chromium from wastewater using green alga *Ulva lactuca* and its activated carbon," *Journal of Hazardous Materials*, vol. 148, no. 1-2, pp. 216–228, 2007.

[11] S. Karthikeyan, R. Balasubramanian, and C. S. P. Iyer, "Evaluation of the marine algae Ulva fasciata and *Sargassum* sp. for the biosorption of Cu(II) from aqueous solutions," *Bioresource Technology*, vol. 98, no. 2, pp. 452–455, 2007.

[12] K. Masakorala, A. Turner, and M. T. Brown, "Influence of synthetic surfactants on the uptake of Pd, Cd and Pb by the marine macroalga, *Ulva lactuca*," *Environmental Pollution*, vol. 156, no. 3, pp. 897–904, 2008.

[13] P. J. Say, I. G. Burrows, and B. A. Whitton, "Enteromorpha as a monitor of heavy metals in estuaries," *Hydrobiologia*, vol. 195, pp. 119–126, 1990.

[14] P. Malea, J. W. Rijstenbil, and S. Haritonidis, "Effects of cadmium, zinc and nitrogen status on non-protein thiols in the macroalgae *Enteromorpha* spp. from the Scheldt Estuary (SW Netherlands, Belgium) and Thermaikos Gulf (N Aegean Sea, Greece)," *Marine Environmental Research*, vol. 62, no. 1, pp. 45–60, 2006.

[15] A. Lin, S. Shen, G. Wang et al., "Comparison of chlorophyll and photosynthesis parameters of floating and attached *Ulva prolifera*," *Journal of Integrative Plant Biology*, vol. 53, no. 1, pp. 25–34, 2011.

[16] C. Yarish, T. Chopin, R. Wilkes, A. C. Mathieson, X. G. Fei, and S. Lu, "Domestication of Nori for northeast America: the Asian experience," *Bulletin of Aquaculture Association of Canada*, vol. 1, pp. 11–17, 1999.

[17] J. R. Xia, Y. J. Li, J. Lu, and B. Chen, "Effects of copper and cadmium on growth, photosynthesis, and pigment content in *Gracilaria lemaneiformis*," *Bulletin of Environmental Contamination and Toxicology*, vol. 73, no. 6, pp. 979–986, 2004.

[18] Q. Zheng, Z. Liu, G. Chen, Y. Gao, Q. Li, and J. Wang, "Comparison of osmotic regulation in dehydrationand salinity-stressed sunflower seedlings," *Journal of Plant Nutrition*, vol. 33, no. 7, pp. 966–981, 2010.

[19] D. P. Häder, M. Lebert, and E. W. Helbling, "Effects of solar radiation on the Patagonian macroalga *Enteromorpha linza* (L.) J. Agardh—Chlorophyceae," *Journal of Photochemistry and Photobiology B*, vol. 62, no. 1-2, pp. 43–54, 2001.

[20] K. Kamer and P. Fong, "Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*," *Marine Ecology Progress Series*, vol. 218, pp. 87–93, 2001.

[21] F. B. Wu, J. Dong, Q. Q. Qiong, and G. P. Zhang, "Subcellular distribution and chemical form of Cd and Cd-Zn interaction in different barley genotypes," *Chemosphere*, vol. 60, no. 10, pp. 1437–1446, 2005.

[22] M. B. Gilliam, M. P. Sherman, J. M. Griscavage, and L. J. Ignarro, "A spectrophotometric assay for nitrate using NADPH oxidation by *Aspergillus* nitrate reductase," *Analytical Biochemistry*, vol. 212, no. 2, pp. 359–365, 1993.

[23] Q. Zhou and B. Yu, "Changes in content of free, conjugated and bound polyamines and osmotic adjustment in adaptation of vetiver grass to water deficit," *Plant Physiology and Biochemistry*, vol. 48, no. 6, pp. 417–425, 2010.

[24] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[25] J. J. Irigoyen, D. W. Emerich, and M. Sanchezdiaz, "Water-stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants," *Plant Physiology*, vol. 84, no. 1, pp. 55–60, 1992.

[26] J. Song, G. Feng, C. Y. Tian, and F. S. Zhang, "Osmotic adjustment traits of *Suaeda physophora, Haloxylon ammodendron* and *Haloxylon persicum* in field or controlled conditions," *Plant Science*, vol. 170, no. 1, pp. 113–119, 2006.

[27] J. W. Markham, B. P. Kremer, and K. R. Sperling, "Cadmium effects on growth and physiology of *Ulva lactuca*," *Helgoländer Meeresuntersuchungen*, vol. 33, no. 1–4, pp. 103–110, 1980.

[28] D. Liu, J. K. Keesing, Z. Dong et al., "Recurrence of the world's largest green-tide in 2009 in Yellow Sea, China: *Porphyra yezoensis* aquaculture rafts confirmed as nursery for macroalgal blooms," *Marine Pollution Bulletin*, vol. 60, no. 9, pp. 1423–1432, 2010.

[29] H. A. Baumann, L. Morrison, and D. B. Stengel, "Metal accumulation and toxicity measured by PAM—Chlorophyll fluorescence in seven species of marine macroalgae," *Ecotoxicology and Environmental Safety*, vol. 72, no. 4, pp. 1063–1075, 2009.

[30] M. P. Benavides, S. M. Gallego, and M. L. Tomaro, "Cadmium toxicity in plants," *Brazilian Journal of Plant Physiology*, vol. 17, no. 1, pp. 21–34, 2005.

[31] S. Hu, C. H. Tang, and M. Wu, "Cadmium accumulation by several seaweeds," *Science of the Total Environment*, vol. 187, no. 2, pp. 65–71, 1996.

[32] M. T. Milone, C. Sgherri, H. Clijsters, and F. Navari-Izzo, "Antioxidative responses of wheat treated with realistic concentration of cadmium," *Environmental and Experimental Botany*, vol. 50, no. 3, pp. 265–276, 2003.

[33] L. Sanità Di Toppi and R. Gabbrielli, "Response to cadmium in higher plants," *Environmental and Experimental Botany*, vol. 41, no. 2, pp. 105–130, 1999.

[34] F. F. Nocito, L. Pirovano, M. Cocucci, and G. A. Sacchi, "Cadmium-induced sulfate uptake in maize roots," *Plant Physiology*, vol. 129, no. 4, pp. 1872–1879, 2002.

[35] Z. Ciecko, S. Kalembasa, M. Wyszkowski, and E. Rolka, "The effect of elevated cadmium content in soil on the uptake of nitrogen by plants," *Plant, Soil and Environment*, vol. 50, no. 7, pp. 283–294, 2004.

[36] H. Obata and M. Umebayashi, "Effects of cadmium on mineral nutrient concentrations in plants differing in tolerance for cadmium," *Journal of Plant Nutrition*, vol. 20, no. 1, pp. 97–105, 1997.

[37] I. Maksimović, R. Kastori, L. Krstić, and J. Luković, "Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings," *Biologia Plantarum*, vol. 51, no. 3, pp. 589–592, 2007.

[38] T. Ghnaya, I. Slama, D. Messedi, C. Grignon, M. H. Ghorbel, and C. Abdelly, "Effects of $Cd2^+$ on $K^+$, $Ca2^+$ and N uptake in two halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: consequences on growth," *Chemosphere*, vol. 67, no. 1, pp. 72–79, 2007.

[39] R. John, P. Ahmad, K. Gadgil, and S. Sharma, "Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L.," *Plant, Soil and Environment*, vol. 54, no. 6, pp. 262–270, 2008.

[40] S. Verma and R. S. Dubey, "Effect of cadmium on soluble sugars and enzymes of their metabolism in rice," *Biologia Plantarum*, vol. 44, no. 1, pp. 117–123, 2001.

[41] P. K. Singh and R. K. Tewari, "Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassicajuncea* L. plants," *Journal of Environmental Biology*, vol. 24, no. 1, pp. 107–112, 2003.

[42] S. Jana and M. A. Choudhuri, "Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plants," *Water, Air, and Soil Pollution*, vol. 21, no. 1–4, pp. 351–357, 1984.

[43] D. Delmail, P. Labrousse, P. Hourdin, L. Larcher, C. Moesch, and M. Botineau, "Differential responses of *Myriophyllum alterniflorum* DC (Haloragaceae) organs to copper: physiological and developmental approaches," *Hydrobiologia*, vol. 664, no. 1, pp. 95–105, 2011.

[44] R. Munns and M. Tester, "Mechanisms of salinity tolerance," *Annual Review of Plant Biology*, vol. 59, pp. 651–681, 2008.