



Editorial

Advances in Molecular Exploration of Crop Plants under Environmental Stresses from Genetic and Genomic Perspectives

Fengjie Sun ^{1,*}  and Gustavo Caetano-Anollés ^{2,*} 

¹ Department of Biological Sciences, School of Science and Technology, Georgia Gwinnett College, Lawrenceville, GA 30043, USA

² Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

* Correspondence: fsun@ggc.edu (F.S.); gca@illinois.edu (G.C.-A.)

It is well known and has been evidently indicated by numerous studies that the demand for food and energy supply has been rapidly increasing for many years worldwide [1,2]. In agricultural practice, it has become enormously important and technically challenging to develop effective and efficient techniques for improving soil properties, cultivating crop plants, and growing livestock with a transforming planetary landscape and changing global climate [3–5]. Agricultural products are also continuously and commonly utilized in multiple fields and systems for various purposes, including developing products necessary and important for engineering and medicines [6–9]. Fortunately, facilitated by the advent of next-generation DNA sequencing technology and rapid advancement in the multiple “omics” techniques, our knowledge of the interactions among crop plants, soil properties, and soil inhabitants has been significantly advanced from a wide range of perspectives [10–12]. Therefore, it is necessary and important to further strengthen the worldwide advancements of the molecular investigations of crop plants and their interactions with the surrounding environments, especially those based on genetic and genomic analyses, and to identify their effective applications and challenges.

We here summarize the main results of a total of 16 publications that appeared in the Special Issue entitled “Recent progress in molecular investigations of crop plants and crop plant–environmental interactions from genetic and genomic perspectives: application and challenges—Series I and II” of the journal *Agronomy*. Our goals of organizing this Special Issue were to widely explore the advances in not only the traditional research areas, such as plant physiology, development, ecology, and bioinformatics, but also cutting-edge, modern techniques, such as the molecular, genetic, and genomic investigations of crop plants or other model plant systems, crop-sustaining rhizospheric microbes, and agricultural products, including medicinal plants in a greenhouse or field. These goals were well achieved, as demonstrated by the outcome of the final acceptance and publication of a total of sixteen articles contributed by over 100 authors, with four research papers based on soybean, one research paper based on wild soybean, *Physalis*, *Eutrema*, *Chlorella*, *Mortierella*, wheat, tobacco, corn, olive, *Anemone*, and castor bean, respectively, and one review article based on barley.

First, there are a total of four articles focused on soybean [*Glycine max* (L.) Merr.], with two contributed by the Cui laboratory at the Jilin Agricultural University [13,14] and the other two by the Li Laboratory at the Nanjing Agricultural University [15,16]. Most of the results derived from these studies were verified using *Arabidopsis thaliana*. Due to the severe effects of drought stress and high soil salinization on soybean yield and quality, Chen et al. [13] investigated the alleviation effects of overexpressing the transcription factor GmTGA15 on the damages caused by drought stress using both transgenic soybean hairy roots and plants of *Arabidopsis thaliana*, showing that the expression levels of GmTGA15 were significantly increased by the treatments of saline, PEG6000, and four types of plant hormones, i.e., salicylic acid (SA), gibberellic acid (GA), abscisic acid (ABA), and methyl



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jasmonate (MeJA). The authors reported that under drought stress, the soybean hairy roots with *GmTGA15* overexpressed were revealed with increased levels of both chlorophyll and proline, whereas the level of malondialdehyde (MDA) was significantly decreased, in comparison to the wild type (WT). These results indicated that *GmTGA15* promoted drought tolerance in soybean. The authors further verified these results using *Arabidopsis* plants, providing strong experimental evidence to support the further functional analysis of soybean TGA transcription factors under drought stress and the molecular breeding of drought-resistant crop plants. Chen et al. [14] characterized the molecular properties of phospholipid N-methyltransferase (PLMT) in soybean (*GmPLMT*) and the effects of the expression of *GmPLMT* on the production of lipid metabolites in soybean. The results showed that *GmPLMT* was abundantly expressed in soybean leaves during both seedling and flowering stages, whereas in transgenic *Arabidopsis thaliana*, the highest and lowest expression levels of *GmPLMT* were revealed during flowering and maturity stages, respectively. The contents of both phosphatidylcholine (PtdCho) and total phospholipids showed similar patterns. In particular, the contents of total phospholipids and PtdCho were significantly increased in the seeds of transgenic *A. thaliana*, suggesting that the overexpression of *GmPLMT* enhanced the production of lipid metabolites. This study provided strong experimental evidence to support further molecular breeding based on *GmPLMT* to enhance soybean quality. Due to the important roles that the DNAJ proteins play as chaperones in plants responding to a variety of abiotic and biotic strains, Jin et al. [15] further characterized the function of a DNAJ gene, i.e., *GmDNAJC7*, in soybean tolerance to abiotic stresses. The overexpression of *GmDNAJC7* improved the alkaline salt tolerance in composite soybean plants treated with NaHCO_3 and promoted the germination rate and average root length in *Arabidopsis thaliana* compared to both wild type and *dnajc7* mutant under the treatments of NaHCO_3 , NaCl, and mannitol, respectively. These results indicated that the ectopic expression of *GmDNAJC7* enhanced the alkaline salt and drought tolerance in *Arabidopsis*. In summary, these findings evidently suggested that *GmDNAJC7* was closely associated with alkaline salt and drought tolerance in both *Arabidopsis* and soybean. This study provided novel evidence to verify the roles of DNAJ proteins in plant tolerance to various abiotic stresses, such as accelerating the tolerance to soil alkalization and salinization as well as water deficit. Aluminum toxicity is well known as an obstruction to soybean production in acid soils. However, investigations on the genes involved in aluminum tolerance in soybean are sparse. Liu et al. [16] performed comparative transcriptome analyses based on both aluminum-tolerant and aluminum-sensitive soybean varieties to identify the target genes and further evaluate their functions in tolerance to aluminum toxicity in soybean. A total of 1411 differentially expressed genes (DEGs) were revealed with significant up-regulation in aluminum-tolerant soybean varieties than that in aluminum-sensitive soybean varieties under aluminum stress, and they were significantly annotated in two Gene Ontology (GO) terms, i.e., peroxidase (POD) activity and transporter activity, including the known aluminum-tolerance-related ABC transporter, aluminum-activated malate transporter (ALMT), multidrug and toxic compound extrusion (MATE), and four families of transcription factors (i.e., AP2, C3H4, MYB, and WRKY). In particular, the expression levels of seven *POD* genes were up-regulated by aluminum stress, while the H_2O_2 pretreatment significantly improved aluminum tolerance in aluminum-tolerant soybean varieties, which was probably due to the increased POD activity induced by H_2O_2 . This study suggested that PODs played important roles in soybean tolerance to aluminum toxicity. The list of candidate genes involved in aluminum tolerance identified in aluminum-tolerant soybean varieties provided valuable molecular components for further exploration of the aluminum tolerance mechanisms. Finally, it is well known that the wild soybean (*Glycine soja* Siebold and Zucc.) germplasm is widely distributed in northeastern China, in particular Heilongjiang Province. Li et al. [17] evaluated a significant amount of variations in a total of 242 accessions of wild soybean collected from four agricultural divisions in Heilongjiang Province based on a total of 14 traits (6 qualitative and 8 quantitative). In particular, the results of the multivariate analysis revealed significant differences

among samples in the quantitative traits collected, i.e., 100-seed weight, seed weight per plant, number of seeds per plant, number of effective pods, and number of invalid pods. Based on the identification of a total of 27 germplasms and the abundant wild soybean resources detected in the northern site, it was concluded that the wild lines of soybean in Heilongjiang Province should be protected in situ. The rich genetic diversities revealed in wild soybeans of different sites provided important evidence to support the conservation of the wild soybean resources in Heilongjiang Province, suggesting the potentially significant utilization of the wild soybean resources in genetic improvement and breakthrough for soybean breeding.

Second, two contributions explored chloroplast genome evolution in *Physalis* [18] and *Eutrema* [19]. Zhang et al. [18] characterized the molecular structures of two completely sequenced chloroplast genomes of *P. macrophysa* Rydb. and *P. ixocarpa* Brot. Ex Hornem, two species of medicinal and ornamental plants, and further investigated the phylogenetic relationships among the taxa in the tribe Physaleae. These analyses were important for further studies of species circumscription, phylogeny and evolution, and exploitation of germplasm resources in *Physalis*. Li et al. [19] sequenced the complete chloroplast genome of Japanese horseradish [*Eutrema japonicum* (Miq.) Koidz., sym. *Wasabi japonica* (Miq.) Mastum.], a vegetable of Brassicaceae and the source for wasabi, a rare condiment extracted from the rhizomes. They further characterized the molecular structures of this chloroplast genome and explored the phylogenetic relationships among the plants in *Eutrema*. These analyses were important for molecular breeding, genetic transformation, and the development of the germplasm resources of plants in *Eutrema*.

Third, two studies explored the molecular mechanism regulating the lipid accumulation and composition of two microalgal species of *Chlorella* (*C. pyrenoidesa* and *C. vulgaris*) and a fungal species *Mortierella alpina*, respectively. Since the modified germplasm and heterotrophic cultivation could promote the microalgal biomass and lipid content, ultimately decreasing the production costs, Xing et al. [20] investigated the effect of three different types of irradiation, i.e., a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, a helium–neon (He–Ne) laser, and a semiconductor (SC) laser, and heterotrophic cultivation on lipid productivity and content as well as biomass in two species of *Chlorella*. Varied levels of the highest biomass and lipid contents were obtained based on different laser irradiations and treatment durations, suggesting that laser mutagenesis could be a potentially effective method for generating economically important oleaginous microalgal strains. Conversely, Ren et al. [21] performed transcriptomic analysis based on three experimental groups of *M. alpina* cultured under three different temperature conditions to investigate the molecular mechanisms underlying the molecular response to cold stress and the changes in the lipid composition of *M. alpina*. A total of 1552 DEGs were identified in the microalgae treated under cold stress and mainly involved in the metabolic pathways of glycolysis, fatty acid synthesis, pentose phosphate, glycerolipid, the tricarboxylic acid (TCA) cycle, and glycerophospholipid. A quantitative real-time PCR (qRT-PCR) analysis was performed to confirm the expression patterns of a group of six DEGs involved in lipid metabolism. This study provided solid experimental evidence to further support the metabolic engineering of cold tolerant strains of *M. alpina*.

Fourth, the following six articles were each based on one of six taxa, including corn, wheat, tobacco, castor bean, olive, and *Anemone*, respectively. In *Arabidopsis thaliana*, Chen et al. [22] studied the gene expression patterns of *AtAAPs*, which encoded amino acid permeases (AAPs), the vital transmembrane proteins that are closely associated with both the absorption and transport of amino acids in plants. The results of qRT-PCR analysis revealed differential expression patterns of *AtAAP* genes in various organs of the plants. In particular, a group of five genes (including *AtAAP1*, *AtAAP2*, *AtAAP6*, *AtAAP7*, and *AtAAP8*) were detected at relatively high levels in two organs, i.e., flowers and siliques, indicating the shared roles among these genes in the accumulation of amino acids. The authors further verified these findings in transgenic plants of maize to overexpress gene *AtAAP1*. The results showed that the levels of amino acid were higher in the reproductive

organs (i.e., flowers and fruits) than those of the vegetative organs. To summarize, this study evidently demonstrated that the overexpression of the endosperm-specific promoter significantly enhanced the production of amino acids in the reproductive organs and promoted the efficient consumption of organic nitrogen in plants.

Zhang et al. [23] investigated the correlations between a total of 12 agronomic traits, most related to the spike, e.g., grain number per spike (GNPS), in a total of 259 pre-breeding and elite commercial varieties of wheat. The main findings revealed that the GNPS was highly correlated with the kernel number per spikelet (KNS), suggesting that the high GNPS phenotype in these pre-breeding lines of wheat was largely influenced by the grain set number per spikelet. In particular, a main locus was identified on chromosomes 4BS, which contributed to the high GNPS phenotype, and an efficient PCR-based diagnostic allele specific marker AX-109286577 next to the 4BS locus was established for the convenient selection of large spike phenotype. These findings indicated that the variations in these untapped rare alleles detected in these pre-breeding varieties of wheat could be useful for increasing the yield phenotypes in wheat, e.g., the set grain number per spike.

To examine the level of host resistance in tobacco, which is generally considered to be one of the most successful approaches of preventing the black shank in tobacco caused by the oomycete plant pathogen *Phytophthora nicotianae*, Jin et al. [24] performed an RNA-seq analysis based on two strains of *P. nicotianae*, which were adapted to the susceptible tobacco genotype Hicks and the partially resistant genotype K 326 Wz/Wz, respectively, to detect the DEGs during their pathogenic activities in their hosts. Interestingly, most of the DEGs were involved in pathogenicity in the K 326 Wz/Wz-adapted isolate under the infection of its host. This study broadened our understanding of the molecular mechanisms regulating the adaptation of *P. nicotianae* to its partial resistance in tobacco, i.e., the pathogen recruited pathogenicity-associated genes to ultimately influence the biological activities of the host.

Kim et al. [25] explored the genetic diversity in one of the non-edible oilseed crop plants, castor bean (*Ricinus communis* L.), based on a total of 54 samples (3 wild and 51 cultivated) collected worldwide using two types of molecular markers, i.e., inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD). The authors identified a total of 61 (74.53%) polymorphic bands among 83 bands with high resolution based on a total of nine ISSR primers. Additionally, a total of 56 (60.98%) polymorphic bands were identified out of 90 bands obtained based on 11 out of 20 RAPD primers with the amplification of unique polymorphic products. This study indicated that the cultivated varieties of castor contained a narrow genetic diversity. Therefore, the authors recommended that wild castor genetic resources should be considered for the molecular breeding of novel castor varieties with targeted features.

Qu et al. [26] studied the molecular mechanisms underlying the biosynthesis of oil in olive (*Olea europaea* L.), which is one of the most important crop plants for edible oil. Indeed, its commercial product “virgin olive oil” is well known worldwide for containing high-content fatty acids and many types of secondary metabolites. In particular, transcriptomic analysis was performed based on two olive cultivars, i.e., Kalinjot (JZ) and Coratina (KLD), with fruit samples collected during three developmental stages. The authors identified a total of 170 DEGs involved in five main metabolic pathways, including photosynthesis, amino acid biosynthesis, response to stress, energy metabolism, and, in particular, fatty acid metabolism, with their biological functions in oil biosynthesis being explicitly revealed. These analyses provided novel experimental evidence to facilitate the molecular breeding of olive varieties to increase the oil contents.

Chang et al. [27] investigated the metamorphic features of the male floral organs (i.e., stamens) in *Anemone barbulata* Turcz., which was previously recognized as *A. rivularis* Buch.-Ham. Ex DC. var. *flore-minore* Maxim., using both morphological and histological analyses. The transformation of stamens was revealed with successive variations, including two extreme forms, i.e., the white, sepaloïd structures, and more frequently green leaflike organs. Based on the extreme forms of metamorphic stamens characterized by the leaflike structure composed of three lobes attached to a long stalk, which was largely in accordance

with the morphological features of the typical leaves of the plants, it was hypothesized that the connective tissue and the two pollen sacs of the anther were transformed into the three lobes of the metamorphosed stamen, respectively. To summarize, the authors provided multiple lines of evidence based on various sequential forms of metamorphic stamens to support the hypothesis of the origin of flowers from metamorphic leaves in the progressive development mode during the evolution of floral organs.

Lastly, in their review article, Chen et al. [28] summarized recent advances in the improvements in the efficient use of nitrogen based on barley (*Hordeum vulgare* L.) breeding. Due to the importance of reducing the various types of cost and identifying effective solutions to the environmental problems (e.g., increased carbon footprint) resulting from the excess application of nitrogen fertilizers, both high nitrogen use efficiency or tolerance to low nitrogen fertilization are considered to be the ideal strategies to deal with the issues of the application of nitrogen fertilization worldwide. As a model plant for cereal crops, barley was selected due to its advantages, i.e., high adaptability, rapid growing season, and high resistance or tolerance to environmental stress, ultimately investigating into promoting the efficient use of nitrogen in barley beneficial for other types of cereal crop plants. In this review, the recent advancement has been summarized in various aspects related to nitrogen fertilization in barley, including the molecular response to nutritional nitrogen in barley, evaluation of nitrogen use efficiency or tolerance to low-nitrogen stress, quantitative trait loci (QTL) mapping and gene cloning, facilitating the improvement in the nitrogen use efficiency, and the breeding of highly nitrogen-efficient varieties of barley. Additionally, several biotechnological tools were introduced for exploring the underlying molecular mechanisms or breeding for promoting barley nitrogen use efficiency, including the genome-wide association study (GWAS), “omics,” and gene editing, as well as some promising research directions in the relevant areas.

In summary, we believe that this Special Issue of *Agronomy* significantly promotes our appreciation of the cutting-edge and innovative techniques that could help address agriculture-related issues worldwide. Furthermore, this Special Issue outlines numerous avenues for future research, encompassing both practical applications and technical challenges. The wealth of identified genetic and genomic elements contributing to the growth of crop plants, soil health, and beneficial interactions among plants, soil, and microbes, coupled with the rapid advancement of “omics” technologies, strongly foreshadows significant achievements for the agricultural field. We believe that these advancements align with the core themes highlighted in this Special Issue, underscoring their significance.

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