Chapter 7
Allium Functional Genomic Development for Future Climatic Changes

Mostafa Abdelrahman

7.1 Introduction

Allium plants represent the most economically important and representative genus of the Alliaceae family. References to these plants in the Quran and Bible reflect their significance to ancient civilizations both as flavorful foods and healing herbs. *Allium* is a huge genus (850 species) that is spread widely across the Northern Hemisphere from the boreal zone to the dry subtropics. A region of high species diversity spreads from the Mediterranean Basin to Central Asia, and a second smaller center of species diversity is located in North America (Kamenetsky and Rabinowitch 2006; Fritsch et al. 2010; Abdelrahman et al. 2016, Abdelrahman et al. 2017d) (Fig. 7.1). The *Allium* species have adapted to diverse ecological niches, which led to the development of several distinct morphotypes, resulting in difficulties in classification and taxonomy of *Allium* (Gregory et al. 1998; Abdelrahman et al. 2015). A multidisciplinary approach, including morphological and anatomical examinations, and systematic studies using molecular and biochemical markers have led to an infrageneric classification of *Allium* species into six subgenera (*Melanocrommyum*, *Rhizirideum*, *Caloscordum*, *Bromatorrhiza*, *Amerallium*, and *Allium*) and 43 sections (Hanelt et al. 1992; Hanelt and Fritsch 1994; Khassanov 1996; Friesen et al. 1999; Fritsch and Friesen 2002; Ricroch et al. 2005; Fritsch et al. 2010). Many species of *Allium* genus have high economic importance, including vegetables [bulb onion (*A. cepa*), shallot (*A. cepa* L. Aggregatum group), Japanese bunching onion (*A. fistulosum*), garlic (*A. sativum*), leek, kurrat, and great-headed garlic (*A. ampeloprasum*), chives (*A. schoenoprasum*), Chinese chives (*A. tuberosum*)], and ornamentals [(*A. giganteum*, *A. aflatunense*, *A. karataviense*)]. Also, about two dozens of *Allium*

M. Abdelrahman (✉)
Arid Land Research Center, Tottori University, Tottori 680-0001, Japan
e-mail: meettoo2000@tottori-u.ac.jp; meettoo2000@ige.tohoku.ac.jp

Botany Department, Faculty of Sciences, Aswan University, Aswan 81528, Egypt

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_7

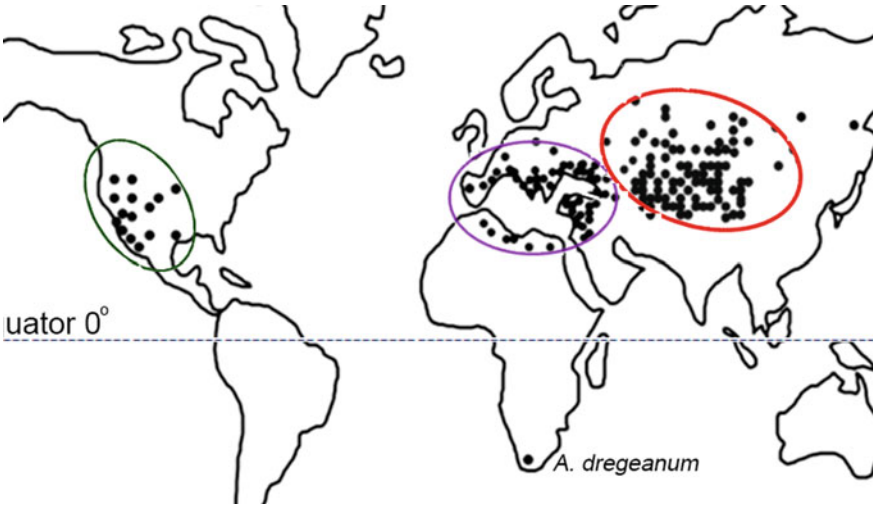

Fig. 7.1 Schematic diagram of the geographical distribution of *Allium* species based on literature. Color circles indicate the diversity centers extending from Central Asia to the Mediterranean Basin and Western North America

species are locally collected or cultivated as highly valued seasonings and medicinal plants. However, detailed information about the widespread use of these species remains incomplete (Khassanov 1996; Friesen et al. 2000; Fritsch and Friesen 2002; Keusgen et al. 2006).

During initial domestication, many immediate ancestors of *Allium* species have either been changed or lost. For instance, increasing numbers of different repetitive DNAs or retrotransposons, the lower GC content together with a minor DNA restructuring by point mutation is accountable for the enormousness size and complexity of the genome of many *Allium* crop species. For example, 2C DNA amounts per genome in 75 *Allium* species range between 16.93 and 63.57 pg (Ohri and Pistrick 2001), and onion has 16,415 megabase pairs (MbPs) of DNA per 1C nucleus which is 6 times higher than maize (*Zea mays*) and 16 times than rice (*Oryza sativa*), while garlic has ~15, 901 Mbp (Orhi et al. 1998). Also, the GC content of onion DNA is 32%, which is considered as the lowest among angiosperms (Kirk et al. 1970). Such a complicated genome reorganization is estimated to cause the speciation of *Allium*, which is the primary factor for reproductive isolation followed by the enlargement of habitat range. Genetic shifts and severe unbalanced selection pressure by breeders and farmers resulted in the loss of many useful agronomic traits for modern agriculture; therefore, genes of potentially useful characteristics were lost or are not readily available for crop improvement (Friesen et al. 2000; Fritsch and Friesen 2002; Kamenetsky and Rabinowitch 2006; Keusgen et al. 2006; Abdelrahman et al. 2014; Abdelrahman et al. 2018).

With the rise of next-generation sequencing (NGS) technologies, an increase in the speed and efficiency of DNA sequencing with higher throughputs and greater genome

coverage became achievable in many plant species including *Allium* (Abdelrahman et al. 2017a, b, c, d, f; Valliyodan et al. 2017; Yuan et al. 2017). These technologies led to the initial waves of crop genome sequences and facilitated the development of gene expression atlases and increased our understanding of the signaling pathways involved in the responses of plants to abiotic and biotic stressors (Rothberg et al. 2011; Pavlovich 2017; Abdelrahman et al. 2019b). The *Allium* international research community has developed several types of genetic stocks and applied these stocks to the latest modern technologies, which will be a milestone to accelerate *Allium* functional genomic studies. In this chapter, we will discuss the recent studies in *Allium* functional genomics as an innovative means of targeting the gene and bioactive metabolites responsible for the development of elite *Allium* varieties with unique chemical constituents and, subsequently, improved plant stress tolerance and human health benefits.

7.2 Organosulfur Compounds: A Prospective Active Ingredient for *Allium* Breeding

The *Allium* consumption as ethnomedicine or food ingredient is mostly associated with its nutritional and functional properties, which is mainly attributed to a variety of secondary metabolites (Caruso et al. 2014; Abdelrahman et al. 2016; Abdelrahman et al. 2019a). Among these secondary metabolites, organosulfur compounds are essential substances in terms of both biological activity and chemotaxonomic value of *Allium* species (Rose et al. 2005; Mostafa et al. 2013). There are four representatives of organosulfur compounds in *Allium* species, including (+)-S-(propyl)-L-cysteine sulfoxide (Propiin), (+)-S-(1-propenyl)-L-cysteine sulfoxide (Isoalliin), (+)-S-methyl-L-cysteine sulfoxide (Methiin), and (+)-S-(2-propenyl)-L-cysteine sulfoxide (Alliin) (Freeman and Whenham 1975; Hashimoto et al. 1984) (Fig. 7.2). These compounds are characteristics of each species and are generated by chemical transformation and cleavage of odorless, S-alk(en)yl cysteine sulphoxide precursors by the enzymes alliinase and lachrymatory-factor synthase (Jones et al. 2004). While S-alk(en)yl cysteine sulphoxides are found in the cytosol of the mesophyll tissue, alliinase is located in the vacuole of the vascular bundle sheath (Lancaster and Collin 1981). Once tissue being damaged by crashing or cutting alliinase is released and contact with S-alk(en)yl cysteine sulphoxides to cleave the C–S bond and generate sulfenic acid which is rapidly converted to thiosulfinates by non-enzymatic self-condensation (Yoshimoto and Saito 2017). Methiin is present in most of the *Allium* species and some Brassicaceae, alliin is characteristic of garlic, isoalliin is characteristic of onion and chive, while propiin is characteristic of onion, but it can be found in a minor content in most of the *Allium* species. Although methiin is present in less than 20% of total precursors in *A. cepa*, *A. sativum*, *A. ampeloprasum*, *A. proliferum*, *A. galanthum*, and *A. tuberosum*, however, some *Allium* species have a high content of methiin, which make them inappropriate for human consumption due to the strong

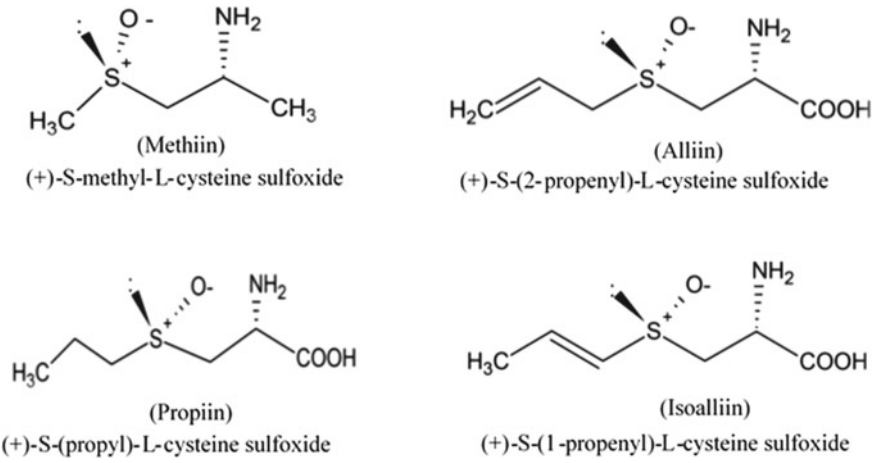

Fig. 7.2 Chemical structure of the four major cysteine sulfoxide compounds in *Allium* species

pungent smell (Kamenetsky and Rabinowitch 2006; Pratt 2010, Ramirez et al. 2017; Putnik et al. 2019).

Furthermore, chemotaxonomy of 40 *Allium* species from different subgenera revealed at least seven different chemotypes and showed specific arrays of volatile organosulfur compounds in the rhizomatous species (Storsberg et al. 2003; Kamenetsky and Rabinowitch 2006). This classification can contribute to a better selection of wild species for breeding experiments to improve taste, aroma, and medicinal properties of interspecific *Allium* hybrids. For instance, a recent study using chromosomal addition lines revealed an increase in organosulfur compounds, including gamma-glutamyl-PrenCS, S-2-carboxypropyl glutathione (2-CPGTH) and methiin, cycloalliin in *A. fistulosum* with extra chromosome 2A from shallot (Abdelrahman et al. 2019a).

Differences between cultivar and species in flavor characteristics mostly arose from variability in their sulfur uptake and metabolism, and the availability of sulfur is one of the factors that control the biosynthesis of each flavor precursor (Fritsch 2001; Storsberg et al. 2003; Kamenetsky and Rabinowitch 2006). The *Allium* plants synthesize organic sulfur compounds using the inorganic sulfate absorbed from the soil. Sulfate is converted into sulfite by adenosine 5′-phosphosulfate reductase (EC 1.8.4.9), and the latter converted into sulfide by sulfite reductase enzyme (EC 1.8.7.1). Sulfide is incorporated into cysteine, which subsequently undergoes two conversions, glutamylation and glycosylation, to yield glutathione (Turnbull et al. 1980; Kodera et al. 2017). Although the biosynthetic pathway of the flavor precursors, including (+)-S-alk(en)yl cysteine sulfoxides and their γ-glutamyl peptide relatives, has been published (Lancaster and Shaw 1989), there is still ambiguity about several stages, and whether the same pathway is followed in all tissues. It has been proposed that the biosynthesis of the flavor precursors in *Alliums* started with S-alk(en)ylation of the cysteine in glutathione, followed by transpeptidation to remove the glycyl group,

oxidation to the cysteine sulfoxides, and, finally, removal of the glutamyl group to yield cysteine sulfoxides (Lancaster and Shaw 1989; Lancaster et al. 1989; Block 1992; Prince et al. 1997). An alternative biosynthetic pathway ignores glutathione in favor of straight thioalk(en)ylation of O-acetyl serine or alk(en)ylation of cysteine, followed by oxidation to a sulphoxide. In both of the pathways, few of the proposed enzymes involved in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides from *Alliums* have not been identified. A recent subcellular localizations and kinetic properties of three γ-Glutamyl transpeptidases (GGT; EC 2.3.2.2) genes including *AsGGT1*, *AsGGT2*, and *AsGGT3* isolated from garlic suggested that these genes may contribute differently to the biosynthesis of alliin in garlic (Yoshimoto et al. 2015). To date, several studies have been conducted to characterize and identify GGTs in *Allium* plants, for instance, AcGGT partially purified from onion showed high substrate specificity to γ-glutamyl compounds, a putative intermediates of S-alk(en)yl-L-cysteine sulfoxide biosynthesis, suggesting the involvement of AcGGT in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides in onion (Lancaster and Shaw 1994). A partial cDNA of *AsGGT*, which has high sequence homology to *AcGGT*, was isolated from garlic, and its expression profiles suggested that AsGGT may play a role in synthesizing S-alk(en)yl-L-cysteine sulfoxides in garlic cloves during cold storage (Cho et al. 2012). Future investigations of the in vivo functions of different GGT will offer a better understanding of the molecular mechanisms underlying the biosynthesis of alliin and other cysteine sulfoxide compounds in *Allium*, which can be applied to future metabolic engineering of crop plants.

The role of organosulfur compounds in *Allium* abiotic stress tolerance is still unclear; however, some few evidences reported that *Allium* species and landraces grown under stress conditions exhibited high level of organosulfur compounds. For example, a comparative targeted metabolite profiling and transcriptome landscapes of tropical shallot doubled haploid (stress-tolerant) and cultivated onion doubled haploid, and their F_1 hybrid revealed several key genes and metabolites related to organosulfur were introgressed in abiotic stress response were upregulated shallot and F_1 genotypes as compared onion (Abdelrahman et al. 2015). Also the additional chromosome from shallot to Japanese bunching onion induced organosulfur compound accumulation under summer conditions (Masamura et al. 2011). Similarly, shallot landraces derived from Indonesia possessed high levels of methiin and isoalliin in comparison with different onion varieties (Ariyanti et al. 2018). In addition, a comparative study of antioxidant activities and organosulfur compounds in garlic, elephant garlic, and onion demonstrated a significant positive correlation between organosulfur compounds and antioxidant capacity in *Allium* crops (Kim et al. 2018). Nevertheless, there are no direct studies that addressed the in-depth role of organosulfur compounds in *Allium* abiotic stress tolerance, which remain to be a future task.

7.3 Steroidal Saponins in *Allium* Species: Anticancer, Antimicrobial, and Biosynthesis Pathway

Although organosulfur compounds have been considered a key component of *Allium* plants' medicinal properties, various researchers tend to attribute the prospective medicinal benefits of *Allium* plants to other constituents, such as polyphenolic compounds, especially flavonoids, steroidal saponins as well as sugars (Lanzotti 2005; Stajner et al. 2006; Lanzotti et al. 2012; Abdelrahman et al. 2017e). The genus *Allium* is a rich source of steroidal saponins, which can be classified into spirostanol, furostanol, and cholestane saponins based on their sapogenin structure (Challinor and De Voss 2013; Mostafa et al. 2013; Abdelrahman et al. 2017d). Apart from the Amaryllidaceae family, steroidal saponins are also broadly spread in other monocot families, such as Costaceae, Asparagaceae, Liliaceae, Dioscoreaceae, Melanthiaceae, and Smilacaceae. These saponin compounds have also been reported in some dicotyledonous angiosperms: Zygophyllaceae, Plantaginaceae, Solanaceae, and Fabaceae. The earliest reports on *Allium* saponins date back to the 1970s through the identification of alliogenin in the bulbs of *A. giganteum* (Khristulas et al. 1970) and diosgenin in *A. albidum* (Kereselidze et al. 1970), which was followed by first chemical survey of saponins from the *Allium* genus by Kravets in (Kravets et al. 1990), and Lanzotti in (Lanzotti 2005), (Kravets et al. 1990, Lanzotti 2005). Since then, a huge number of new saponin compounds have been revealed. The *Allium* saponins are mainly bi- or mono-desmosides; however, a tri-desmodic cholestane glycoside has been described in the bulbs of *A. macleanii* (Inoue et al. 1995). The sugar chain in *Allium* saponins consists of branched or linear chains made up most often of glucose, galactose, rhamnose, arabinose, and xylose units (Mostafa et al. 2013; Sobolewska et al. 2016).

Saponins are considered accountable for various pharmacological properties of several plants, and they are recognized as bioactive constituents of *Allium* species (Sobolewska et al. 2016). There have been several reports addressing the pharmacological activities of steroidal saponins, including cytotoxic, antithrombotic, antifungal, anti-inflammatory, and immunomodulatory effects (Sparg et al. 2004; Sun et al. 2009; Lanzotti et al. 2012; Abdelrahman et al. 2017e). Saponins are potential anticancer molecules, and the induction of apoptosis by saponins has been defined in several studies, including inhibition of cancer migration (Sun et al. 2010; Zhao et al. 2014) and proliferation (Beit-Yannai et al. 2011; Zhang et al. 2012). Steroidal saponins isolated from different *Allium* species displayed amazing cytotoxic activities against different animal and human cancer cell lines, such as 4T1 breast carcinoma, B16 melanoma, hepatocellular carcinoma HepG2, fibroblast 3T3-L1, and pheochromocytoma PC12 cell lines (Chen et al. 2009; Luo et al. 2011; Yu et al. 2015). In vitro examination of the cytotoxic activity of Cepa2 steroidal saponin, isolated from the dry roots of shallot against P3U1 myeloma cancer cell line showed its high efficiency as an anticancer with 91.13% reduction in P3U1 cell viability (Abdelrahman et al. 2017e). The reduction of cell viability was correlated with the increase in reactive oxygen species levels in Cepa2-treated P3U1 cells (Abdelrhaman

et al. Abdelrahman et al. 2017e). Similarly, Tuberoside M isolated from the seeds of *A. tuberosum* and F-gitonin isolated from the fresh bulbs of *A. jesdianum* inhibited the cancer cells growth, with $IC_{50} = 6.8$ and 1.5 μg/mL, respectively (Sang et al. 2001; Mimaki et al. Mimaki et al. 1999). More recently, the cytotoxic substance of *A. chinense* saponins (ACSs) inhibited the proliferation, cell migration, and colony formation of 4T1 and B16 cells in a dose-dependent manner (Yu et al. 2015). These studies above provide clear evidence for the anticancer activities of the natural saponin compounds isolated from *Allium* plants, and a strong basis for in-depth investigations for the development of novel anticancer drugs.

With increasing concern about the negative impacts of climate change on the development of plant disease epidemics and altering the interactions between plant and pathogens, greater effort toward improving plant disease resistance became a mandate. Although many steroidal saponin compounds isolated from diverse plant species have been reported to have antifungal activity, unfortunately, only a few studies have been performed so far on *Allium* steroidal glycosides antifungal properties (Mostafa et al. 2013; Sobolewska et al. 2016). Antifungal activity of *Allium* saponins is controlled by both the number and structure of the sugar residue and sapogenin type. Generally, saponins with spirostanol skeleton exhibited higher antifungal activity than furostanols (Mostafa et al. 2013). Lanzotti et al. (2012) provided a strong evidence for the significant differences in the potency of saponin compounds belonging to spirostane relative to furostane groups. For instance, gitogenin 3-*O*-tetrasaccharide and gigenin 3-*O*-trisaccharide, isolated from the bulbs of *A. sativum* var. Voghiera, were more active against *Trichoderma harzianum* and *Botrytis cinerea* than furostanol voghierosides isolated from the same plant (Lanzotti et al. 2012). Also, the sprirostanol Aginoside isolated from *A. nigrum* at 400 ppm completely inhibited the growth of *Botrytis squamosa* and *C. gloeosporioides*, and partially inhibited *F. oxysporum* f. sp. *cepae* and *F. oxysporum* f. sp. *radicis-lycopersici* (Mostafa et al. 2013). The influence of the structure of the sugar chain on the observed antifungal activity of Alliospirosides A isolated from the roots of shallot, inhibited a wide range of plant pathogenic fungi, including *Alternaria* ssp., *Botrytis* ssp., *Colletotrichum* spp., *Curvularia lunata*, *Epicoccum nigrum*, and *Fusarium* ssp. (Teshima et al. 2013). However, Alliospirosides A activity against *Fusarium* pathogens was relatively low in comparison with other phytopathogens (Teshima et al. 2013). Despite a large number of saponin compounds being isolated from different *Allium* species, little efforts have been invested in their antifungal activity. One of the main reasons for such drawback is the limited amount of the isolated pure compounds which are mostly being consumed through the identification and chemical structure elucidation methods by mass spectrometry and nuclear magnetic resonance (NMR).

In plants, steroidal saponins are mostly synthesized from lanosterol and cycloartenol via cholesterol and sitosterol, respectively. However, the steroidal saponin biosynthesis pathway in *Allium* has not been reported yet. Differential expression analysis of *Asparagus racemosus* fruit, leaves, and roots showed that expression of the transcripts involved in steroidal saponin biosynthesis is mainly upregulated in the leaf and root tissues, whereas triterpene saponins was dominated

in fruit and leaf tissues (Srivastava et al. 2018). In a recent study, Abdelrahman et al. (2017d) were able to isolate and identify Alliospiroside A saponin compound in *A. fistulosum* (FF) with additional chromosome 2A (FF2A) from shallot (AA) with potent role in defense mechanism against *Fusarium* pathogens. In addition, differential gene expression analyses of AA and FF2A as compared to FF (as a control) revealed a strong upregulation of the saponin downstream pathway, including glycosyltransferase, cytochrome P450, and beta-glucosidase in chromosome 2A (Abdelrahman et al. 2017d). An understanding of the biosynthesis-related genes and saponin compounds would facilitate the development of plants with unique saponin content and, subsequently, improved disease resistance.

7.4 Metabolomic and Transcriptomic Landscapes of *Allium* Crops Under Environmental Stress

The heavy yield losses in primary crops due to global warming and the increasing demand for food mean that there is a crucial need to improve food security (Abiala et al. 2018; Zhang et al. 2019). However, the development of abiotic stress-resilient crops requires an in-depth information about the biological processes that enable plants to survive in stressful environments, and this information can be achieved from "omic" studies, such as metabolomics, proteomics, transcriptomics, and genomics (Hirata et al. 2016; Abdelrahman et al. 2017b; Abdelrahman et al. 2018a, b; Galsurker et al. 2018; Wang et al. 2018). Unfortunately, there are limited studies addressing the *Allium* metabolome and transcriptome profiling in response to environmental stress, and thus the *Allium* international community needs further efforts in this regard. Transcriptome analysis between inner and outer scales of commercial brown onion cv. "Orlando" in response to the heat stress demonstrated that oxidation and lipid metabolism pathways, as well as cell-wall modification were highly expressed in the onion outer scale under heat stress (Galsurker et al. 2018). However, defense response-related genes such as those encoding antioxidative stress defense, heat shock proteins, or production of osmo-protectant metabolites were highly induced in the inner scale (Galsurker et al. 2018). These transcriptomic data led to a conceptual model that suggests consecutive processes for the development of desiccation and browning of the outer scale versus processes associated with defense response and heat tolerance in the inner scales (Galsurker et al. 2018). Transcriptome-based sequencing of cold-tolerant and cold-susceptible genotypes of onion under freezing and cold conditions indicated that several genes were significantly induced by freezing and cold stress in tolerant lines relative to susceptible genotype (Han et al. 2016). Among these transcript, genes encoding hypothetical proteins, zinc finger (ZIP) proteins, heat shock proteins (HSPs), and CBL-interacting protein kinase (CIPK), in addition to subset of transcription factors, particularly those that function as activators including dehydration-responsive element (DRE)-binding (DREB),

CBL, MYB, bZIP, zinc finger of *Arabidopsis thaliana* (ZAT), HSPs and basic helix-loop-helix (bHLH) were drastically changed during freezing and cold conditions (Han et al. 2016). Similarly, genome-wide transcriptome profiling analysis of garlic under low temperature stress indicated that enzyme-encoding genes, which significantly enriched the pathway "proteasome," are potentially involved in the garlic discoloration under low temperature stress, such as γ-glutamyltranspeptidase-, δ-aminolevulinic acid dehydratase-, and alliinase-encoding genes (Li et al. 2018). These stress-responsive genes are possibly responsible for the low-temperature-induced garlic discoloration (Li et al. 2018). Effects of salinity stress on the growth parameters and K^+/Na^+ ratio of *Allium* vegetables (Welsh onion and Wakegi) using diverse concentrations of seawater demonstrate stunting of plants; however, the rate of growth reduction under salinity stress varies widely among different *Allium* plants (Arakaki et al. 2014). The chlorophyll content as in term of SPAD values of the leaves of the Welsh onion decreased, whereas the SPAD value of the two types of Wakegi cultivars increased (Arakaki et al. 2014). In addition, the total sugar and phenolic contents increased significantly compared with the respective controls under seawater treatment (Arakaki et al. 2014). Environmental stress affects plant growth, thus identification of stress biomarkers is a major prerequisite for the breeding of stress-tolerant crops. In this regard, because of its high adaptability to subtropical and tropical environment, shallots are recognized as an important genetic resource for the breeding of common onion (Abdelrahman et al. 2015). Using liquid chromatography quadruple-mass spectrometer (LC-QqQ-MS), the bulb onion double haploid, shallot double haploid, and its F_1 hybrid were evaluated. In total, 113 targeted metabolites were detected, and the principal component analysis and volcano plot analysis clearly showed genotype-specific metabolites, which can be used as metabolic markers of environmental tolerance (Abdelrahman et al. 2015). Similarly, integrated transcriptome and metabolome analysis of *A. fistulosum* with additional chromosome 5A from shallot revealed an accumulation of several flavonoid compounds which are majorly involved in abiotic and biotic stress tolerances (Abdelrahman et al. 2019a). Also the increase in flavonoid pool in *A. fistulosum* with additional chromosome 5A from shallot was consistent with the upregulation of many upstream and downstream flavonoid biosynthesis and regulatory genes (Abdelrahman et al. 2019a). The above results confirmed that shallot can be a potential genetic resource for the improvement of onion stress tolerance. Likewise, Zhang et al. (2018) used transcriptome analysis of two contrasting dark-red and white onion cultivars, revealing that both flavonoid 3′,5′-hydroxylase (*F3′,5′H*) and dihydroflavonol 4-reductase (*DFR*) genes play major role in the biosynthesis of dark-red bulbs, and the expression levels of flavonol synthase (*FLS*) and *DFR* genes may act to block blue pigmentation. In addition, the positive variation in the F3′,5′H/F3′H ratio also affects onion bulb color diversity (Zhang et al. 2018). A recent study using comparative transcriptome analysis of cold-tolerant and sensitive bulb onions provides further information regarding the transcriptional changes underlying cold and freezing tolerance mechanisms in addition to molecular markers that would facilitate gene mapping and genetic diversity analysis (Han et al. 2016).

7.5 Future Aspects for *Allium* Functional Genomics

Allium transcriptomics and metabolomics will elucidate characteristic metabolites and their biosynthesis or regulatory related genes within different accessions, landraces, and cultivars. The individual bio-resource-specific metabolic patterns can be used for molecular breeding of *Allium* crops while the broad metabolic profiles of *Allium* bioresources can be used for integrated omics approaches. Further integrated omics approaches, e.g., correlation analysis between transcriptome and metabolome, linkage mapping, can elucidate the gene-to-metabolite networks in environmental responses or stress. In this regard, *Allium* transcriptome database (*Allium* TDB; http://alliumtdb.kazusa.or.jp/) provides a comprehensive information of the transcriptome analysis in different *Allium* species that can be used for further genetic and molecular breeding studies. The integration of metabolomics and transcriptomics will provide insight into the molecular mechanism of *Allium* metabolite biosynthesis, which can be used for elucidation of the molecular architecture underlying environmental responses and stress tolerance in *Allium*.

References

Abdelrahman M, Abdel-Motaal F, El-Sayed M, Jogaiah S, Shigyo M, Ito S, Tran LS (2016) Dissection of *Trichoderma longibrachiatum* induced-defense in onion (*Allium cepa* L.) against *Fusariumoxysporum* f. sp. *cepa* by target metabolite profiling. Plant Sci 246:128–138

Abdelrahman M, Burritt DJ, Gupta A, Tsujimoto H, Tran LSP (2019b) Heat stress effect on source-sink relationships and metabolome dynamics in wheat. J Exp Bot erz296

Abdelrahman M, Burritt DJ, Tran LP (2017a) The use of metabolomic quantitative trait locus mapping and osmotic adjustment traits for the improvement of crop yields under environmental stresses. Semin Cell Dev Biol 83:86–94

Abdelrahman M, El-Sayed M, Jogaiah S, Burritt DJ, Tran LP (2017b) The "STAY-GREEN" trait and phytohormone signaling networks in plants under heat stress. Plant Cell Rep 36:1009–1025

Abdelrahman M, El-Sayed M, Sato S, Hirakawa H, Ito SI, Tanaka K, Mine Y, Sugiyama N, Suzuki M, Yamauchi N, Shigyo M (2017d) RNA-sequencing-based transcriptome and biochemical analyses of steroidal saponin pathway in a complete set of *Allium fistulosum-A. cepa* monosomic addition lines. PLoS One 12:0181784

Abdelrahman M, Jogaiah S, Burritt DJ, Tran LP (2018a) Legume genetic resources and transcriptome dynamics under abiotic stress conditions. Plant, Cell Environ 41:1972–1983

Abdelrahman M, El-Sayed MA, Hashem A, AbdAllah EF, Alqarawi AA, Burritt DJ, Tran LP (2018b) Metabolomics and transcriptomics in legumes under phosphate deficiency in relation to nitrogen fixation by root nodules. Front Plant Sci 9:922

Abdelrahman M, Hirata S, Sawada Y, Hirai MY, Sato S, Hirakawa H, Mine Y, Tanaka K, Shigyo M (2019a)Widely targeted metabolome and transcriptome landscapes of *Allium fistulosum–A. cepa* chromosome addition lines revealed a flavonoid hot spot on chromosome 5A. Sci Rep 9:3541

Abdelrahman M, Hirata S, Ito SI, Yamauchi N, Shigyo M (2014) Compartmentation and localization of bioactive metabolites in different organs of *Allium roylei*. Biosci Biotechnol Biochem 78:1112–22

Abdelrahman M, Mahmoud HYAH, El-Sayed M, Tanaka S, Tran LS (2017e) Isolation and characterization of Cepa2, a natural alliospiroside A, from shallot (*Allium cepa* L. Aggregatum group) with anticancer activity. Plant Physiol Biochem 116:167–173

Abdelrahman M, Mitoma M, Ikeuchi T, Mori M, Murakami K, Ozaki Y, Matsumoto M, Uragami A, Kanno A (2017c) Differential gene expression analysis and SNP/InDel marker discovery in resistant wild *Asparagus kiusianus* and susceptible *A. officinalis* in response to *Phomopsis asparagi* infection. Data Brief 21:2117–2121

Abdelrahman M, Sawada Y, Nakabayashi R, Sato S, Hirakawa H, El-Sayed M, Hirai MY, Saito K, Yamauchi N, Shigyo M (2015) Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. Mol Breed 35:195

Abdelrahman M, Suzumura N, Mitoma M, Matsuo S, Ikeuchi T, Mori M, et al. (2017c) Comparative de novo transcriptome profiles in *Asparagus officinalis* and *A. kiusianus* during the early stage of *Phomopsis asparagi* infection. Sci Rep 7:2608

Abiala MA, Abdelrahman M, Burritt DJ, Tran LP (2018) Salt stress tolerance mechanisms and potential applications of legumes for sustainable reclamation of salt-degraded soils. Land Deg Dev 29:3812–3822

Ariyanti NA, Torikai K, Kirana RP, Hirata S, Sulistyaningsih E, Ito SI, Yamauchi N, Kobayashi N, Shigyo M (2018) Comparative study on phytochemical variations in Japanese F_1 varieties of bulb onions and South-East Asian shallot landraces. J Jpn Soc Hort Sci 87:63–72

Arakaki M, Takahashi M, Hossain MDA, Wada K (2014) Changes in sugar content and antioxidant activity of *Allium* vegetables by salinity-stress. Food Sci Technol Res 20:705–710

Beit-Yannai E, Ben-Shabat S, Goldschmidt N (2011) Antiproliferative activity of steroidal saponins from *Balanites aegyptiaca*: an in vitro study. Phytochem Lett 4:43–47

Block E (1992) The organosulfur chemistry of the genus *Allium* implications for the organic chemistry of sulfur. Angew Chem 31:1135–1178

Caruso G, Conti S, Villari G, Borrelli C, Melchionna G, Mintutolo M, Russo G, Amalfitano C (2014) Effects of transplanting time and plant density on yield, quality and antioxidant content of onion (*Allium cepa* L.) in southern Italy. Sci Hort 166:111–120

Challinor VL, De Voss JJ (2013) Open-chain steroidal glycosides, a diverse class of plant saponins. Nat Prod Rep 30:429–454

Chen HF, Wang GH, Luo Q (2009) Two new steroidal saponins from *Allium macrostemon* bunge and their cytotoxicity on different cancer cell lines. Molecules 14:2246–2253

Cho J, Park M, Choi D, Lee SK (2012) Cloning and expression of γ-glutamyl transpeptidase and its relationship to greening in crushed garlic (*Allium sativum*) cloves. J Sci Food Agri 92:253–257

Fattorusso E, Lanzotti V, Taglialatela-Scafati O, Di Rosa M, Ianaro A (2000) Cytotoxic saponins from bulbs of *Allium porrum* L. J Agri Food Chem 48:3455–3462

Freeman GG, Whenham RJ (1975) A survey of volatile components of some *Allium* species in terms of S-alk(en)yl-L-cysteine sulphoxides presents as flavor precursors. J Sci Food Agri 26:186–1886

Friesen N, Fritsch RM, Pollner S, Blattner FR (2000) Molecular and morphological evidence for an origin of the aberrant genus Milula within Himalayan species of *Allium* (Alliaceae). Mol Phylogenet Evol 17:209–218

Friesen N, Pollner S, Bachmann K, Blattner FR (1999) RAPDs and non-coding chloroplast DNA reveal a single origin of the cultivated *Allium fistulosum* from *A. altaicum* (Alliaceae). Amer J Bot 86:554–562

Fritsch R (2001) Taxonomy of the genus *Allium*: Contribution from IPK Gatersleben. Herbertia 56:19–50

Fritsch RM, Blattner FR, Gurushidze M (2010) New classification of *Allium* L. subg. Melanocrommyum (Webb & Berthel) Rouy (Alliaceae) based on molecular and morphological characters. Phyton 49:145–220

Fritsch RM, Friesen N (2002) Evolution, domestication and taxonomy. In: Rabinovitch HD, Currah L (eds) *Allium* crop science: recent advances. CAB international, Wallingford, UK, pp 5–27

Galsurker O, Doron-Faigenboim A, Teper-Bamnolker P, Daus A, Lers A, Eshel D (2018) Differential response to heat stress in outer and inner onion bulb scales. J Exp Bot 69:4047–4064

Gregory M, Fritsch RM, Friesen NW, Khassanov FO, McNeal DW (1998) Nomenclator alliorum. Allium Names and Synonyms a World Guide, Royal Botanic Gardens, Kew

Han J, Thamilarasan SK, Natarajan S, Park J-I, Chung M-Y, Nou I-S (2016) De Novo assembly and transcriptome analysis of bulb onion (*Allium cepa* L.) during cold acclimation using contrasting genotypes. PLoS One 11(9):0161987

Hanelt P, Fritsch RM (1994) Notes on infrageneric taxa in *Allium* L. Kew Bull 49:559–564

Hanelt P, Schultze-Motel J, Fritsch RM, Kruse J, Maass HI, Ohle H, Pistrick K (1992) Infrageneric grouping of *Allium*. The Gatersleben approach. In: Hanelt P, Hammer K, Knupffer H (eds) The genus *Allium*-taxonomic problems and genetic resources. Proceedings of an international symposium held at Gatersleben, Germany, pp 11–13. Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, pp 107–123

Hashimoto S, Miyazawa M, Kameoka H (1984) Volatile flavor components of *Allium grayi* Regel. J Sci Food Agri 35:353–356

Hirata S, Abdelrahman M, Yamauchi N, Shigyo M (2016) Characteristics of chemical components in genetic resources of garlic *Allium sativum* collected from all over the world. Genet Res Crop Evol 63:35–45

Inoue T, Mimaki Y, Sashida Y, Nishino A, Satomi Y, Nishino H (1995) Steroidal glycosides from *Allium macleanii* and *A. senescens*, and their inhibitory activity on tumor promoter-induced phospholipid metabolism of HeLa cells. Phytochemistry 40:521–525

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA (2004) Biosynthesis of the flavour precursors of onion and garlic. J Exp Bot 55:1903–1918

Kamenetsky R, Rabinowitch HD (2006). The genus *Allium*: a developmental and horticultural analysis. Hort Rev 32:329–378

Kereselidze EV, Pkheidze TA, Kemertelidze EP (1970) Diosgenin from *Allium albidum*. Chem Nat Comp 6:378

Keusgen M, Fritsch RM, Hisoriev H, Kurbonova PA, Khassanov FO (2006) Wild *Allium* species (Alliaceae) used in folk medicine of Tajikistan and Uzbekistan. J Ethnobiol Ethnomed 2:18

Khassanov FO (1996) Conspectus of the wild growing *Allium* species of Middle Asia. In: Öztürk M, Seçmen Ö, Görk G (eds) Plant Life in Southwest and Central Asia. EGE University Press, Izmir, pp 141–159

Khristulas FS, Gorovits MB, Luchanskaya VN, Abubakirov NK (1970) A new steroid sapogenin from *Allium giganteum*. Chem Nat Comp 6:489

Kim S, Kim DB, Jin W, Park J, Yoon W, Lee Y, Kim S, Lee S, Kim S, Lee OH, Shin D, Yoo M (2018) Comparative studies of bioactive organosulphur compounds and antioxidant activities in garlic (*Allium sativum* L.), elephant garlic (*Allium ampeloprasum* L.) and onion (*Allium cepa* L.). Nat Prod Res 32:1193–1197

Kirk JTO, Rees H, Evans G (1970) Base composition of nuclear DNA with the genus *Allium*. Heredity 25:507–512

Kodera Y, Ushijima M, Amano H, Suzuki JI, Matsutomo T (2017) Chemical and biological properties of S-1-Propenyl-l-Cysteine in aged garlic extract. Molecules 22:570

Kravets SD, Vollerner YS, Gorovits MB, Abubakirov NK (1990) Steroids of the spirostan and furostan series from plants of the genus *Allium*. Chem Nat Comp 26:359–373

Lancaster JE, Collin HA (1981) Presence of alliinase in isolated vacuoles and of alkyl cysteine sulphoxides in the cytoplasm of bulbs of onion (*Allium cepa*). Plant Sci Lett 22:169–176

Lancaster JE, Reynolds PHS, Shaw ML, Dommisse EM, Munro J (1989) Intracellular localization of the biosynthetic pathway to flavour precursors in onion. Phytochemistry 28:461–464

Lancaster JE, Shaw ML (1989) γ-glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. Phytochemistry 28:455–460

Lancaster JE, Shaw ML (1994) Characterization of purified γ-glutamyl transpeptidase in onions: evidence for in vivo role as a peptidase. Phytochemistry 36:1351–1358

Lanzotti V (2005) Bioactive saponins from *Allium* and Aster plants. Phytochem Rev 4:95–110

Lanzotti V, Barile E, Antignani V, Bonanomi G, Scala F (2012) Antifungal saponins from bulbs of garlic, *Allium sativum* L. var. Voghiera. Phytochemistry 78:126–134

Li N, Qiu Z, Lu X, Shi B, Sun X, Tang X, Qiao X (2018) Comparative transcriptome analysis of temperature-induced green discoloration in garlic. Intl J Genom 2018:6725728

Luo H, Huang J, Liao WG (2011) The antioxidant effects of garlic saponins protect PC12 cells from hypoxia-induced damage. Brit J Nutr 105:1164–1172

Masamura N, Yaguchi S, Ono Y, Nakajima T, Masuzaki S, Imai S, Yamauchi N, Shigyo M (2011) Characterization of amino acid and S-alk(en)yl-L-cysteine sulfoxide production in Japanese bunching onion carrying an extra chromosome of shallot. J Jpn Soc Hort Sci 80:322–333

Mimaki Y, Kuroda M, Sashida Y (1999) Steroidal saponins from the bulbs of *Allium aflatunense*. Nat Med 53:88–93

Mostafa A, Sudisha J, El-Sayed M, Ito SI, Ikeda T, Yamauchi N, Shigyo M (2013) Aginsodie saponin, a potent antifungal compound, and secondary metabolite analyses from *Allium nigrum* L. Phytochem Lett 6:274–280

Ohri D, Pistrick K (2001) Phenology and genome size variation in *Allium* L.-a tight correlation? Plant Biol 3:654–660

Orhi D, Fritsch RM, Hanelt P (1998) Evolution of genome size in *Allium* (Alliaceae). Plant Syst Evol 210:57–86

Pavlovich (2017) Computing in biotechnology: Omics and beyond. Computation and Modeling 35:479–480

Pratt DA (2010) Garlic and other *Alliums*. The lore and the science. By Eric Block. Angewandte Chemie International Edition, Wiley. Royal Society of Chemistry, Cambridge, 454, pp 7162–7162

Prince CL, Shuler ML, Yamada Y (1997) Altering flavor profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis. Biotechnol Progr 13:506–510

Putnik P, Gabrić D, Roohinejad S, Barba FJ, Granato D, Mallikarjunan K, Lorenzo MJ, Kovačević DB (2019) An overview of organosulfur compounds from *Allium* spp.: from processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chem 276:680–691

Ramirez DA, Locatelli DA, González RE, Cavagnaro PF, Camargo AB (2017) Analytical methods for bioactive sulfur compounds in *Allium*: an integrated review and future directions. J Food Comp Analys 61:4–19

Ricroch A, Yockteng R, Brown SC, Nado S (2005) Evolution of genome size across some cultivated *Allium* species. Genome 48:511–520

Rose P, Whiteman M, Moore PK et al (2005) Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: the chemistry of potential therapeutic agents. Nat Prod Rep 22:351–368

Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M et al (2011) An integrated semiconductor device enabling non-optical genome sequencing. Nature 475:348–352

Sang SM, Zou M, Xia Z, Lao A, Chen Z, Ho CT (2001) New spirostanol saponins from Chiense chives (*Allium tuberosum*). J Agri Food Chem 49:4780–4783

Sobolewska D, Michalska K, Podolak I, Grabowska K (2016) Steroidal saponins from the genus *Allium*. Phytochem Rev 15:1–35

Sparg SG, Light ME, Van Staden J (2004) Biological activities and distribution of plant saponins. J Ethnopharm 94:219–243

Srivastava PL, Shukla A, Kalunke RM (2018) Comprehensive metabolic and transcriptomic profiling of various tissues provide insights for saponin biosynthesis in the medicinally important *Asparagus racemosus*. Sci Rep 8:9098

Stajner D, Milic´ N, Canadanović-Brunet J, Kapor A, Stajner M, Popović BM (2006) Exploring *Allium* species as a source of potential medicinal agents. Phytother Res 20:581–584

Storsberg J, Schulz H, Keller ERJ (2003) Chemotaxonomic classification of some *Allium* wild species on the basis of their volatile sulphur compounds. J Appl Bot 77:160–162

Sun HX, Xie Y, Ye YP (2009) Advances in saponin-based adjuvants. Vaccine 27:1787–1796

Sun L, Lin S, Zhao R (2010) The saponin monomer of dwarf lilyturf tuber, DT-13, reduces human breast cancer cell adhesion and migration during hypoxia via regulation of tissue factor. Biol Pharm Bull 33:1192–1198

Teshima Y, Ikeda T, Imada K et al (2013) Identification and biological activity of antifungal saponins from shallot (*Allium cepa* L. Aggregatum group). J Agri Food Chem 61:7440–7445

Turnbull A, Galpin IJ, Collin HA (1980) Comparison of the onion plant (*Allium cepa*) and onion tissue culture. III. Feeding of ^{14}C lS1PC has two forms of isomersabeled precursor of the flavor precursor compounds. New Phytol 85:485–487

Valliyodan B, Ye H, Song L, Murphy M, Shannon JG, Nguyen HT (2017) Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans. J Exp Bot 68:1835–1849

Wang C, Jogaiah S, Zhang WY, Abdelrahman M, Fang JG (2018) Spatio-temporal expression of miRNA159 family members and their GAMYB target gene during the modulation of gibberellin-induced grapevine parthenocarpy. J Exp Bot 69:3639–3650

Yoshimoto N, Saito K (2017) Biosynthesis of S-Alk(en)yl-cysteine sulfoxides in *Allium*: Retro prospective. In: De Kok LJ, Hawkesford MJ, Haneklause SH, Schnug E (eds) Sulfur metabolism in higher plants-fundamentals, environmental and agricultural aspects. Processing of International Plant Sulfur Workshop, Springer, Switzerland AG, pp 49–60

Yoshimoto N, Yabe A, Sugino Y, Murakami S, Sai-ngam N, Sumi SI, Tsuneyoshi T, Saito K (2015) Garlic γ-glutamyl transpeptidases that catalyze deglutamylation of biosynthetic intermediate of alliin. Front Plant Sci 5:758

Yu Z, Zhang T, Zhou F, Xiao X, Ding X, He H, Rang J, Quan M, Wang T, Zuo M, Xia L (2015) Anticancer Activity of Saponins from *Allium chinense* against the B16 melanoma and 4T1 breast carcinoma cell. Evid-Based Comp Alter Med 2015:725023

Yuan Y, Bayer PE, Batley J, Edwards D (2017) Improvements in genomic technologies: application to crop genomics. Trend Biotechnol 35:558–574

Zhang W, Chen G, Deng CQ (2012) Effects and mechanisms of total *Panax notoginseng* saponins on proliferation of vascular smooth muscle cells with plasma pharmacology method. J Pharm Pharmacol 64:139–145

Zhao RP, Lin SS, Yuan ST (2014) DT-13, A saponin of dwarf lilyturf tuber, exhibits anticancer activity by down-regulating C-C chemokine receptor type 5 and vascular endothelial growth factor in MDA-MB-435 cells. Chin J Nat Med 12:24–29

Zhang C, Li X, Zhan Z, Cao L, Zeng A, Chang G, Liang Y (2018) Transcriptome sequencing and metabolism analysis reveals the role of cyanidin metabolism in dark-red onion (*Allium cepa* L.) bulbs. Sci Rep 8:14109

Zhang W, Abdelrahman M, Jiu S, Guan L, Han J, Zheng T, Jia H, Song C, Fang J, Wang C (2019) VvmiR160s/VvARFs interaction and their spatio-temporal expression/cleavage products during GA-induced grape parthenocarpy. BMC Plant Biol 19:111