SEMINAR REPORT

Repressor proteins' role in abiotic stress response in different plants

By

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CERTIFICATE

This is to certify that the seminar report entitled **"Repressor proteins' role in abiotic stress response in different plants"** has been solely prepared by **Keanapalle Sunny Babu (2018-11-141)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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1. Introduction

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.(1.1)

Plants are subjected to a wide range of environmental stresses which reduces and limits the productivity of agricultural crops. Two types of environmental stresses are encountered to plants which can be categorized as (1) Abiotic stress and (2) Biotic stress. The abiotic stress causes the loss of major crop plants worldwide and includes radiation, salinity, floods, drought, extremes in temperature, heavy metals, etc. On the other hand, attacks by various pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores are included in biotic stresses. As plants are sessile in nature, they have no choice to escape from these environmental cues. Plants have developed various mechanisms in order to overcome these threats of biotic and abiotic stresses. They sense the external stress environment, get stimulated and then generate appropriate cellular responses. They do this by stimuli received from the sensors located on the cell surface or cytoplasm and transferred to the transcriptional machinery situated in the nucleus, with the help of various signal transduction pathways. This leads to differential transcriptional changes making the plant tolerant against the stress. The signalling pathways act as a connecting link and play an important role between sensing the stress environment and generating an appropriate biochemical and physiological response. (1.2)

2. Abiotic stress and types (1.2)

The negative impact of non-living factors on living organisms in a specific environment

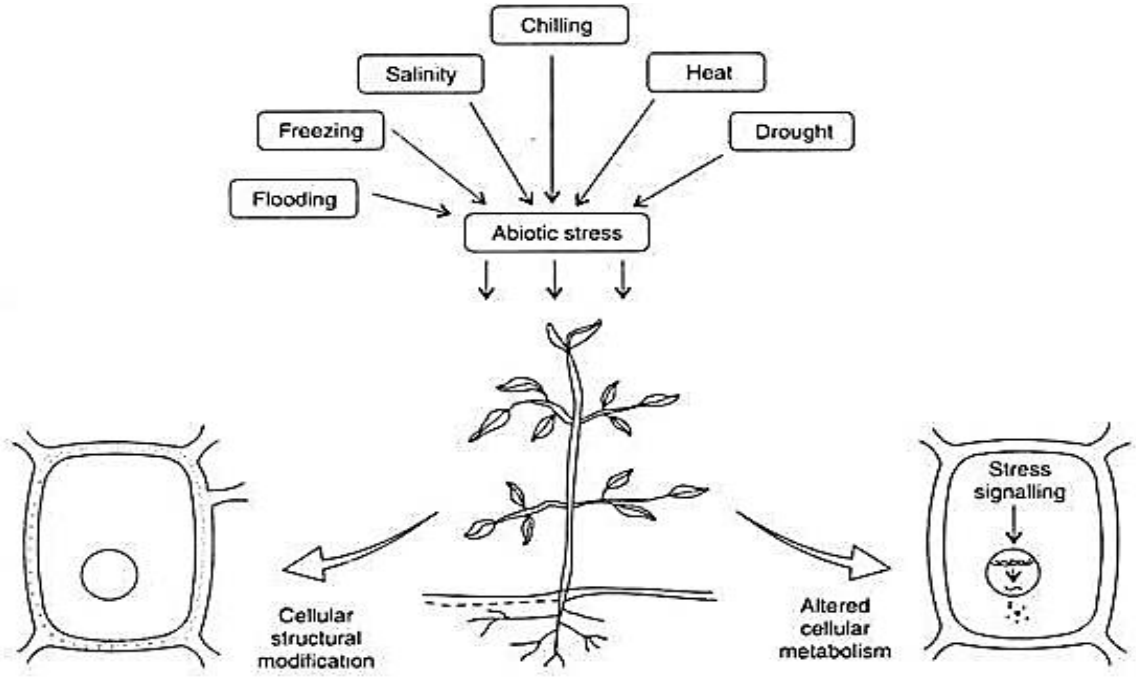

Fig .1

Plants are encountered by number of abiotic stresses which impact on the crop productivity worldwide. These abiotic stresses are interconnected with each other and may occur in form of osmotic stress, malfunction of ion distribution and plant cell homeostasis. The growth rate and productivity is affected by a response caused by group of genes by changing their expression patterns. So, the identification of responsive genes against abiotic stresses is necessary in order to understand the abiotic stress response mechanisms in crop plants. The abiotic stresses occurring in plants include.

2.1 Cold

Cold stress as abiotic stress has proved to be the main abiotic stresses that decrease productivity of agricultural crops by affecting the quality of crops and their post-harvest life. Plants being immobile in nature are always busy to modify their mechanisms in order to prevent themselves from such stresses. In temperate conditions plants are encountered by chilling and freezing conditions that are very harmful to plants as stress. In order to adopt themselves, plants acquire chilling and freezing tolerance against such lethal cold stresses by a process called as acclimation. However many important crops are still incompetent to the process of cold acclimation. The abiotic stress caused by cold affect the cellular functions of plants in every

aspect. Several signal transduction pathways are there by which these cold stresses are transduced like components of ROS, protein kinase, protein phosphate, ABA and Ca2+, etc. and among these ABA proves to be best.

2.2 Salt

Soil salinity poses a global threat to world agriculture by reducing the yield of crops and ultimately the crop productivity in the salt affected areas. Salt stress reduces growth of crops and yield in many ways. Two primary effects are imposed on crop plants by salt stress; osmotic stress and ion toxicity. The osmotic pressure under salinity stress in the soil solution exceeds the osmotic pressure in plant cells due to the presence of more salt, and thus, limits the ability of plants to take up water and minerals like K+ and Ca2+. These primary effects of salinity stress causes some secondary effects like assimilate production, reduced cell expansion and membrane function as well as decreased cytosolic metabolism.

2.3 Drought

Nowadays climate has changed all around the globe by continuously increase in temperature and atmospheric CO2 levels. The distribution of rainfall is uneven due to the change in climate which acts as an important stress as drought. The soil water available to plants is steadily increased due severe drought conditions and cause death of plants prematurely. After drought is imposed on crop plants growth arrest is the first response subjected on the plants. Plants reduce their growth of shoots under drought conditions and reduce their metabolic demands. After that protective compounds are synthesized by plants under drought by mobilizing metabolites required for their osmotic adjustment.

2.4 Heat

Increase in temperature throughout the globe has become a great concern, which not only affect the growth of plants but their productivity as well especially in agricultural crops plants. When plants encounter heat stress the percentage of seed germination, photosynthetic efficiency and yield declines. Under heat stress, during the reproductive growth period, the function of tapetal cells is lost, and the anther is dysplastic.

2.5 Toxin

The increased dependence of agriculture on chemical fertilizers and sewage waste water irrigation and rapid industrialization has added toxic metals to agriculture soils causing harmful effects on soil-plant environment system.

3. Repressor proteins and types

In molecular genetics, a repressor is a DNA- or RNA-binding protein that inhibits the expression of one or more genes by binding to the operator or associated silencers **Nicholas, 1997**

3.1. DNA binding repressors

- Block the binding of RNA polymerase to the promoter
- The gene is prevented from being transcribed into mRNA

3.2. RNA-binding repressors

- Bind to mRNA, preventing protein translation

3.3. Mechanisms of repression

4. Different repressors

4.1. AP2/ERF Factors:

Dynamic environmental changes such as extreme temperature, water scarcity and high salinity affect plant growth, survival, and reproduction. Plants have evolved sophisticated regulatory mechanisms to adapt to these unfavourable conditions, many of which interface with plant hormone signalling pathways. Abiotic stresses alter the production and distribution of phytohormones that in turn mediate stress responses at least in part through hormone- and stress-responsive transcription factors. Among these, the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) family transcription factors (AP2/ERFs) have emerged as key regulators of various stress responses, in which they also respond to hormones with improved plant survival during stress conditions. Apart from participation in specific stresses, AP2/ERFs are involved in a wide range of stress tolerance, enabling them to form an interconnected stress regulatory network. Additionally, many AP2/ERFs respond to the plant hormones abscisic acid (ABA) and ethylene (ET) to help activate ABA and ET dependent and independent stress responsive genes. While some AP2/ERFs are implicated in growth and developmental processes mediated by gibberellins (GAs), cytokinins (CTK), and brassinosteroids (BRs).

The involvement of AP2/ERFs in hormone signalling adds the complexity of stress regulatory network. AP2/ERFs are characterized by an APETALA2 (AP2)/Ethylene Responsive Element Binding Factor (EREB) domain, which consists of 40–70 conserved amino acids involved in DNA binding. AP2/ERFs contain the four major subfamilies: APETALA2 (AP2), RELATED TO ABSCISIC ACID INSENSITIVE 3/VIVIPAROUS 1 (RAV), DEHYDRATION-RESPONSIVE ELEMENT BINDING proteins (DREBs) (subgroup A1– A6) and ETHYLENE RESPONSIVE FACTORS (ERFs) (subgroup V-X). As transcription factors, AP2/ERFs regulate genes involved in diverse biological processes such as growth, development, hormone and stress responses through several mechanisms including transcriptional and post-translational control. In many cases, AP2/ERFs expression is regulated by the conserved cis-elements present in their promoter regions, or a combination of multiple responsive element. Histone modifications such as phosphorylation, ubiquitination, methylation, and acetylation can either activate or repress transcription by creating more “open” or “closed” chromatin configurations.

4.1.1. AP2/ERF REGULATORY NETWORKS IN ABIOTIC STRESSES

AP2/ERFs regulate numerous abiotic stresses such as cold, drought, heat, salt, and freezing. Although many AP2/ERFs are proposed to form an abiotic stress-specific regulatory network, the ability of AP2/ERFs to respond to multiple stimuli and regulate different stresses enable them to form a more complex stress response network. In this network, AP2/ERFs also respond to abiotic stresses with varying dynamic patterns: some AP2/ERFs are induced quickly and continuously, whereas others are regulated by prolonged stress, which indicates they might have mutual influence on each other's function (Van den Broeck et al., 2017). However, the detailed mechanisms of how different AP2/ERFs cooperate or antagonize with each other are yet to be established. Therefore, in addition to studying the function of individual transcription factors in this family, it is also necessary to study the relationship between different AP2/ERFs in abiotic stress responses.

4.1.2. DREBs: Major Regulators in Cold, Drought, Heat, and Salt Stress Responses

DREBs have been extensively examined in abiotic stresses, where they respond to and positively regulate cold, drought, heat and salt tolerance by directly regulating stress-responsive genes. Among these, DREB1s (DREB-A1 subgroup) containing several C-Repeat-Binding Factors (CBFs) play major roles in acquisition of freezing tolerance (Chinnusamy et al., 2003). CBFs together with another major cold responsive transcription factor, Inducer of CBF Expression (ICE), establish a central cold response pathway to activate a majority of DRE containing Cold Responsive Genes (CORs) in Arabidopsis (Zhao et al., 2016; Liu J. et al., 2018). CORs encode Late Embryogenesis Abundant (LEA) proteins and enzymes for sugar metabolism and fatty acid desaturation that provide the protection for plants from cold stress (Maruyama et al., 2009). The roles of Arabidopsis CBFs in stress responses have been characterized by genetics using cbf mutants generated by CRISPR/Cas9 genome editing techniques and CBFs overexpression lines, as well as transcriptome analysis (Park et al., 2015; Zhao et al., 2016). CBFs are also reported to positively regulate plant drought and salt tolerance, which might due to a common set of stress responsive genes (Kasuga et al., 1999; Zhao and Zhu, 2016). Despite having a clear function in cold response pathways, how CBFs regulate different stresses and the mechanisms by which they confer stress tolerance are still unknown. Environmental changes are usually multifactorial and several stresses often occur simultaneously. Therefore, the multiple roles of CBFs in abiotic stresses might be necessary for plants to overcome stresses and it will be interesting to examine how CBFs regulate genes

under different stress conditions. In addition to CBFs, transcriptome profiling in Arabidopsis also identified several other regulators that are activated during cold acclimation, including members in the DREB-A5 group (Fowler and Thomashow, 2002). The DREB-A5 group contains six members with EAR motifs (DEARs) acting as transcriptional repressors on DRE motif containing genes (Nakano et al., 2006), which provides a repression effect in the DREB-regulated abiotic stress network. Among these, DEAR1 likely acts upstream of CBFs, while DEAR6/RAP2.1 acts downstream of CBFs (Tsutsui et al., 2009; Dong and Liu, 2010). Overexpressed DEAR1 suppressed the cold induced expression of CBFs and displayed reduced plant freezing tolerance (Tsutsui et al., 2009). RAP2.1 is induced by cold, as well as in plants constitutively overexpressing CBFs, but the induction of RAP2.1 by cold has a later onset than the induction of CBF2 (Dong and Liu, 2010). RAP2.1 was first identified as a downstream CBFs subregulon and negatively modifies plants cold tolerance (Fowler and Thomashow, 2002). The presence of the DRE motif in the RAP2.1 promoter region also suggests that RAP2.1 might be a direct target of CBFs (Dong and Liu, 2010). Although the integration of DEAR1 and RAP2.1 in the CBF pathway still needs to be examined using genetics, this negative regulation mechanism might provide checks and balances that minimize the adverse effects of prolonged stress responses. In addition to the negative role of RAP2.1 in cold stress, it also negatively regulates drought tolerance, in that overexpression of RAP2.1 resulted in sensitivity to drought (Dong and Liu, 2010). However, how RAP2.1 regulates drought response, whether it regulates other abiotic stresses and what other DEARs function in abiotic stresses are interesting questions to answer in the future. The checks and balances of DREBs' function in stress were also revealed in Brassica napus, where two groups DREBs (Group I and Group II) regulate cold stress responsive genes sequentially. The early induced Group I DREBs activate cold stress response pathways, whereas the Group II DREBs which are expressed later competitively inhibited Group I DREBs function (Zhao et al., 2006). DREB2s from DREB-A2 are mostly involved in plant drought and heat tolerance, which has been reviewed in detail elsewhere (Mizoi et al., 2012). Briefly, DREB2s are induced upon drought and heat, and positively regulate DRE containing drought responsive genes such as LEAs, and heat responsive genes such as heat chaperons (Maruyama et al., 2009). Moreover, members in DREB-A4 family such as HARDY (HRD) and in DREB-A6 family such as ERF53, RAP2.4, and TG/RAP2.4A also positively regulate drought and salt tolerance (Karaba et al., 2007; Lin et al., 2008; Cheng et al., 2012; Zhu et al., 2014). Overexpression of HRD in Arabidopsis or rice remarkably improved plant drought and salt tolerance (Karaba et al., 2007). Overexpression of TRANSLUCENT GREEN (TG) resulted in vitrified leaves with increased water content in cells, leading to increased drought tolerance (Zhu et al., 2014). TG regulates

cell water homeostasis mainly by directly activating several aquaporin genes (Rae et al., 2011; Zhu et al., 2014), as well as Ascorbate Peroxidases (APx) genes encoding chloroplast peroxidases that functions to protect against photo-oxidative stresses caused by Reactive Oxygen Species (ROS) (Rudnik et al., 2017).

ERF, AP2, and RAV Subfamily Members in Freezing, Hypoxia, and Salt Stress Responses

Members in ERF subfamily also contribute to abiotic stress responses (Licausi et al., 2013). Recently, two groups of ERFs have emerged as central players of abiotic stress regulation in Arabidopsis (**Figure 2**). CYTOKININ RESPONSE FACTORS (CRFs) in ERF-VI subfamily are induced by CTK as well as multiple abiotic stresses to positively regulate osmotic and freezing tolerance (Rashotte et al., 2006; Rashotte and Goertzen, 2010). CRF6, whose induction is dependent on the perception of CTK, alleviated the H2O2 damage on plants to positively regulate oxidative response (Zwack et al., 2016b). CRF4, one of several CRFs not transcriptionally regulated by CTK, positively regulates freezing tolerance by promoting CORs expression (Zwack et al.,2016a). However, the mechanisms by which CRFs confer stress tolerance remain to be determined. Members of ERF-VII subfamily in Arabidopsis as well as rice have been demonstrated to play major roles in flooding, low oxygen (hypoxia) and submergence tolerance and their redundant function in hypoxia responses has been reviewed (Bailey-Serres et al., 2012; Bui et al., 2015; Gibbs et al., 2015). For example, rice SUBMERGENCE 1A (SUB1A), and SNORKEL1/2 (SK1/SK2) positively regulate flooding tolerance by two opposite mechanisms: SUB1A mediates a quiescence strategy associated with reduced growth and respiration whereas SK1/SK2 promote a deep-water escape strategy allowing rapid growth of petioles, stems, and vascular changes (Hattori et al., 2009; Locke et al., 2018). In Arabidopsis, ERF-VIIs have conserved N-terminal domains that allow them to be degraded under anoxia conditions though oxygen-dependent N-end rule pathway (Gibbs et al., 2015). Five members including ERF71/HRE2, ERF72/RAP2.3, ERF73/HRE1, ERF74/RAP2.12, and ERF75/ RAP2.2 are induced by limited oxygen. With limited oxygen,

these ERF-VIIs accumulate and positively regulate hypoxia responsive genes involved in sugar metabolism, fermentation and ET biosynthesis to achieve hypoxia tolerance. Apart from hypoxia responses, ERF-VIIs also regulate oxidative and osmotic stresses. Overexpression of RAP2.2, RAP2.3 and RAP2.12 (RAPs) results in a higher survival rate from low oxygen, oxidative and osmotic stresses, while rap2.12-2 rap2.3-1 double mutants are sensitive to these stresses (Papdi et al., 2015; Yao et al., 2017b). RESPIRATORY BURST OXIDASE HOMOLOG D (RbohD), a NADPH oxidase, helps to generate ROS burst (Yao et al., 2017b). It is reported that RAPs regulate abiotic stresses via an RbohD-dependent mechanism. Apart from being a toxic by-product of biochemical processes, ROS serve as signaling molecules to trigger stress responses and transduce signals crossing cells according to its lower molecular weight and fast cell diffusion (Qi et al., 2018). First it was found that the ROS production and RbohD expression were compromised in single erf74 and double erf74 erf75 mutants at an early stage, which resulted in compromised stress responsive gene expression and stress tolerance. Given that ERF74 binds to RbohD promoter to activate its expression, the RbohD dependent ROS activation was essential for ERF74 and ERF75 mediated hypoxia resistance. However, too much ROS can cause cell injury and cell death. To overcome adverse effects of ROS, overexpression of ERF74 promoted increased ROS scavenging enzymes and stress responsive genes at later stage. Therefore, ERF74 acts as an on-off switch to control RbohD-dependent ROS burst in response to different stresses in Arabidopsis (Yao et al., 2017b). This newly identified mechanism provides more details and divides the stress response into early and later stages, as well as ROS balance. One example that rice ERFs OsLG3 induced ROS scavenging to positively regulate stress tolerance was reported recently, suggesting the similar mechanism exists in rice (Xiong et al., 2018). Additionally, many other Arabidopsis ERFs also regulate abiotic stresses. ERF1 and Ethylene- and Salt-inducible ERF genes (ESEs) in ERF-IX group positively regulate plant salinity tolerance by promoting salt responsive gene expression (Zhang et al., 2011). ERF6, another member in ERF-IX group, triggers growth inhibition to confer longterm osmotic stress tolerance (Dubois et al., 2013). RAP2.6L from ERF-X subgroup improves drought and salt tolerance (Yang et al., 2009; Liu et al., 2012). Additionally, Arabidopsis RAVs, especially AP2s, play central roles in developmental processes, such as organ number and size control, shoot and root meristem maintenance, flower initiation and growth (Osnato et al., 2012; Horstman et al., 2014). Members in these subfamilies are also reported to mediate diverse abiotic stress responses. AINTEGUMENTA (ANT) controls organ cell number and size throughout shoot development. ANT also negatively regulates salt tolerance by repressing SOS3- LIKE CALCIUM BINDING PROTEIN 8 (SCABP8/CBL10), a putative Ca2C sensor that protects Arabidopsis shoots against salt stress and maintains ion

homeostasis (Meng et al., 2015a). Overexpression of Arabidopsis RAV1 and RAV2 in cotton increased fiber length and even obtained the same yield under drought stress compared with control conditions (Mittal et al., 2015). Similar as DREBs, ERFs in other plant species like rice, wheat and tomato are also involved in a broad range of abiotic stresses (Abiri et al., 2017; Phukan et al., 2017). Overall, these findings provide the potential of engineering high-efficiency crops under stress conditions. In summary, ERFs function to receive multiple stress signals and control a diverse set of stress responsive genes, where many ERFs have cooperative or antagonistic regulation on stress responses. Therefore, constructing ERFspecific gene regulatory networks would be interesting to provide insight as to how ERFs function as a unit to regulate common downstream genes.

INTEGRATION OF AP2/ERFS WITH HORMONE RESPONSES

In addition to directly regulating abiotic stresses, AP2/ERFs are also involved in hormone signaling and hormone mediatedstress responses. Plant hormones affect abiotic stresses by triggering a wide range of physiological processes (Kazan, 2013, 2015; Colebrook et al., 2014; Khan et al., 2015; Muller and Munne-Bosch, 2015; Tao et al., 2015; Sah et al., 2016; Nolan T. et al., 2017). ABA and ET are major stress hormones that are induced under abiotic stress conditions and regulate stress responses associated with AP2/ERFs (Kazan, 2015; Sah et al., 2016). GAs, CTK, and BRs are growth-related hormones that promote cell growth, proliferation and differentiation. It is becoming increasingly evident that these growth-related hormones also have direct and/or indirect effects on abiotic stresses

AP2/ERFs in ABA-Mediated Stress-Response

The plant hormone ABA is a pivotal hormone that regulates abiotic stress responses including drought, salinity, cold and heat stresses. ABA exerts a protective function through inducing stomata closure, modulating root architecture, and promoting the synthesis of osmolytes (Cutler et al., 2010; Sah et al., 2016). During stress conditions such as water deprivation and osmotic pressure, the rate-limiting ABA biosynthetic enzyme Nine-cis- Epoxy carotenoid Dioxygenase (NCED) is rapidly up-regulated to promote ABA biosynthesis. ABA is then sensed by a large family of PYRABACTIN RESISTANCE1 /PYR1-LIKE/REGULATORY COMPONENTS OF ABA RECEPTORS (PYR/PLY/RCAR) receptors that interact with Protein Phosphatase 2C (PP2Cs) as a ternary complex to release PP2Cs' inhibition on SnRK2 kinases (SnRK2s). The active SnRK2s phosphorylate downstream substrate proteins, including AREBs/ABFs, ion channels, and enzymes such as NADPH oxidases, thereby inducing ABA responses (Finkelstein, 2013; Sah et al., 2016). Generally, transcription factors regulate abiotic stresses through ABAdependent or ABA-independent pathways. A great deal of studies have

shown that DREBs regulate ABA independent abiotic stresses by directly binding to DRE/CRT motifs on stress responsive genes (Gilmour et al., 2004; Matsukura et al., 2010; Lata and Prasad, 2011; Mizoi et al., 2012; Zhu et al., 2014). However, AP2/ERFs are indispensable for ABA-dependent stress responses as well. ANT (Meng et al., 2015b), ERF53 (Hsieh et al., 2013), ERF-VIIs (Papdi et al., 2015; Yao et al., 2017a), RAP2.6L (Liu et al., 2012), and RAP2.6 (Zhu et al., 2010) in Arabidopsis are induced by ABA to up-regulate DRE- and ABRE-element containing genes. Rice OsERF71 positively regulates ABA signaling to alter root architecture and confer drought tolerance (Lee et al., 2017; Li et al., 2018). The combination of abiotic stresses and ABA also led to the further activation of DREB2s and stress inducible genes (Lee et al., 2016). ABA INSENSITIVE 4 (ABI4), a unique one in the DREBA3 group is a key component of the ABA signaling pathway. Upon ABA and ROS accumulation under stress conditions, ABI4 represses CCAAT Binding Factor A (CBFA) (Zhang et al., 2013). CBFA is a subunit of the trimeric transcription complex of Heme Activator Proteins (HAPs). Repression of CBFA then allows other transcription factors to enter the transcription complex and improves the efficiency of stress responsive gene transcription (Zhang et al., 2013). Apart from the positive effect of AP2/ERFs in ABAmediated stress responses, many Arabidopsis studies have shown that AP2/ERFs also interrupt ABA signaling, resulting in reduced sensitivity on root growth inhibition and stomata closure (**Figure 3**). ERF18/ ORA47 activated the PP2C family phosphatase gene ABI2. At the same time ABI1 acted upstream of ORA47 to activate ORA47, leading to an ABI1-ORA47-ABI2 regulation loop that inhibits ABA signaling as well as drought tolerance (Chen et al., 2016). RAV1 inhibites ABA sensitivity on root growth by repressing ABI3, ABI4 and ABI5 expression (Feng et al., 2014). SnRK2.2, SnRK2.3, SnRK2.6 also interact with and phosphorylate RAV1 to inhibit RAV1's transcriptional repression of targets genes (Feng et al., 2014).

AP2/ERFs in Ethylene-Mediated Stress-Response

ET is also reported to regulate abiotic stress responses including salt, cold, and flooding (Kazan, 2015). ET is synthesized from the rate limiting enzymes ACC Synthases (ACSs), a major target for the regulation for ET production under stresses (Tao et al., 2015). The binding of ET with its receptor ETHYLENE INSENSITIVE 1 (ETR1) deactivates CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) kinase activity to release CTR1's inhibition on ETHY INSENSITIVE 2 (EIN2). Then the C-terminal of EIN2 translocates into the nucleus to activate ETHYLENE INSENSITIVE 3 (EIN3) as well as the transcriptional cascade of ethylene-regulated genes (Qiao et al., 2012; Muller and Munne-Bosch, 2015). In stress responses, AP2/ERFs, especially the ERF subfamily, are the major downstream regulatory factors of the ET signaling pathway (Licausi et al., 2013; Gibbs et al., 2015; Kazan, 2015; Muller and

Munne-Bosch, 2015). ET regulates several aspects of Arabidopsis abiotic stress responses, including inhibiting CBFs to negatively regulate cold stress (**Figure 3**). Conversely, ET positively regulates flooding and submergence mediated by ERFVIIs, and improves salt tolerance by activating ERF1 and ESEs. For ET-regulated cold response, the production of ET is inhibited after exposure to cold, which results in compromised cold tolerance (Shi et al., 2012). Consistently, ET insensitive mutants etr1-1, ein2-5, and ein3-1 displayed increased freezing tolerance. EIN3 inhibits the expression of CBFs by directly binding to their promoters. In addition, ET plays crucial roles on plant survival and recovery from flooding, especially in rice. Flooding causes oxygen deficiency, which promotes ET production (Yang et al., 2011) and activates the expression of a set of ERF-VIIs, whose function in hypoxia is discussed above. ET also promotes RAP2.3 nuclear localization and advances ORA59 mediated ethylene responses which is dependent on RAP2.3 (Kim et al., 2018). However, ERF-VIIs regulate hypoxia response partially through ET-independent pathways. The induction of ERF73/HRE1 under hypoxia was not completely abolished in ethylene-insensitive mutants or in the presence of ethylene biosynthesis inhibitors. ERFVIIs
also negatively regulate ET signaling and homeostasis probably via feedback regulations (Hinz et al., 2010; Yang et al., 2011). For instance, HRE1-RNAi seedlings displayed exaggerated triple responses; ACSs was decreased in RAP2.2 overexpression plants, but up-regulated in rap2.2-2 knockout mutant (Hinz et al., 2010). However, how ERF-VIIs control ET homeostasis via negative feedback mechanism under stresses needs further investigation. ET also has complex regulation in salt stress, which has been extensively discussed (Kazan, 2015; Muller and Munne- Bosch, 2015; Tao et al., 2015). In Arabidopsis, ET signaling is required for plant tolerance to salinity stress as EIN3 activates ERF1 and ESEs to activate downstream stress-related genes and promote salinity tolerance. However, knockout mutants of ACSs also led to salt tolerance, leading to an opposite conclusion in terms of ET signaling and salt tolerance. These different conclusions might be due to the different mutants, growth conditions or experimental setups used. Future investigation is necessary to further our understanding of the role of ET in plant salinity response.

AP2/ERFs in GA-Mediated Stress-Response

The plant hormone GAs is known to promote plant growth and development. GAs have also been shown to regulate abiotic stresses, as reduced GA content slows down plant growth upon exposure to several abiotic stresses including cold, salt, and osmotic stresses (Claeys et al., 2012; Colebrook et al., 2014). GAs are synthesized through several key oxidases including GA 20-oxidases (GA20oxs) and GA 3-oxidases (GA3oxs), and catabolized by GA 2-oxidase (GA2ox) that depletes pools of GA precursors to maintain GA homeostasis (Phillips et al.,

1995; Rieu et al., 2008). In the absence of GAs, a group of DELLA proteins (DELLAs) inhibit GA response, and this inhibition can be released by the degradation of DELLAs in the presence of GAs (Claeys et al., 2012). Generally, abiotic stresses cause reduction of GA content and signaling through the inhibition of GA biosynthesis enzymes mediated by Arabidopsis AP2/ERFs (**Figure 3**). DREB1E and DREB1F confer salt stress-induced growth retardation mostly through the repression of GA20oxs (Magome et al., 2004). CBF1 and ERF6 overexpression plants were sensitive to stress-induced growth retardation because of increased GA2oxs expression as well as the accumulation of DELLAs (Achard et al., 2008; Dubois et al., 2013). Conversely, ERF11 promotes plant internode elongation by activating GA biosynthesis, and expression of GA3ox1 and GA20oxs are increased in ERF11 overexpression plants (Zhou et al., 2016). Nevertheless, ERF11 and ERF6 show antagonistic regulation on stress-induced growth inhibition. ERF11 suppresses the extreme dwarf phenotype of ERF6 overexpression plants and represses ERF6-induced gene expression (Dubois et al., 2015). The opposite regulation by ERF6 and ERF11 reveals that dynamic mechanisms must exist in plants to fine-tune and maintain the balance between plant growth and stress responses. In addition to GA regulation in Arabidopsis abiotic stresses, rice regulates flooding coping submergence tolerance by two opposite GA regulations. The first quiescence strategy was that SUB1A increased the accumulation of SLENDER RICE1 (SLR1) and SLENDER RICE1 LIKE1 (SLRL1) (DELLA like proteins in rice) to restrict GA signaling and sensitivity, which resulted in inhibition of plant internode elongation and reduced carbohydrate consumption (Fukao and Bailey-Serres, 2008; Locke et al., 2018; Perata, 2018). The second deep-water escape strategy involves SK1/SK2, which lead to up-regulated GA20oxs and promoted internode elongation to escape submergence in water (Hattori et al., 2009; Ayano et al., 2014).

AP2/ERFs in CTK-Mediated Stress-Response

The plant hormone CTK not only plays diverse roles in plant growth and development, but also has been reported to regulate plant abiotic stresses (Zwack and Rashotte, 2015), one of which is mediate by CRFs (**Figure 3**). CRFs are essential for CTK-mediated embryo, cotyledon, and leaf development, as both single and multiple CRF1/2/3/5/6 mutants displayed cell proliferation deficient phenotypes (Rashotte et al., 2006). The roles of CRF's regulation on CTK-mediated development were further confirmed by the transcriptome analysis of crf 1,2,5 and crf2,3,6 mutants, with or without CTK treatment in Arabidopsis. About 60% of the CTK responsive genes were regulated by both CRFs and type-B ARRs (the typical cytokininresponsive transcription factors), suggesting a model that CRFs acted tandemly with type-B ARRs to mediate CTK response. CRF6 also cooperated with CTK signaling to inhibit stressinduced leaf senescence through a common subset of CTKregulated genes (Zwack et al.,

2013). Apart from the CRFspositive effect in the CTK pathway, CRF6 also represses CTKassociated target genes involved in CTK biosynthesis, signaling and transport, to alleviate the adverse effect of CTK on abiotic stress (Zwack et al., 2016b). The opposite regulation between CRF6 and CTK on stresses and similar regulation on senescence suggest that CRF6 regulates CTK signaling through two subsets of genes: one set of genes alleviate the negative effect of CTK on abiotic stresses, while the other set helps CTK to promote plant development. The detailed mechanisms of CRF regulation in these processes remain to be determined. Identification of CRF target genes and the upstream signaling could allow for a better understanding about ERF-VIs function and how CTK regulates abiotic stresses.

AP2/ERFs in BR-Mediated Stress-Response

The plant hormone BRs play important roles throughout plant development, such as cell elongation, leaf development, pollen tube growth, xylem differentiation, senescence, and photo morphogenesis as well as stress response (Clouse et al., 1996; Ye et al., 2017). BRs are sensed by plasma membrane located receptor kinase BRASSINOSTEROID INSENSITIVE 1 (BRI1) to inhibit negative regulator BRASSINOSTEROID INSENSITIVE 2 (BIN2), leading to accumulation of transcription factors BRASSINAZOLE-RESISTANT 2/BRI1- EMS-SUPPRESSOR 1 (BES1/BZR1) to regulate 1000s of BR responsive genes involved in plant growth and stresses responses (Guo et al., 2013). BR regulates cold and drought responses through several pathways. For example, BR positively regulates cold tolerance partially though CBF-mediated cold response pathway, where BZR1 binds and promotes the expression of CBF1/CBF2 in response to cold. Cold stress also promotes accumulation of the unphosphorylated and active form of BZR1 by unknown mechanisms (Li et al., 2017b; **Figure 3**). However, BR negatively regulates drought tolerance via antagonizing with drought induced transcription factor RD26 on drought responsive genes (Ye et al., 2017). BR also antagonizes with ABA pathway from receptors to transcription factors and regulates the trade-off of plants growth under stress conditions (Nolan T. et al., 2017). Although there are no reports of AP2/ERFs in BR mediated drought response, BES1 and BZR1 target genes include numerous AP2/ERFs, implying that AP2/ERFs have a potential function to integrate the BR pathway with abiotic stresses (Sun et al., 2010; Yu et al., 2011). Future studies in this area will shed light on the mechanisms and roles of AP2/ERFs in BR and stress responses. In addition, AP2/ERFs regulate the BR pathway through different mechanisms. ERF72/RAP2.3 antagonizes BZR1 and AUXIN RESPONSIVE FACTOR 6 (ARF6) to inhibit hypocotyl elongation, while its role in BR regulated stresses response is unknown (Liu K. et al., 2018). The role of ERF72 in controlling growth implies that ERF72 might be a candidate for the study of the integration of BR and stresses. In fact, rice SUB1A mediates GA and BR cross-talk to

control submergence tolerance. SUB1A activates BR biosynthesis and signaling, which in turn induces GA catabolic gene GA2ox7 to lower GAs content, and ultimately promotes rice DELLA protein accumulation (Schmitz et al., 2013). AP2/ERF Transcription Factors in Stress Responses

Aux/IAA Gene Family in Plants

Auxin plays a crucial role in the diverse cellular and developmental responses of plants across their lifespan. Plants can quickly sense and respond to changes in auxin levels, and these responses involve several major classes of auxin-responsive genes, including the Auxin/Indole-3-Acetic Acid (Aux/IAA) family, the auxin response factor (ARF) family, small auxin upregulated RNA (SAUR), and the auxin-responsive Gretchen Hagen3 (GH3) family. Aux/IAA proteins are short-lived nuclear proteins comprising several highly conserved domains that are encoded by the auxin early response gene family. These proteins have specific domains that interact with ARFs and inhibit the transcription of genes activated by ARFs. Molecular studies have revealed that Aux/IAA family members can form diverse dimers with ARFs to regulate genes in various ways. Functional analyses of Aux/IAA family members have indicated that they have various roles in plant development, such as root development, shoot growth, and fruit ripening. In this review, recently discovered details regarding the molecular characteristics, regulation, and protein–protein interactions of the Aux/IAA proteins are discussed. These details provide new insights into the molecular basis of the Aux/IAA protein functions in plant developmental processes. In plants, many developmental processes are finely tuned by auxin, such as vascular tissue formation, adventitious root initiation, tropistic responses, apical dominance, and flower and fruit development. Auxin also affects cellular processes, such as cell division, enlargement, and differentiation [1–4]. Dynamic spatial and temporal changes in auxin levels can trigger gene reprogramming precisely and rapidly, which requires auxin early response genes, such as the Auxin/Indole-3-Acetic Acid (Aux/IAA) family, the auxin response factor (ARF) family, small auxin upregulated RNA (SAUR), aminocyclopropane-1-carboxylic acid synthase (ACS), glutathione-S-transferase (GH2/4-like), and the auxin-responsive Gretchen Hagen3 (GH3) family [3,5,6]. Among these genes, Aux/IAA family members have been identified as short-lived nuclear proteins that play a crucial role in repressing the expression levels of genes activated by ARFs [7,8]. It has also been demonstrated that auxin-mediated transcriptional regulation is exclusively dependent on the functions of Aux/IAA [9]. Aux/IAA proteins have been suggested to bind with ARFs and prevent activation of auxin-responsive genes in the absence of auxin. At high auxin levels, these proteins can be ubiquitinated by interacting with TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX (TIR1/AFB) receptors and subsequently

degraded via the 26S proteasome [10–12]. The released ARFs regulate the expression of auxin-responsive genes [10]. The different TIR1/AFB-Aux/IAA protein combinations usually have differential auxin-binding affinities, and the auxin levels are different in different tissues and developmental phases, which results in distinct auxin-sensing effects [9,11,13,14]. Thus, the spatial–temporal dynamic change in auxin levels can be finely transformed into gene reprogramming signaling, thereby regulating the processes of plant growth and development in a precise manner.mOver the past two decades, mutations in Aux/IAA genes have been intensely studied in Arabidopsis, and these studies have revealed that the members of this family display distinct functions in plant growth and development processes [15–18]. To date, 29 Aux/IAA gene family members in Arabidopsis have been isolated [18–20]. Meanwhile, many candidate genes that potentially regulate the stability of Aux/IAA proteins have also been identified [21,22]. It is noteworthy that a large number of Aux/IAA family members have also been identified in other plants, including Eucalyptus grandis, Solanum lycopersicon, Cucumis sativus, Populus trichocarpa, Zea mays, and Oryza sativa [23–29]. Recently, through the combined application of physiological, genetic, molecular, and biochemical methods, increasingly abundant new information regarding the mechanisms of Aux/IAA in regulating auxin signal transduction and auxin degradation has been obtained, and this information provides valuable opportunities to clarify the developmental processes fine-tuned by auxin signaling in plants. In this review, recent advances in the roles of Aux/IAA in regulating plant growth and development, as well as global and new insights into the underlying molecular mechanisms of these processes, will be presented.

Interaction of Aux/IAA Proteins in Plants

Plants can sense and respond to auxin signaling in a precise and rapid manner, and this response involves a group of genes called the early auxin response genes [44]. The Aux/IAA proteins are well-known as the early auxin response proteins and participate in auxin signaling through interacting with ARF proteins as transcriptional repressors [7,9,62]. The functions of Aux/IAA proteins in auxin signaling have been well summarized [63,64]. In brief, Aux/IAA and ARF have the same type I/II PB1 domain in their homologues, Domain III/IV, and the type I/II PB1 domain carries both positive and negative charges, which facilitate head-to-tail homo- and heterodimerization of Aux/IAA and ARF via electrostatic interactions [58,61,65,66]. At low auxin levels, Aux/IAA proteins directly dimerize with ARF proteins to prevent their physical interaction with transcription initiation complexes and recruit TPL/TPR co-repressors in the EAR domain [52,67]. The recruited TPL/TPR proteins interact with histone deacetylases to induce chromatin condensation [52]. At high auxin levels, the Aux/IAA proteins are brought together with TIR1/AFB proteins by auxin. The F-box in TIR1/AFB proteins can be recognized

by SCF-type ubiquitin protein ligase complexes, and subsequently, the Aux/IAA proteins are poly-ubiquitinated by SCF proteins and degraded through the 26S proteasome proteolytic pathway [12,63,64]. Next, the released ARF proteins may recruit SWITCH/SUCROSE NONFERMENTING (SWI/SNF) to remodel the chromatin into an active state and bind to the auxin response DNA cis-elements (AuxREs) to trigger changes in their target auxin response genes at the transcriptional level [5,62]. Based on this mechanism, auxin signaling can result in changes in gene expression levels. The interaction and regulation of Aux/IAA proteins are closely associated with their modular structure. Domains I and II are well known for their roles in stabilizing Aux/IAA proteins, and Domain II contains a GWPP-core degron sequence, which is the core binding site with TIR/AFB proteins and auxin [68]. Crystal structures have shown that the leucine-rich repeat (LRR) domain of TIR/AFB proteins can form an auxin binding pocket, which allows auxin to anchor at the bottom and "glue" its "GWPP" motif in the degron of Aux/IAA proteins and to occupy the rest of the area of this pocket [12]. Thus, even single-point mutations in the degron region reduce the affinity to TIR/AFB proteins and hamper the degradation of Aux/IAA proteins [69]. In addition, the conserved rate motif, KR, also facilitates the turnover of Aux/IAA proteins, and plants without this rate motif exhibit insensitive auxin response phenotypes similar to plants with degron mutations [54]. Recent evidence showed that auxin can regulate the 26S proteasome-mediated degradation of ubiquitinated Aux/IAA by remobilizing PROTEASOME REGULATOR1 (PTRE1) from the nucleus to the plasma membrane [70,71]. The degradation rates and ubiquitination sites are varied among Aux/IAA proteins, and lysine residues are not necessary for degradation [72]. Variations in the ubiquitylation sites in flexible hotspots of the degron-flanking regions also lead to distinct auxin affinities, which correspond to the degradation dynamics among different IAA proteins [56]. The negative feedback regulation of 26S proteasome activity may contribute to avoiding over-enhancement of auxin signaling and excessive degradation of Aux/IAA proteins [70]. In addition to Domains I and II, Domains III and IV, which are homologous to the carboxy-terminal dimerization domain (CTD) of ARFs, contain the well-known PB1 domain [61]. Based on thermodynamic and structural approaches, a two-pronged electrostatic interaction was identified in the PB1 domain, and the roles of the charged residues on the,basic and acidic faces in stabilizing the protein–protein interaction have been revealed [60]. Although Aux/IAA and ARF can form homo- or hetero-oligomers via their PB1 domains, case studies of ARF5 and IAA17 have revealed that their hetero-oligomers have up to 100-fold greater affinity than their homo-oligomers [73]. These studies shed new light on how these PB1-containing proteins interact with each other at the atomic level to facilitate auxin signaling. Based on large-scale interactome analyses conducted in Arabidopsis using affinity capture-Western/MS, yeast two-

hybrid, reconstituted complex, and protein-fragment complementation assay (PCA) approaches, a comprehensive physical interactome map of Aux/IAA proteins has been developed in recent years (Figure 2) [13,17,20,74]. This interactome map consists of the essential auxin signaling transduction components mediated by Aux/IAA proteins (Figure 2). For instance, 29 Aux/IAA proteins had physical contact with 20 ARF proteins via 544 interactions, and Aux/IAA homo-interactions and Aux/IAA-ARF hetero-interactions occupied 47% and 45% of interactions, respectively (Figure 2). All 29 Aux/IAA proteins interacted with each other with 253 interactions, and only 13 ARF shared 22 homo-interactions (Figure 2). TPL/TPR proteins are co-repressors that bind to the EAR domain of Aux/IAA. In the interaction network, four TPL/TPR proteins were identified, and most Aux/IAA proteins had physical interactions with these four TPL/TPR proteins, except IAA5, IAA6, IAA9, IAA14, IAA15, IAA20, IAA29, IAA30, IAA31, IAA32, IAA33, and IAA34 (Figure 2). Recently, a report demonstrated that the homo-oligomerization of TIR1 in Arabidopsis was another pathway for regulating auxin signaling by affecting Aux/IAA protein turnover [75]. In the interactome map, four out of six TIR1/AFB proteins were involved in this interactome, but only 11 Aux/IAA proteins had direct physical interactions with the four TIR1/AFB proteins (Figure 2). Thus, the large number of homo-interactions among Aux/IAA proteins and the lack of physical interaction with the TIR1/AFB proteins imply that many Aux/IAA proteins may only function as heterodimerization partners and may not participate in ubiquitination and turnover processes. It should also be noted that most interactions in the Aux/IAA interactome map come from in vitro experiments, and the specific roles of these interactions in auxin signaling need to be further verified with in vivo evidence. These identified essential components, as well as potential protein interactors in the Aux/IAA-mediating auxin signaling pathway, provide comprehensive maps of how changes in auxin levels are reflected in gene reprogramming events. heterodimerization partners and may not participate in ubiquitination and turnover processes. It should also be noted that most interactions in the Aux/IAA interactome map come from in vitro experiments, and the specific roles of these interactions in auxin signaling need to be further verified with in vivo evidence. These identified essential components, as well as potential protein interactors in the Aux/IAA-mediating auxin signaling pathway, provide comprehensive maps of how changes in auxin levels are reflected in gene reprogramming events.

Functional Roles of Aux/IAA Genes during Plant Growth and Development Processes

Although there are 29 Aux/IAA proteins in Arabidopsis (with some functional redundancy), most mutations in Aux/IAA proteins display similar phenotypes in which auxin signaling transduction is hampered, leading to defects in diverse developmental processes such as

embryo development, lateral root initiation and elongation, hypocotyl growth, tropisms, flower organ development, and other processes (Figure 4) [17–19]. For example, a gain-of-function mutant, iaa1/axr5, showed defects in root and shoot tropisms [104], but gain-of-function mutations in iaa2 and iaa6 lead to similar phenotypes, which exhibited a short hypocotyl and upcurled leaves, in Arabidopsis [15,105]. IAA3, IAA14, and IAA18 act redundantly in controlling lateral root formation by interacting with two ARF proteins (ARF7 and ARF19). Additionally, phenotypic analysis of transgenic Arabidopsis variants differing in the IAA14 degradation rate indicated that the timing of lateral root initiation is finely tuned by the turnover rate of IAA14 [106]. IAA7/AXR2 controls the morphological responses induced by light, such as inhibiting hypocotyl elongation and promoting leaf development, as observed in iaa7/axr2-1 mutants cultured in the dark [107]. IAA8 can interact with TIR1 to regulate lateral root formation, and overexpression of this gene caused abnormal gravitropism [108]. A mutation in domain II of IAA8 resulted in abnormal flower organs with low jasmonic acid levels, which was mediated by its interactions with ARF6 and AFR8 [17,109]. IAA12 regulates root meristem initiation during early embryogenesis by interacting with the ARF5 protein, the latter of which is essential for embryonic root and embryo patterning [110,111]. Stabilized iaa16 mutants display smaller rosettes, shorter root hairs, fewer lateral roots and shorter anther filament elongations than the wild type, and the homozygous iaa16 mutants are not fertile [16]. IAA17 is involved in some typical phenotypes controlled by auxin signaling, such as hypocotyl elongation, root gravitropism, and root hair and adventitious root formation [112,113]. A recent report showed that IAA17 was also involved in cytosolic glutamine synthetase (GLN1;2)-mediated ammonium assimilation in Arabidopsis root [114]. A gain-of-function analysis verified the roles that IAA18 plays in embryonic apical patterning by repressing the activity of ARF5 and other ARFs [115]. Interestingly, transcripts of IAA18 and IAA28 can be transported from mature leaves to the root via phloem to negatively regulate lateral root formation [116]. IAA19 participated in hypocotyl growth and lateral root formation by interacting with ARF7, and the IAA19 mutant failed to maintain hypocotyl gravitropism and regular lateral roots [117]. Gain-of-function iaa28-1 mutants show a strong auxin phenotype with failure to form lateral roots and a reduction in plant size and apical dominance [118]. Meanwhile, IAA28 also participates in the process of lateral root founder cell identity by negatively regulating the activity of its downstream transcription factor, GATA23 [119]. Overexpression of three non-canonical Aux/IAA genes (IAA20, IAA30, and IAA31) results in similar aberrant phenotypes, and plants with IAA20 overexpression show modified gravitropic root and hypocotyl growth and collapse of root apical meristem after germination [120]. In addition to Arabidopsis, significant achievements have also been made in understanding the roles of Aux/IAA proteins

in auxin signaling in other plants, such as tomato, potato, rice, and poplar [95,121–124]. In tomato, SiIAA3 has been verified as a molecular bridge between auxin and ethylene signaling pathways, and SiIAA3 RNAi plants display decreased petiole epinasty in light-grown seedlings and an exaggerated curvature of the apical hook under dark conditions [121]. SiIAA9 controls multiple processes mediated by auxin signaling, such as apical dominance, leaf morphogenesis, flower organ development, and fruit set and development [125–127]. Mutations in SiIAA9 lead to abnormal leaf shape and parthenocarpic fruits [125–127]. Recently, the CRISPR/Cas9 system was optimized to obtain SlIAA9-crispr in tomato plant (Solanum lycopersicum) mutants, which have phonotypes similar to SiIAA9 mutant plants, e.g., abnormal leaves and parthenocarpy [128]. SlIAA15 plays multiple roles during tomato developmental processes, and SlIAA15-suppressed plants exhibit enhanced lateral root formation, modified development of axillary shoots, decreased trichome number and fruit set, and increased leaf thickness [129]. SiIAA17 plays crucial roles in controlling fruit quality, and SiIAA17-silenced lines display larger fruit with a thicker pericarp than the wild-type line [130]. SiIAA27 RNAi plants display modified reproductive organ anatomy and reduced expression levels of genes involved in chlorophyll synthesis [131]. More recently, SlIAA27 has been shown to positively regulate the formation of arbuscular mycorrhiza and participate in strigolactone biosynthesis by regulating the expression level of NODULATION SIGNALING PATHWAY1 (NSP1) [132]. In potato, StIAA2 repression results in altered shoot morphogenesis, increased plant height, and petiole hyponasty, and transcriptional reprogramming of other StIAA genes [122]. For members of the Aux/IAA gene family in rice, OsIAA1 is induced by auxin and plays a role in light response and coleoptile elongation in rice [133]. Overexpression of OsIAA4 in rice leads to less sensitivity to exogenous auxin, dwarfism, and more tiller angles in comparison with wild-type plants [134]. Overexpression of OsIAA6 enhances tolerance to drought, however, loss-of-function mutants of OsIAA6 display much more tiller outgrowth than does the wild type [135]. Conversely, overexpression of OsIAA10 increases the number of tillers [98]. Degradation of OsIAA11 is essential for initiating lateral root formation, and mutations in the degron of OsIAA11 stabilize this protein and produce defects in the lateral root [95,96,136]. Gain-of-function OsIAA13 mutants also display phenotypes of reduced lateral roots and defects in the gravitropic response, which are closely related to transcriptional reprograming of the set of genes involved in lateral root initiation [137]. OsIAA23 is essential for postembryonic maintenance of the quiescent center by auxin signaling in rice [138]. In maize, IAA10 can repress the expression level of the LATERAL ROOT PRIMORDIA 1 (LRP1) gene by directly binding to its promoter, and the gain-of-function mutant iaa11 (rum1) cannot activate the expression of LRP1 and fails to initiate seminal and lateral roots [139]. In woody plants,

overexpression of PttIAA3m causes global auxin-mediated gene reprogramming, which is closely linked to cambial cell division and secondary xylem development in hybrid aspen [123]. Overexpression of PtrIAA14.1 (a homolog of Arabidopsis IAA7) in Arabidopsis confirms its roles in regulating vascular patterning via interaction with AFR5 [140]. Recently, another study verified the role of Eucalyptus EgrIAA4 in fiber formation and secondary cell wall deposition by heterologous expression of this gene in Arabidopsis [23].

DELLA

The diterpenoid hormone GA controls diverse developmental processes throughout the life cycle of a plant. Physiological and genetic studies show that active GA promotes seed germination and vegetative growth. In some species, GA also induces flowering and regulates flower, fruit, and seed development. This article highlights recent advances in our understanding of the molecular mechanisms of GA metabolism, transport, perception, and signaling, and the regulatory circuit between the GA pathway and other pathways to control plant growth and development in response to internal and external cues.

GA METABOLISM AND TRANSPORT

The biochemical pathway of GA biosynthesis and catabolism in plants is well defined, and genes encoding most enzymes in this pathway have been identified (Yamaguchi, 2008). Although many GA derivatives are present in a given plant, only very few GAs are biologically active. GA biosynthesis appears to be tightly linked to the site of GAresponses as bioactiveGAs have been found to be more abundant in rapidly growing tissues (Yamaguchi, 2008). This idea is also supported by recent studies on the expression of GA metabolism genes and phenotypic characterization of GA-deficient mutants (Sun, 2008; Yamaguchi, 2008). However, transport of GA intermediates or active GAs may also play an important role in modulating GA responses. For example, the cereal aleurone is well known for its response to embryo-produced GA (namely expression of hydrolytic enzyme genes in this tissue). In Arabidopsis (Arabidopsis thaliana), GA4 (the major bioactive GA in this plant) can be transported from rosette leaves to the shoot apex to facilitate flower initiation in Arabidopsis (Eriksson et al., 2006). This was demonstrated by feeding labeled GA4 to leaves and then measuring GA4 levels in the shoot apical meristem. GA 3-oxidase (GA3ox) catalyzes the final step for bioactive GA production. Studies of GA3ox mutants suggest that bioactive GAs made in the stamens and/or flower receptacles are transported to petals to promote their growth in Arabidopsis (Hu et al., 2008).

In developing siliques, active GAs are transported from the seed endosperm to the surrounding maternal tissues where they promote fruit growth. In germinating seeds, a subset of the GA-responsive genes are expressed in different cell types from those of GA3ox genes, also suggesting that GAs need to be transported between cells to regulate gene expression (Ogawa et al., 2003). It will be important to investigate the molecular mechanism of GA transport and how GA metabolism and transport are coordinated to modulate the local GA levels in response to the internal developmental program and environmental cues. Development of gene markers and/or fluorescent sensors to monitor bioactive GA molecules in planta will greatly facilitate these studies.

GA RECEPTOR AND EARLY EVENTS IN GA SIGNALING

Recent genetic, biochemical, and structural studies have elucidated the molecular mechanism of GA perception and initial steps in GA signaling in plants (Fig. 1; Ueguchi-Tanaka et al., 2007; Sun, 2008). The GA signal is perceived by the GA receptor GID1 (for GA INSENSITIVE DWARF1), which is a soluble protein that is localized to both cytoplasm and nucleus. DELLA proteins are nuclear transcriptional regulators, which repress GA signaling and restrict plant growth presumably by causing transcriptional reprogramming. Binding of GA to GID1 enhances the interaction between GID1 and DELLA, resulting in rapid degradation of DELLAs via the ubiquitinproteasome pathway. A specific ubiquitin E3 ligase complex (SCFSLY1/GID2) is required to recruit DELLA for polyubiquitination and subsequent degradation by the 26S proteasome. Recently, crystal structures of GAGID1 and GA-GID1-DELLA complexes have been determined (Murase et al., 2008; Shimada et al., 2008). Without GA binding, the N-terminal extension (N-Ex) of GID1 has a flexible structure that is highly sensitive to protease treatment. Binding of GA to the C-terminal domain of GID1 induces a conformational switch of its N-Ex to cover the GA-binding pocket (like closing the lid), as well as creates hydrophobic surfaces for DELLA binding (Fig. 1). Although there is no direct contact between DELLA and GA, DELLA binding further stabilizes the GA-GID1-DELLA complex. These studies indicate that bioactive GA is an allosteric inducer of its receptor GID1. The current model also suggests that binding of GA-GID1 to DELLA enhances recognition of DELLA by the F-box protein of the ubiquitin E3 ligase SCF complex (SLY1 in Arabidopsis and GID2 in rice [Oryza sativa]; Fig. 1). This is different from the mechanism of auxin perception: Auxin functions as a molecular glue that brings the F-box protein (TIR1 and its homologs) and its substrate protein (IAA/AUX) together without altering the conformations of these proteins (Tan et al., 2007). In addition to GA-dependent proteolysis, recent studies also suggest that DELLA activity may be modulated by other mechanisms. Overexpression of GID1 could inactivate DELLA by direct interaction without protein degradation (Ariizumi et

al., 2008). Posttranslational modifications such as glycosylation and phosphorylation may also affect DELLA activity. SPINDLY (SPY), an O-linked GlcNAc (O-GlcNAc) transferase (OGT), is a GA signaling repressor (Olszewski et al., 2002). In animal systems, O-GlcNAc modification of Ser or Thr residues of target proteins by OGT often interferes with phosphorylation by protein kinases. Epistasis analysis between spy and della and twodimensional gel-blot analysis of DELLA modifications suggest that SPY activates DELLA by GlcNAc modification, whereas phosphorylation of DELLA by an unknown GA-activated protein kinase may compete with SPY and inactivate DELLA (Shimada et al., 2006; Silverstone et al., 2007). However, EARLIER FLOWERING1 (EL1), encoding a casein kinase in rice, has been shown recently to function as a repressor of GA signaling (Dai and Xue, 2010). The results of this study also suggested that phosphorylation of DELLA by EL1 is required for DELLA activity and stability. This recent finding seems to challenge the current working model described above, although it is possible that phosphorylations at distinct sites by two different protein kinases may have opposite effects on DELLA activity. Direct evidence for O-GlcNAc modification of DELLA by SPYand the effects of GlcNAc modification and phosphorylation on DELLA function will require further investigation.

MOLECULAR MECHANISM OF DELLA-REGULATED GROWTH

DELLA proteins belong to the GRAS family of plant-specific nuclear proteins, which do not contain any canonical DNA-binding domain and therefore, are likely to regulate expression of their target genes by interacting with other transcription factors. To investigate the mechanism of DELLA-mediated growth repression, several putative DELLA direct targets in Arabidopsis were identified by expression microarrays, and DELLA was shown to be associated with several promoters of its target genes by chromatin immunoprecipitation-quantitative PCR analysis (Zentella et al., 2007). Interestingly, DELLA induces expression of upstream GA biosynthetic genes and GA receptor genes, suggesting that DELLA functions in maintaining GA homeostasis via a feedback mechanism (Fig. 2). Other DELLA-induced target genes encode transcription factors/regulators and RINGtype ubiquitin E3 ligases. One of the RING ubiquitin E3 ligases, XERICO, is important for abscisic acid (ABA) accumulation. Thus, DELLA inhibits GAmediated responses (e.g. seed germination) in part by up-regulating ABA levels through XERICO. Sequence comparison and chromatin immunoprecipitation-quantitative PCR using promoters of DELLA targets did not uncover any conserved DELLAresponsive cis-elements, suggesting that DELLA interacts with different transcription factors to regulate expression of target genes. The best-elucidated molecular mechanism of DELLA-mediated growth regulation came from studies on the interaction between light and GA pathways. In etiolated Arabidopsis seedlings, PHYTOCHROMEINTERACTING

FACTORs (PIFs) belonging to the subfamily 15 of bHLH transcription factors promote hypocotyl elongation. During deetiolation, phytochromes inhibit hypocotyl elongation by causing PIF degradation and also inhibiting GA accumulation and in turn increasing DELLA protein levels (Achard and Genschik, 2009). Two recent studies (de Lucas et al., 2008; Feng et al., 2008) reveal that DELLA inhibits hypocotyl elongation by binding directly to PIF3 and PIF4, and preventing expression of PIF3/PIF4 target genes. Therefore, in addition to light regulation of GA metabolism, cross talk between light and GA signaling pathways occurs through protein-protein interaction between PIF and DELLA (Fig. 2). Three additional bHLH subfamily-15 members PIF1 (PIL5), SPT, and PIL2 also interact with DELLA in yeast two-hybrid assays (Gallego-Bartolome et al., 2010). It will be important to determine whether DELLA regulates the functions of other members in this bHLH subfamily by direct protein-protein interactions. DELLA may also modulate gene expression via interaction with other classes of transcription factors and/or chromatin modification complexes. Future identification of additional DELLA interacting proteins by yeast two-hybrid assays and/or proteomic approaches will provide more complete understanding of the molecular mechanisms of DELLA function. Interaction between light and GA also occurs during seed germination. In contrast to their antagonistic effects on hypocotyl elongation, both light and GA promote germination (Fig. 2). In the dark, PIL5 (PIF1) inhibits germination, in part, by binding to and activating transcription of promoters of two AtDELLA genes (RGA and GAI; Oh et al., 2007). PIL5 also reduces GA accumulation and increases ABA levels via SOMNUS (a nuclear zinc-finger protein; Kim et al., 2008). Interestingly, ABAwas shown to promote transcription of another AtDELLA gene (RGL2; Piskurewicz et al., 2008). In the light, phytochromesmediate light-induced germination by causing PIL5 degradation and GA accumulation, the combined effects of which allow down-regulation of DELLA activity (by reducing transcription and increasing protein turnover). In addition to mediating the cross talk between GA and light signaling pathways, DELLA plays a major role in modulating plant growth in response to internal cues (other hormone signals) and external biotic and abiotic stresses (Fig. 2; Achard and Genschik, 2009; Bari and Jones, 2009; Harberd et al., 2009). In most cases, DELLA stability is indirectly affected by other pathways through alteration of GA metabolism and bioactive GA levels. For example, auxin induces root and stem elongation, at least in part, by upregulating GA biosynthetic genes (GA3ox) and downregulating GA catabolism genes (GA2ox). During cold and salt stresses, AP2 transcription factors CBF1 and DDF1, respectively, induce expression of GA2ox genes.

Similarly, stabilization of DELLA by ABA treatment is achieved by reduction of GA accumulation. In a GA biosynthesis mutant background, ABA pretreatment failed to inhibit

GA-induced DELLA degradation (Zentella et al., 2007). In conclusion, the GA-GID1-DELLA signaling module is regulated at multiple levels to achieve proper growth: (1) The amounts of bioactive GAs are affected via altered expression of GA metabolic genes by internal and external cues. GA transport may also play a role, although the regulatory mechanism is unknown; (2) elevated GA signals will activate GID1 to induce rapid proteolysis of DELLA; (3) in addition to protein degradation, DELLA activity is also regulated by transcription, posttranslational modifications, and direct protein-protein interactions; and (4) DELLA may play a role in maintaining GA homeostasis by feedback regulation of expression of GA biosynthesis and receptor genes. Further elucidation of how DELLA coordinates GA and other signaling activities will come from functional studies of DELLA modifications, DELLA target genes, and identification and functional characterization of additional DELLA interacting proteins.

SPATIAL AND TEMPORAL CONTROL OF THE GA-GID1-DELLA SIGNALING MODULE

To understand how the GA-GID1-DELLA module regulates plant growth and development, it will be necessary to monitor the sites and timing of the actions of this module. In the root, expression of a GAresistant (gain-of-function) DELLA mutant protein in the endodermis (but not other cell types) appears to inhibit root elongation, suggesting that the primary site of GA-induced DELLA degradation for primary root elongation is in the endodermis (Ubeda-Tomas et al., 2008). Interestingly, recent studies showed that different phytohormones seem to act in distinct and not completely overlapping cell types (Jaillais and Chory, 2010). This is not too surprising because defects in different hormone pathways cause distinct phenotypes. With the available knowledge of the metabolic pathway and early signaling pathway for individual phytohormone, systems biology approach will help to visualize the complex regulatory webs (both transcriptional networks and protein interactomes) among these pathways in specific cell types to modulate different developmental events.

The JAZ family

Jasmonates are essential phytohormones for plant development and survival. However, the molecular details of their signalling pathway remain largely unknown. The identification more than a decade ago of COI1 as an F-box protein suggested the existence of a repressor of jasmonate responses that is targeted by the SCFCOI1 complex for proteasome degradation in response to jasmonate. Here we report the identification of JASMONATE-INSENSITIVE 3 (JAI3) and a family of related proteins named JAZ (jasmonate ZIM-domain), in Arabidopsis

thaliana. Our results demonstrate that JAI3 and other JAZs are direct targets of the SCFCOI1 E3 ubiquitin ligase and jasmonate treatment induces their proteasome degradation. Moreover, JAI3 negatively regulates the key transcriptional activator of jasmonate responses, MYC2. The JAZ family therefore represents the molecular link between the two previously known steps in the jasmonate pathway. Furthermore, we demonstrate the existence of a regulatory feed-back loop involving MYC2 and JAZ proteins, which provides a mechanistic explanation for the pulsed response to jasmonate and the subsequent desensitization of the cell. Life on earth relies on a complex equilibrium of biotic and abiotic interactions. Plant small signalling molecules such as the jasmonates, structurally similar to prostaglandins in metazoans1,2, are essential for plant survival in nature, and thus contribute to modulation of this equilibrium. Jasmonates (jasmonic acid and other oxylipin derivatives) are key regulators of plant responses to environmental stresses and biotic challenges, such as ozone exposure, wounding, water deficit, and pathogen and pest attack1–4. They are also involved in important plant developmental processes, such as root growth, tuberization, fruit ripening, tendril coiling, reproductive development and senescence1–4. Their importance beyond the plant kingdom has been recently highlighted by their suggested anti-cancer activity in humans5. More than four decades after the discovery of methyl jasmonate as a major lipid constituent of the jasmine scent6, understanding of the biosynthetic (octadecanoid) pathway of jasmonates from linolenic acid is now well established7. In contrast, our current knowledge about the jasmonate signalling pathway lags behind3,8 Efforts to dissect the signalling pathway have defined two steps. The first one comprises components and regulators of SCF (Skip/ Cullin/Fbox) E3 ubiquitin ligase complexes (SCFCOI1)9–13. The second step is defined by transcription factors, such as MYC2 and ERF1, which orchestrate the expression of jasmonate-related effector genes14–16. However, these two steps remain unlinked and major questions about the molecular details of the jasmonate signalling pathway remain unanswered. For example, the jasmonate receptor is unknown, the link between jasmonate perception and SCFCOI1 is unidentified, and the connection between this SCF complex and the activation of transcription factors remains unresolved. The existence of these two types of jasmonate-signalling components has led to the hypothesis that activation of jasmonate responses by transcription factors requires ubiquitin-mediated degradation of a repressor that is targeted to the 26S proteasome by the SCFCOI1 after jasmonate perception. However, more than a decade after the molecular identification of COI1, the F-box component of the SCFCOI1 complex, the identity of this hypothetical repressor remains unknown. Here we report the identification of JASMONATE-INSENSITIVE 3 (JAI3) and show that it belongs to a novel family of jasmonateregulated nuclear targets of SCFCOI1, named JAZ (jasmonate ZIMdomain) proteins. JAI3 and other

JAZs physically interact with COI1, and jasmonate treatment induces their SCFCOI1-dependent proteasome degradation. The jai3-1 allele encodes a mutant protein resistant to degradation that also inhibits degradation of the wild-type JAI3 and other JAZs, explaining its dominant jasmonate-insensitive phenotype. Additionally, JAI3 and JAZ1 interact with MYC2, the key transcriptional activator of jasmonate-regulated gene expression, suggesting a model of JAI3/JAZ action as repressors of MYC2. Our results demonstrate that JAZs are direct targets of the SCFCOI1, linking ubiquitin-mediated protein degradation to transcriptional activation of jasmonate responses. Moreover, our results show that MYC2 and JAZs are involved in a negative regulatory feed-back loop that provides a mechanistic explanation for the pulsed response to jasmonate and the subsequent desensitization of the cell. Thus, the activation of jasmonate responses is regulated through a negative feed-back regulatory loop involving MYC2 and JAZ proteins. This provides a mechanistic explanation of the pulsed hormonal response and the subsequent desensitization of the cell to jasmonate23. Binding of the hormone to its receptor induces SCFCOI1-dependent proteasome degradation of JAZ proteins liberating MYC2 and allowing transcriptional activation of jasmonate responses (Supplementary Fig. 6). Because JAZ genes are transcriptional targets of MYC2, their rapid expression induced by this transcription factor contributes to the self-repression of MYC2. In the jai3-1 mutant, partial sequestration of COI1 by JAI3-1 prevents degradation of the other JAZs after jasmonate perception (Fig. 4b), which keep repressing MYC2 and therefore confer the jasmonate-insensitive phenotype. Consistent with this model, the majority of genes downregulated in the jai3-1mutant (as compared with wild-type) following jasmonate treatment seem to be targets of MYC2 (Fig. 3e). The identification of the JAZ family may also help us to understand jasmonate perception. Since the identification of TIR1 as the auxin receptor24–26, the sequence similarity of COI1 and TIR1 indicated that the SCFCOI1 might be the jasmonate receptor (see ref. 22 for results supporting this hypothesis). It has been previously proposed that jasmonate is unlikely to be the active hormone21. However, several jasmonate analogues, such as JA–Ile and coronatine, displaying an intriguing similarity in their chemical structure21,27,28, show overlapping as well as specific COI1-dependent activities29–31. It is tempting to speculate that different receptor complexes may be formed by the combination of COI1 with different members of the JAZ family. This combinatory mechanism would provide a molecular explanation for the overlapping and specific activities of these active jasmonate analogues. The identification of JAZ proteins will allow testing of this hypothesis. Finally, transcriptional reprogramming in response to jasmonate cannot be exclusively attributed to MYC2, because loss-of-function mutations in this gene do not completely impair jasmonate sensitivity 3,14,15. The identification of this family of repressors provides a new molecular

tool to dissect the transcriptional network regulating jasmonate responses, and to understand how different signalling modules (SCFCOI1–JAZ–transcription factors) fine-tune cellular responses to specific challenges.

5. Case study

Tobacco transcription repressors NtJAZ: Potential involvement in abiotic stress response and glandular trichome induction

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Members of the Jasmonate ZIM domain (JAZ) proteins act as transcriptional repressors in the jasmonate (JA) hormonal response. To characterize the potential roles of JAZ gene family in plant development and abiotic stress response, fifteen JAZs were identified based on the genome of Nicotiana tabacum. Structural analysis confirmed the presence of single Jas and TIFY motif. Tissue expression pattern analysis indicated that NtJAZ-2, -3, -5, and -10 were highly expressed in roots and NtJAZ-11 was expressed only in the cotyledons. The transcript level of NtJAZ-3, -5, -9, and -10 in the stem epidermis was higher than that in the stem without epidermis. Dynamic expression of NtJAZs exposed to abiotic stress and phytohormone indicated that the expression of most NtJAZs was activated by salicylic acid, methyl jasmonate, gibberellic acid, cold, salt, and heat stresses. With abscisic acid treatment, NtJAZ-1, -2, and -3 were not activated; NtJAZ-4, -5, and -6 were up-regulated; and the remaining NtJAZ genes were inhibited. With drought stress, the expression of NtJAZ-1, -2, -3, -4, -5, -6, -7, and -8 was up-regulated, whereas the transcript of the remaining genes was inhibited. Moreover, high concentration MeJA (more than 1mM MeJA) had an effect on secreting trichome induction, but inhabited the plant growth. Nine NtJAZs may play important role in secreting trichome induction. These results indicate that the JAZ proteins are convergence points for various phytohormone signal networks, which are involved in abiotic stress responses.

Effect of phytohormone on induction of glandular trichomes

Tobacco Introduction 1112 (N. tabacum) characterized by non secreting trichomes was developed by the Oxford Tobacco Research Station. Fourth-leaf T.I.1112 plants were separately sprayed with 1.0, 3.0 and 5.0mM methyl jasmonate (MeJA), or control solution (0.8% ethanol and water) every seven days for a total of three applications. After three weeks, stem epidermis were stained using 2% rhodamine, and photographed using a microscope. The experiment was in triplicates. Experiments were performed in triplicate. For 5.0mM MeJA treatment and control, the stem epidermis was removed for qPCR analysis at 6, 12, and 24 h after MeJA treatment.

Tissue-specific expression analysis

Six tissues of tobacco plants (N. tabacum L. 'Kentucky 326'), viz., the cotyledon, leaf, root, seed, stem epidermis, and stem without epidermis, were collected for tissue-specific expression analysis. Stem with its epidermis removed was collected as stem without epidermis. Reverse transcription-polymerase chain reaction (RT-PCR) was used to determine the tissue expression patterns of NtJAZs in tobacco. The transcript of tobacco ribosomal protein gene L25 was used as a reference gene.

Abiotic stress and hormone treatments

For abiotic stress treatment, ten four-leaf seedlings were separately stressed with 300mM NaCl solution, PEG-6000 (−0.5 MPa) solution, and low (4 °C) and high (42 °C) temperatures. For exogenous hormone treatment, ten four-leaf seedlings were separately sprayed with 100 μM MeJA, 150 μMGA, 100 μM abscisic acid (ABA) and 2.0mM salicylic acid (SA). Controls were cultured without any treatment. Leaves were sampled at 0, 1, 3, 6, 12, 24, 48, and 72 h post treatment for RNA isolation. Experiments were performed in triplicate.

Expression pattern of the NtJAZ genes

Total RNA was extracted from the samples using the TRIzol method, and then treated with Nase-free DNase (Takara, China). Complementary DNA was synthesized using the reverse transcription kit (Takara, China). The expression pattern of NtJAZs in various tobacco tissues was evaluated using the reverse transcription-polymerase chain reaction (RT-PCR). The quantitative real-time PCR (q-PCR) was performed to detect the transcription level of NtJAZ under different abiotic stresses and phytohormone treatments. Quantitative assays were performed in triplicates for each sample with the SYBR Green Master Mix (Takara, China) on an ABI PRISM 7000 system. The relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The CT (cycle threshold) value is the mean of the triplicate independent PCRs. The L25 transcript was used to quantify the relative transcript levels. Gene-specific primers are listed in Table S1.

Results

Jasmonic acid had an effect on secreting trichome induction

To address the effect of JA, SA and GA on the induction of glandular trichomes, tobacco T.I.1112 with no long-stalked glandular trichomes were separately sprayed with different phytohormone (0–5 mM/L). The results showed that SA or GA had no significant effect on the induction of glandular trichomes (data not shown). Under high concentration of MeJA treatment from 600 to 900 μM, a few long-stalk glandular trichomes were induced, whereas low concentration MeJA (less than 600 μM MeJA) had no effect on glandular trichome induction (Fig. S2). When treated using high concentration MeJA (more than 1mM MeJA), long-stalk glandular trichomes were mostly induced, and their density was positively related to the MeJA concentration; this was not the case for the short stalked glandular trichome (Fig. 4A and B). Rhodamine staining patterns showed that application of 5mM MeJA to tobacco plants induced obvious screting trichomes after treatment, and had an increased glandular trichome density. However, the plant growth and development were obviously inhibited by high concentration MeJA. Analyzing the expression level of the NtJAZ genes in the stem epidermis showed that nine NtJAZs (NtJAZ-1, -3, -5, -7, -8, -9, -10, -12 and -15) were up-regulated under 5.0mM MeJA application (Fig. 4C, Table S2). Notably, the transcription level of NtJAZ-7 increased rapidly and strongly after MeJA treatment. NtJAZ-5, -8 and -9 were induced gradually, while NtJAZ-1, -3, -10, -12 and -15 were up-regulated weakly compared with other four NtJAZs.

Tissue expression pattern of the NtJAZ genes

To assess the potential functions of NtJAZs in tobacco development, the expression profiles of all 15 NtJAZ genes were investigated in six tissues. As shown in Fig. 5A, no trichome was existed on cotyledons, while lots of trichomes were present on the surface of leaf and stem epidermis. Tissue expression pattern analysis showed that three NtJAZ genes, namely, NtJAZ-4, NtJAZ-14, and NtJAZ-15, showed no significant tissue-related differences in expression, which might play a more ubiquitous role in tobacco (Fig. 5B). NtJAZ-1 was constitutively expressed in tobacco, with high expression in the cotyledon, seed, root, and stem without epidermis. NtJAZ-2, NtJAZ-3, NtJAZ-5, and NtJAZ-10 were expressed at high levels in the roots compared with those in other tissues. Compared with that in other tissues, the expression of four NtJAZs—NtJAZ-7, NtJAZ-8, NtJAZ-12, and NtJAZ-13—were weak in the cotyledons, whereas, NtJAZ-11 was expressed only in the cotyledons. NtJAZ-6 was weakly expressed in the stem epidermis. However, the transcript level of NtJAZ-3, NtJAZ-5, NtJAZ-9, and NtJAZ-10 in the stem epidermis was higher than that in the stem without epidermis, indicating that the four NtJAZs might be specifically associated with epidermis development.

Expression pattern of the NtJAZ genes under abiotic stress and phytohormone treatments

In order to obtain the expression profiling of NtJAZ genes under phytohormone and abiotic stresses, qRT-PCR was performed to gain the relative expression pattern of each NtJAZ gene. As shown in Fig. 6, the transcripts of most NtJAZ genes increased significantly, whereas NtJAZ-1 was only marginally up-regulated with SA treatment. The treatment with ABA influenced the expression dynamics of NtJAZ genes, except NtJAZ-1, -2, and -3, which were not activated. The expression of three NtJAZ genes, namely, NtJAZ-4, -5, and -6, was up-regulated by ABA treatment, but the expression of the remaining NtJAZ genes were inhibited by ABA treatment. With 100 μM MeJA, the expression pattern of most NtJAZ genes in leaves was consistent with those in stem epidermis under 5.0mM MeJA application. The transcripts of ten NtJAZs (NtJAZ- 1, -2, -3, -5, -7, -8, -9, -10, -12 and -15) were activated by MeJA treatment. With GA treatment, the transcript of NtJAZ-5 was inhibited, whereas the expression level of other NtJAZs was increased. These results revealed that all the NtJAZ genes may be involved in intricate signaling pathways, and each gene has acquired distinct regulatory properties. With high salinity treatment, the expression of all the NtJAZ genes was up-regulated. Moreover, the transcript level of NtJAZ-1, -10, -12, and -15 was high at 24–72 h post treatment (Fig. 6, Table S3). Under drought stress, the expression of eight NtJAZ genes (NtJAZ-1, -2, -3, -4,-5, -6, -7, and -8) was up-regulated, whereas the transcript level of the remaining genes was inhibited by drought stress. Under cold stress, all NtJAZ genes were activated. The expression of five NtJAZ genes (NtJAZ- 6, -7, -8, -10, and -11) was up-regulated considerably by cold stress. Compared with that of other stresses, the transcripts of all NtJAZ genes were increased under high (42 °C) temperature stress. In addition, the transcript level of five genes (NtJAZ-2, -6, -7, -8, and -9) was high at each sampling time after treatment.

7. Summary

Photosynthesis in crop plants having C_3 cycle of carbon fixation can be increased by different approaches. Synthetic biology being a multidisciplinary field is promising tool to do so. Improvement in catalytic property of Rubsico help to inhibit oxygenation reaction site and

ultimately increases the photosynthetic yield and biomass. Complete inhibition of photorespiration is detrimental for plant as it has some role in other metabolic processes. Incorporating synthetic photorespiration bypass reduces energy requirement for fixing CO_2 lost during these process without causing detrimental effects to plant. Carbon concentrating mechanism helps to increase CO_2 concentration around Rubisco in C_4 plants as well as in cyanobacteria. These mechanism can be transferred to C_3 plants to increase photosynthesis. C_4 rice project a having ultimate aim to transfer whole C_4 pathway into rice will be great achievement towards adequately feeding rapidly growing population of world.

8. Future thrust

- Plants with higher photosynthetic efficiency with normal phenotype can be developed
- Fine tuning of genes expression can achieved by using new generation genome editing technologies.
- Plants with higher water use efficiency (WUE) and nitrogen use efficiency (NUE)
- Plants incorporated with entire C_4 pathway can be developed.

9. Discussion

1. Which is best strategy amongst these?

Ans. All strategies has few limitation combination of strategy will result good still improvement in catalytic property of Rubisco is better as it does not proves detrimental to plant

2. Does synthetic biology approaches produce abnormal phenotypes as it is synthetic way of improvement?

Ans. Synthetic biology is a combination of different fields of biology. During initial stage of experiments there might be chances of plant showing abnormal phenotypes due to overexpression or low expression of a gene. These problems can be tackled by using tissue specific promoters or enhancers. After series of experiment we can get a plant with normal phenotype having our interested genes.

3. Why tobacco Rubsico activase is more thermostable than Arabidopsis?

Ans. Thermo stability of Rubisco activase may be due to adaptation of plant to high temperature conditions and during course of evolution as tobacco plant grows at temperate condition.

4. Does transfer of gene coding for catalase, a H_2O_2 scavenger affect defence response of plants towards biotic stresses?

Ans. No, it will not affect. The copies of gene coding for catalase inserted into plant are less as compared to generation of H_2O_2 in plant and it is produced at different location while genes are incorporated under promoter acting in plant tissues.

5. Why overexpression of C_4 pathway genes caused abnormal phenotype in rice?

Ans. It was due to difference in their activity regulation, location of expression, kinetic property and absence of special anatomical structures in rice.

6. Why Rubisco has dual activity?

Ans. When life came into existence on earth there was no oxygen or very limited. Hence Rubico had a carboxylation activity. As time passed the carbon concentration drastically reduced at same time concentration of oxygen increased and due to this it Rubisco in evolution emerged with oxygenase activity.

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