



Roles of cytokinins in root growth and abiotic stress response of *Arabidopsis thaliana*

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Abstract

The root is an important vegetative plant organ. The root growth directly determine the growth and development of the shoots and affect the yield of crops. As one of the classic phytohormones, cytokinins, which promote cell division and regulate nutrient transport, are primarily synthesized in plant root tips. In recent years, increasing studies have found that cytokinins have different effects on growth and development of plant roots through signal transduction pathways or related receptors. Furthermore, cytokinins play an indispensable role in the abiotic stress response. This review provides a general overview of current progress in understanding the regulation mechanisms of cytokinins on the root growth and development, as well as abiotic responses of *Arabidopsis thaliana*, to establish a foundation for future scientific research.

Keywords Cytokinins · Abiotic stress · *Arabidopsis thaliana* · Root · Growth and development

Introduction

As an important organ by which plants maintain their normal growth and development, plant root mainly plays role in fixing plants, as well as absorbing and transporting water and nutrients from the soil (Abdi et al. 2019; Hodge et al. 2009; Martinez-Arias et al. 2020). Roots usually acquire vital resources from the soil and can directly feel changes in various environmental conditions in the soil and subsequently respond in real time (Armstrong et al. 1991; Chapman et al. 2020). Whether the root system grows well it can directly affects the growth status of the aboveground parts of the plant, that is, the yield of crops, but the problem of food yield still perplexes many countries throughout the world.

Phytohormones primarily refer to chemical substances that can regulate the growth and development of plants. These phytohormones are synthesized in plants and act as molecular signals at very low concentrations. Although their contents in plants are low, they play an indispensable regulatory role in the process of plant growth and development. Cytokinins (CKs), as one of the classic plant hormones, can promote cell division, accelerate the growth of collaterals, delay ageing, and regulate the transportation of nutrients (Collier et al. 2000; Jones and Schreiber 1997; Kurepa et al. 2018; Wang et al. 2020a, b; Werner et al. 2003; Zhang et al. 2005). At present, the effects of CKs on plant root growth and development, as well as the response of plants to abiotic stress, have been reported (Gao et al. 2014), which is primarily due to the important regulatory role played by the CKs signal transduction pathway and its components on plant roots. CKs have a fine regulation mechanism on roots and play a unique role in the growth and development of plant roots under abiotic stress. Additionally, CKs can combine with plant hormones, such as auxin (IAA) and abscisic acid (ABA), to regulate root growth and development (Albacete et al. 2008; Nguyen et al. 2018), but to date, there is no systematic summary.

With the progress of science and technology, researchers have made great progress in the area of plant hormone regulation of root growth and development and the abiotic stress response. This paper briefly describes the basic situation of

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Arabidopsis roots and CKs and summarizes the progress in the field of CKs regulation of the plant root response to abiotic stress. Based on the work in recent years, this article summarizes the regulatory mechanisms of CKs and its combination with other plant hormones on *Arabidopsis* root growth and development, as well as the abiotic stress response to provide certain information for researchers, which is conducive to performing follow-up research.

Basic characteristics of the *Arabidopsis* root

The root systems of plants can be divided into taproot and fibrous root systems according to their basic configuration (Fig. 1a). The axial root of the taproot system clear and well developed, and the direction is vertical downward. However, the fibrous root system has no obvious taproot and lateral roots (LRs), and the shape of the whole root system is flocculent. Generally, the root systems of dicotyledons are mostly taproot, while the roots of monocots are usually fibrous. The following is mainly elaborated by using the root system of *Arabidopsis thaliana*, a dicotyledonous model

plant in plant biology, to briefly introduce the structure of plant root systems and the process of LRs development.

Basic structure of the *Arabidopsis* root

The root of *Arabidopsis* is a typical taproot system, composed of the axial and LRs, which could be clearly distinguished in appearance. According to the cell morphology and structure of the root, it can be divided into the root cap, meristem, elongation, and maturation zone. The root cap and meristem zone together constitute the root apex, and cells in the elongation zone finally cannot elongate longitudinally to become mature cells, which are usually accompanied by root hair (Fig. 1b) (Dello Ioio et al. 2007). The cells at the junction of the root cap and meristem do not divide; thus, it is called quiescent center (QC). Various types of stem cells surround the QC, which can maintain root growth through asymmetric division. Generally, the QC can generate small scale signals to control the stem cells around it. The signal area covered by the QC is usually called the stem cell niche. The reason why stem cells can produce offspring cells is that they exceed the range of stem cell niches before they begin to differentiate (Fig. 1c) (Azpeitia et al. 2010, 2013).

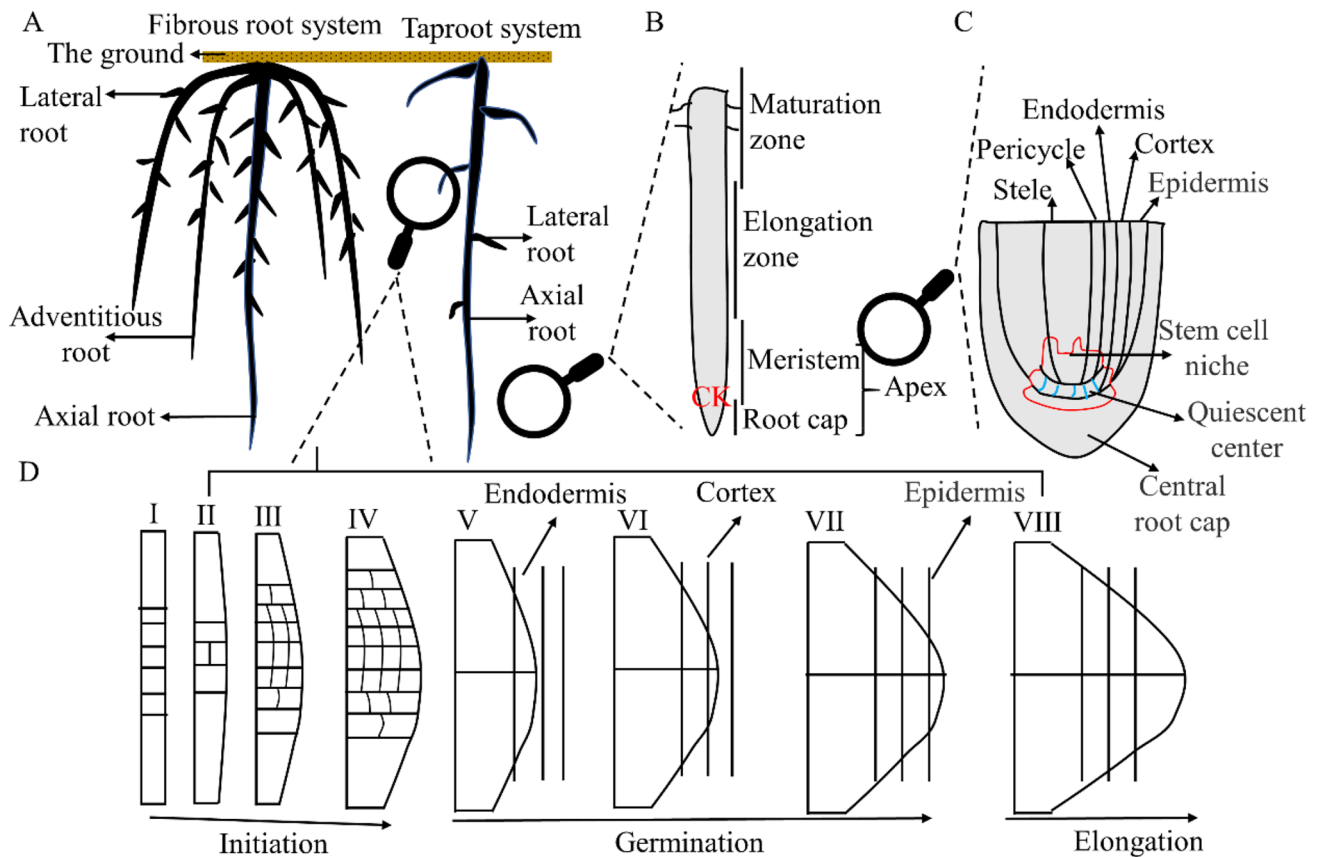


Fig. 1 Overview of the *Arabidopsis thaliana* root

The *Arabidopsis* transcription factor SHORT-ROOT (SHR) can affect cell division by regulating the microenvironment of active oxygen in the root tip (Li et al. 2018). CKs are primarily produced in the root tip, and CK mutants *ahk2/3/4* and *arr1/10/12* will disrupt root tip QC specialization, as well as stem cell maintenance and differentiation, and reduce the de novo rate of root growth, which shows that in the absence of CKs, the process of the de novo guidance and initiation of roots can occur, while the specialization of QC in the root apical meristem and the differentiation of stem cells in the distal stem cannot proceed normally (Sun et al. 2016; Yokoyama et al. 2007; Zhang et al. 2013). Taken together, these findings indicate that the function of CKs is region-specific.

Development of the *Arabidopsis* LRs

The LRs of *Arabidopsis* usually originate from the pericycle cells near the xylem pole, which is different from the monocotyledonous where LRs generally derive from the pericycle cells near the phloem pole. However, only a few of the producing cells can eventually develop into LRs. LRs have an essential role in increasing the water absorption area of the root system and maintaining the overall structure of the root system. The development of LRs has been systematically divided into 8 detailed stages from initiation, germination, and elongation in recent years (Fig. 1d) (Malamy and Benfey 1997). In the initial stage, the two adjacent generating cells undergo unequal vertical division followed by radioactivity to expand the cell layer, gradually showing a conical shape. In the germination phase, the LR primordium further divides and expands, breaking through the endodermis, cortex, and epidermis in turn. Finally, the LR primordium enters the elongation stage and develops into mature LRs under the regulation of the newly formed LR meristem (Benkova and Bielach 2010). It has been reported that the two receptor-like protein kinases MUSTACHES (MUS) and MUSTACHES-LIKE (MUL) of *Arabidopsis* rich in leucine-rich repeat (LRR) sequences can regulate cell wall synthesis and remodelling to control the early development of *Arabidopsis* LR primordium (Xun et al. 2020). Usually, there are few atypical aspartic proteases (APs) in plants, but recently, it was found that overexpression of APs in *Arabidopsis* roots inhibited the development of LRs, suggesting that APs have a previously unknown biological role (Soares et al. 2019). The signal peptide treatment of CK oxidase 2 (CKX2) affects its enzymatic activity, thereby determining the degradation of CKs in natural *Arabidopsis*. The CK signal interferes with the growth of the upper LR flanks, thereby preventing downward bending (Waidmann et al. 2019). We can see that there are many ways to adjust the development of LRs in *Arabidopsis*, which involve different proteins and signalling molecules.

However, there are still many unknown ways or patterns to be discovered.

Synthesis, signal transduction, and molecular mechanism in the regulation of *Arabidopsis* root growth by CKs

CKs are widely distributed in higher plants, and their content is in range of 1–1000 ng/g. Although the content is very low, its role is integral. CKs are usually distributed in the apex, stem tips, germinated seeds, and growing fruits of plants. The root tip is the primary place for CKs synthesis, which are primarily transported upward through the xylem (Aloni et al. 2005; Cedzich et al. 2008). The following principally introduces the synthesis of CKs, signal transduction pathway, and molecular mechanism in regulating the growth and development of the *Arabidopsis* root system.

Synthesis of CKs

Currently, there are two known synthetic pathways of CKs, the de novo synthetic pathway is the primary one, and the tRNA decomposition pathway is the auxiliary one. Among them, the de novo synthetic pathway catalysed by isopentenyl transferase (IPT) is a conserved mechanism in evolution. The IPT protein encoded by the 7 *AtIPT* genes of *Arabidopsis* takes part in the de novo synthesis of CKs. Additionally, overexpressing the *IPT* gene can facilitate the synthesis of CKs (Galichet et al. 2008; Sakakibara and Takei 2002; Takei et al. 2004). The de novo synthetic pathway begins through the combination of dimethylallylpyrophosphate (DMAPP) and adenine ribonucleotide (AMP) or adenosine triphosphate (ATP)/adenine nucleoside diphosphate (ADP). Isopentenyl nucleoside is formed under the catalysis of cytochrome p450 monooxygenase (CYP735A), which is capable of converting isoamylene-type CK into zeatin nucleotide-type CK, but the CK at this stage does not have physiological activity and needs to be activated under the action of phosphoribosyl hydrolase (LOG) (Kakimoto 2003; Zurcher and Muller 2016). Nine LOG encoding gene families have been identified in *Arabidopsis* (Tokunaga et al. 2012). Another tRNA decomposition pathway is catalysed by tRNA-IPT, and the 3 ends of some tRNA anticodons are prenylated, which decomposes tRNA and finally produces *cis*-zeatin (*cZT*) (Mok and Mok 2001). Studies have demonstrated that the *Arabidopsis AtIPT2/9* gene can encode these, and the *cZT* content is significantly reduced after mutation (Miyawaki et al. 2006; Xu et al. 2016). Currently, the research on the synthesis of CKs is relatively clear, and considerable progress has been made in the related research on the signal transduction pathway, but it is not very profound yet.

Signal transduction pathway of CKs

The signal transduction pathway of CKs is a multi-step phosphotransfer reaction (Fig. 2). CKs bind to the CHASE domain of CKs receptors (AHK2, AHK3 and AHK4/WOL1/CRE1), which are located on the plasma membrane, thereby activating their histidine kinase activity (Ishikawa et al. 2002; Pas et al. 2004). The phosphate group is transferred to the aspartate receptor domain connected to it, and it is subsequently transferred to the histidine of the histidine-phosphotransfer protein (AHP) and phosphorylated. The phosphorylated AHP protein enters the nucleus and transfers the phosphate groups to the type-A ARR and type-B ARR receptor domains in the nucleus. The phosphorylation of type-B ARR can activate the output domain and induce the transcription of the *type-A ARR* gene. The activated type-B ARR output domain is also able to affect downstream transcriptional regulation by affecting CKs. At the same time, type-A ARR can also be directly phosphorylated by the AHP protein to cause a CKs response (Haberer and Kieber 2002; Horak et al. 2008; Jeon and Kim 2013). For the research of the CKs signal transduction pathway, the core issue is the exploration of its new receptors. At present, it still only has several known receptors. In the future, we will focus on exploring and discovering new receptors. Going forward, the discovery of new receptors will inevitably become the focus and difficulty of foreign research.

Mechanism of CKs in regulating the root growth of *Arabidopsis*

Root growth is regulated by a variety of factors, including plant hormones, transcription factors, and small RNAs (miRNAs) (Chen et al. 2020; Hernandez et al. 2020; Lin and Sauter 2020; Wang et al. 2020a, b; Ye et al. 2020; Zhou et al. 2020a). To date, researchers have performed various studies in these areas. The root tip of *Arabidopsis* is one of the staple places where CKs are produced. The CK content in the plant is usually increased by externally applying CKs or genetically modified technology. Compared with the promotion effect on the above-ground part (Riefler et al. 2006), for the growth of the underground part, CKs mainly play a negative regulatory role, which can affect the growth and development process of the plant's axial root elongation and LR development. The following mainly summarizes the role of CKs in growth and development of the axial and LR of *Arabidopsis* from different aspects.

Mechanism of CKs affecting the growth of the axial roots of *Arabidopsis*

The effect of CKs on growth and development of *Arabidopsis* axial roots can mainly be affected by the regulators and receptor proteins in the CKs signalling pathway. Although the main physiological function of CKs is to affect cell

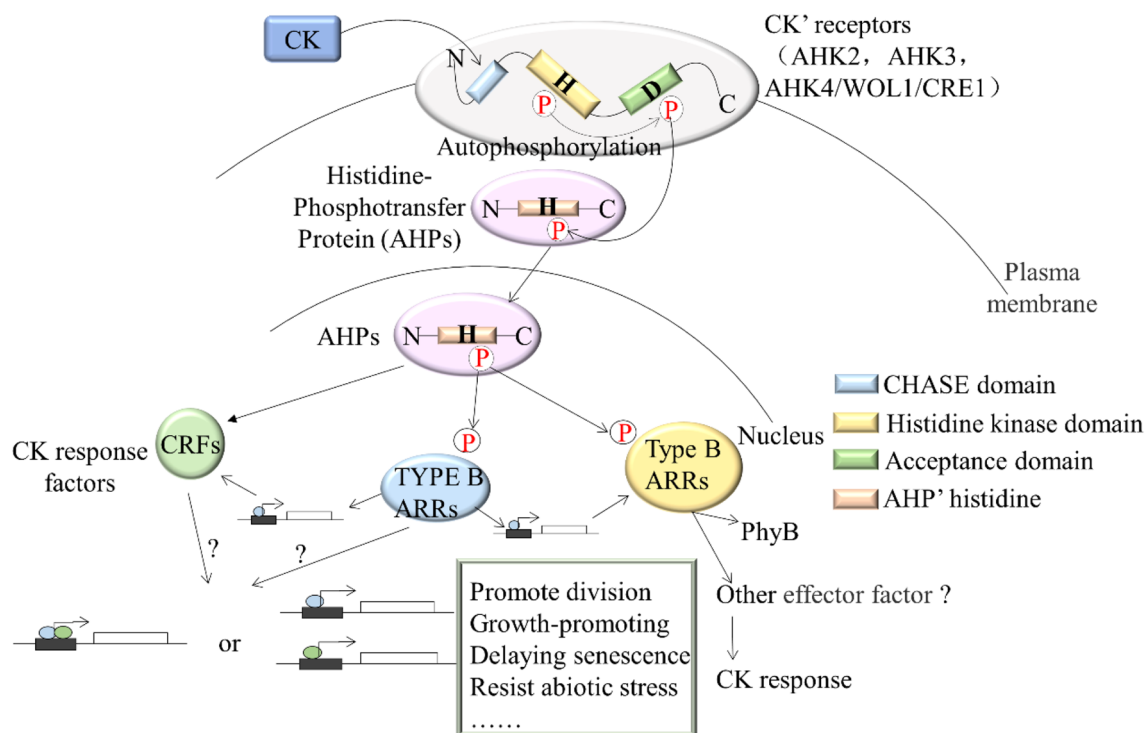


Fig. 2 Signal transduction pathway of *Arabidopsis thaliana* cytokinin

division, regulation of taproot growth is not achieved by directly affecting the division of cells in the meristem area but by affecting the rate of differentiation. The meristematic region of taproot became smaller after exogenous application of CKs. But, if the key genes in CKs signaling pathway were mutated, the opposite phenotype appeared (Li et al. 2006). Additionally, after mutating all of the CK receptors, it was found that the rate of cell division in the root meristem decreased significantly (Higuchi et al. 2004; Nishimura et al. 2004). Nitrate can be used as one of the effective indicators to regulate the growth and development of plant roots. CKs sensing and biosynthesis mutants have shorter taproots than wild-type plants when nitrate is the only source of nitrogen, and the root tip tissue scientific analysis showed that the cell division and elongation of the double mutant of CK receptor *ahk2/ahk4* were reduced compared with wild-type plants under sufficient nitrate conditions (Naulin et al. 2020). This indicates that nitrate can stimulate the growth of the primary roots by increasing the meristem activity and CKs signal. The balance between cell division and differentiation is mainly achieved by the *SHORT HYOPCOTYL2 (SHY2)* gene through the regulation of IAA and CKs signalling pathways. SHY2 protein is one of the key points for plant IAA, brassinosteroids, and CKs to regulate root meristem development (Li et al. 2020). Besides, according to Dello Ioio that an AHK3/ARR1, AHK3/ARR12 two component cytokinin signaling pathway mediates control of root-meristem size at the transition zone (Dello Ioio et al. 2007).

Except for these regulators and receptor proteins in the CKs signalling pathway can affect the axial root, the miRNAs can also have great impact on the axial root. The miRNAs are a type of endogenous non-coding RNA with regulatory functions that exist in eukaryotes, which can participate in multiple processes of plant growth and development, and their length is usually 22 nucleotide. The miR156 and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* genes have opposite expression patterns during the primary root (PR) growth of *Arabidopsis*. Plants with a higher level of miR156 exhibit decreased size of the meristem, leading to the shortening of the main root. In contrast, plants with reduced levels of miR156 showed higher meristem activity. Meristem activity is regulated by *SPL10* probably through the reduction of cytokinin responses, via the modulation of ARR1 expression (Barrera-Rojas et al. 2020).

Mechanism of CKs affecting the growth of *Arabidopsis* LRs

The growth and development of LRs are also regulated by many factors. CKs play an indispensable role in the distribution direction of LRs as well as the initiation and extension of LRs. IAA can promote the downward bending of the lower root organs, but the CKs signal, as a unique anti-gravity component of the LR, can promote the radial distribution

of the root system. Among these factors, cytokinin oxidase 2 (CKX2) mainly determines the degradation of CK in natural *Arabidopsis*. The CK signal interferes with the growth of the upper root side and prevents downward bending (Waidmann et al. 2019). At present, most studies believe that CKs act as an antagonist of IAA during the formation of LRs, and CKs inhibit the initiation of LRs (Chang et al. 2013). The reason may be that CKs inhibit cyclin (CYC) and the cell cycle during the initiation of LRs. The expression level of protein-dependent kinase (CDK) can inhibit the extension of LRs by increasing the length of epidermal cells, and this pathway is regulated by IAA-dependent pathways. Mutations in CKs receptors AHK2 and AHK3 can increase the sensitivity of IAA to LR formation. With regards to the functional relevance of IAA-CKs interaction during LR formation, in addition to IAA-dependent pathways, LR formation may also be achieved by promoting CYC expression activity (Chang et al. 2013). Abscisic acid insensitivity factor 4 (ABI4) mediates the inhibition of ABA and CKs on the formation of LRs by reducing IAA transport, leading to the reduction of IAA in the roots and ultimately inhibiting the development of LRs (Shkolnik-Inbar and Bar-Zvi 2010). It can be seen that CKs can not only affect the initiation and extension of *Arabidopsis* LRs but also work together with other plant hormones. In this process, the factors in the CKs signalling pathway also play a vital role.

Effect of CKs on plant roots under abiotic stress

As the external environmental conditions change, the phytohormone content in plants often changes accordingly. In previous studies, it was found that ABA plays an important role in regulating the adaptation of plants to adversity; thus, it is also called the adversity hormone (Ma et al. 2018; Wu et al. 2020; Yu et al. 2020). In recent years, it has been discovered that CKs also play a unique role in plant resistance to adversity (Bielach et al. 2017; Cortleven et al. 2019; Ryu and Cho 2015). CKs can affect the growth and development of plant roots under drought stress, cold stress, salt stress, and various chemical element stresses. The influence of CKs on the plants growth and development under abiotic stress in recent years is summarized in Table 1. The related literature on root growth and development can provide a systematic understanding of the role of CKs in roots under different abiotic stresses. On the whole, CKs mainly affects the growth of plant roots under abiotic stress through its interaction with other phytohormones.

Based on the research results in the table, it can be seen that there has been significant progress in CKs regulation of root growth and development under abiotic stress. While different types of stress are involved, the research objects

Table 1 Function of cytokinins in growth and development of plant root under abiotic stress

Stresses	Species (Latin name)	Functions	References
Drought stress	<i>Populus</i>	The ABA/CK and aquaporins of the local roots of poplars regulate the response to mild drought stress independently of the ectomycorrhizal fungus <i>Laccaria bicolor</i>	Calvo-Polanco et al. (2019)
Drought stress	<i>Zea mays</i>	In the process of drought rewatering, the concentration of CKs induced at deep roots affects the compensatory growth of maize	Wang et al. (2018a, 2018b, 2016)
Drought stress	<i>Hordeum vulgare</i>	By promoting the degradation of CKs in the roots, a transgenic barley with a larger root system is generated, thereby improving the drought resistance of the crop	Ramireddy et al. (2018)
Drought stress	<i>Agrostis stolonifera</i>	Overexpression of <i>IPT</i> promotes CKs synthesis and activates ROS scavenging system to reduce the inhibition of drought stress on root growth	Xu et al. (2016)
Osmotic stress	<i>Arabidopsis thaliana</i>	CKs regulates <i>Arabidopsis</i> root growth under osmotic stress through a hormone network that interacts with ABA, ethylene and IAA	Gujjar and Supaibulwatana (2019), Rowe et al. (2016)
Cold stress	<i>Arabidopsis thaliana</i>	CKs response factors CRF2 and CRF3 encode APETALA2 transcription factors and play an important role in regulating the initiation of <i>Arabidopsis</i> LR _s under cold stress	Jeon et al. (2016)
Salt stress	<i>Centaurium erythraea Rafn</i>	The <i>AtCKX</i> transgenic line reduces the CK content in the root system, affects the growth of the root system, and enhances salt tolerance	Trifunović-Momčilov et al. (2020)
Salt stress	<i>Oryza sativa</i>	The application of GA ₃ or CKs biosynthesis inhibitors can replenish the phenotypes of CYP71D8L-OE and CYP71D8L root dysplasia and show higher salt tolerance	Zhou et al. (2020b)
High boron stress	<i>Arabidopsis thaliana</i>	Under high boron stress, 26S proteasome maintains the viability of root tip meristem by regulating the response of IAA and CKs	Sakamoto et al. (2019)
Low nitrogen stress	<i>Zea mays</i>	Under low nitrogen stress, CK, ethylene and ABA play an antagonistic effect, while BR and IAA play a synergistic effect to regulate root elongation	Lv et al. (2020), Sun et al. (2020)

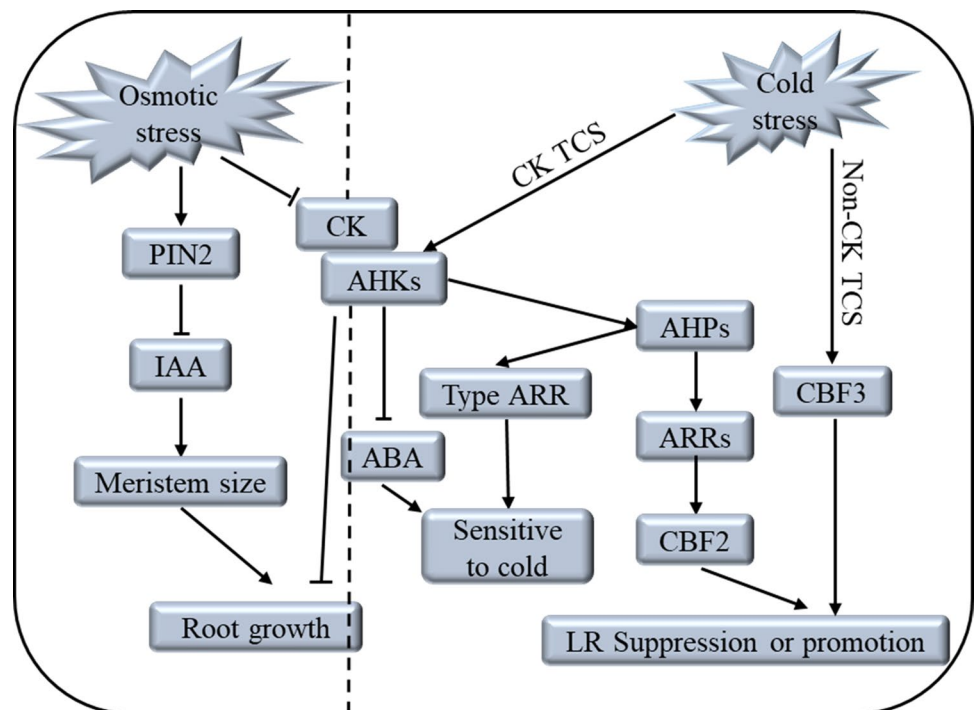
are not limited to *Arabidopsis* but also in maize and rice. Corresponding research work has also been performed in other crops. At present, especially under cold stress and osmotic stress, the research on the effect of cytokinin on root system is more comprehensive. Cytokinin-responsive factors CRF2 and CRF3 play a significant role in regulating the initiation of *Arabidopsis* LR_s at low temperatures, which mainly include two types of regulatory pathways, namely, the two-component signal transduction pathway (TCS) dependent on CK to induce the expression of CRF2, and it does not rely on the TCS pathway to induce CRF3 upregulation, thereby inhibiting or promoting the growth of LR_s (Jeon et al. 2016). Additionally, CKs bind to receptors AHK2 and AHK3, and the expression of type-A ARR at this time has a negative regulatory effect on cold stress. At the same time, CKs binds to receptors to inhibit the response of ABA to cold stress (Fig. 3) (Jeon and Kim 2013; Jeon et al. 2010; Nishimura et al. 2004; Xia et al. 2009). Under osmotic stress, this response inhibits the synthesis of CKs, thereby inhibiting the growth and development of the root system.

At the same time, osmotic stress promotes the high expression of PIN2 protein and inhibits the synthesis of IAA. The size of the root meristem increases with the decrease of the IAA concentration, thereby affecting the normal growth of the root system (Fig. 3) (Hussain et al. 2015; Lakehal et al. 2019; Rowe et al. 2016). It can be seen that CKs are indispensable in regulating of root growth and development under abiotic stress.

Conclusion and prospects

In recent years, researchers have conducted a series of in-depth studies on plant roots using molecular biology and genetics. These researchers have realized that plant roots play a critical role in plant growth and development, as well as the response of the plant to abiotic stress. Much outstanding progress has also been made in the field, especially in the related research represented by the dicotyledonous model

Fig. 3 Pathway of CKs regulating root growth under cold and osmotic stress



plant *Arabidopsis*, which has notable reference significance for subsequent research on other plants.

Recently, many advances have been made in understanding the role of plant hormone abiotic stress and other factors in the root growth and development of *Arabidopsis*, but many problems still need to be further understood and explored as follows: (1) Although it has been reported that various phytohormones regulate growth and development of *Arabidopsis* roots through the mutual interaction, are there other factors that work together in the interaction between hormones? The dominating factors and the roles they play need to be further explored. (2) When plant hormones regulate the growth and development of *Arabidopsis* roots, they usually act through the signal transduction pathway. New signalling pathways and receptors based on pathways are need to be explored along with enriching the regulatory pathways and signal networks of phytohormones on *Arabidopsis* roots. (3) Future research needs to fully integrate the growth and development of the above-ground and underground parts of plants to systematically explore the mutual regulation mechanisms and signal transduction pathways between them.

In brief, further strengthening the research on the regulation mechanism of plant hormones and root growth and development and the response to abiotic stress can motivate our in-depth understanding of the molecular regulation mechanism and signal network of plant hormone regulation of plant root growth and development. These results may help achieve greater research progress and

then provide a solid technology and information basis for crop breeding and cultivation, as well as mechanized intensive planting in the future.

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Authors' contributions Yu Wu had the idea for this review article. Yu Wu and Huimin Liu performed the literature search and data analysis. Yu Wu drafted, Qing Wang and Genfa Zhang critically revised the work.

Declarations

Conflicts of interest The authors declare no conflict of interest.

References

- Abdi E, Saleh HR, Majnonian B, Deljouei A (2019) Soil fixation and erosion control by *Haloxylon persicum* roots in arid lands, iran. *J Arid Land* 11:86–96
- Albacete A, Albacete A, Ghanem ME, Martinez-Andujar C, Acosta M, Sanchez-Bravo J, Martinez V, Lutts S, Dodd IC, Perez-Alfocea F (2008) Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. *J Exp Bot* 59:4119–4131
- Aloni R, Langhans M, Aloni E, Dreieicher E, Ullrich CI (2005) Root-synthesized cytokinin in *Arabidopsis* is distributed in the shoot by the transpiration stream. *J Exp Bot* 56:1535–1544
- Armstrong W, Justin S, Beckett P, Lythe S (1991) Root adaptation to soil waterlogging. *Aquat Bot* 39:57–73

- Azpeitia E, Benitez M, Vega I, Villarreal C, Alvarez-Buylla ER (2010) Single-cell and coupled grn models of cell patterning in the *Arabidopsis thaliana* root stem cell niche. *BMC Syst Biol* 4:1–19
- Azpeitia E, Weinstein N, Benitez M, Mendoza L, Alvarez-Buylla ER (2013) Finding missing interactions of the *Arabidopsis thaliana* root stem cell niche gene regulatory network. *Front Plant Sci* 4:110
- Barrera-Rojas CH, Rocha GHB, Polverari L, Brito DAP, Batista DS, Notini MM, da Cruz ACF, Morea EGO, Sabatini S, Otoni WC, Nogueira FTS (2020) Mir156-targeted spl10 controls *Arabidopsis* root meristem activity and root-derived de novo shoot regeneration via cytokinin responses. *J Exp Bot* 71:934–950
- Benkova E, Bielach A (2010) Lateral root organogenesis—from cell to organ. *Curr Opin Plant Biol* 13:677–683
- Bielach A, Hrtyan M, Tognetti VB (2017) Plants under stress: Involvement of auxin and cytokinin. *Int J Mol Sci* 18:1427
- Calvo-Polanco M, Armada E, Zamarreno AM, Garcia-Mina JM, Aroca R (2019) Local root aba/cytokinin status and aquaporins regulate poplar responses to mild drought stress independently of the ectomycorrhizal fungus *Laccaria bicolor*. *J Exp Bot* 70:6437–6446
- Cedzich A, Stransky H, Schulz B, Frommer WB (2008) Characterization of cytokinin and adenine transport in *Arabidopsis* cell cultures. *Plant Physiol* 148:1857–1867
- Chang L, Ramireddy E, Schmulling T (2013) Lateral root formation and growth of *Arabidopsis* is redundantly regulated by cytokinin metabolism and signalling genes. *J Exp Bot* 64:5021–5032
- Chapman K, Ivanovici A, Taleski M, Sturrock CJ, Ng JLP, Mohd-Radzman NA, Frugier F, Bennett MJ, Mathesius U, Djordjevic MA (2020) CEP receptor signalling controls root system architecture in *Arabidopsis* and *Medicago*. *New Phytol* 226:1809–1821
- Chen QJ, Deng BH, Gao J, Zhao ZY, Chen ZL, Song SR, Wang L, Zhao LP, Xu WP, Zhang CX, Ma C, Wang SP (2020) A mirna-encoded small peptide, vvi-mipep171d1, regulates adventitious root formation. *Plant Physiol* 183:656–670
- Collier MD, Huitson S, Hanke DE (2000) Altering n nutrition changes the cytokinin content of leaves of woody plants. *Phyton-Ann Rei Bot A* 40:13–16
- Cortleven A, Leuendorf JE, Frank M, Pezzetta D, Bolt S, Schmulling T (2019) Cytokinin action in response to abiotic and biotic stresses in plants. *Plant, Cell Environ* 42:998–1018
- Dello Ioio R, Linhares FS, Scacchi E, Casamitjana-Martinez E, Heidstra R, Costantino P, Sabatini S (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr Biol* 17:678–682
- Galichet A, Hoyerova K, Kaminek M, Gruissem W (2008) Farnesylation directs atipt3 subcellular localization and modulates cytokinin biosynthesis in *Arabidopsis*. *Plant Physiol* 146:1155–1164
- Gao SP, Fang J, Xu F, Wang W, Sun XH, Chu JF, Cai BD, Feng YQ, Chu CC (2014) CYTOKININ OXIDASE/DEHYDROGENASE4 integrates cytokinin and auxin signaling to control rice crown root formation. *Plant Physiol* 165(3):1035–1046
- Gujjar RS, Supaibulwatana K (2019) The mode of cytokinin functions assisting plant adaptations to osmotic stresses. *Plants-Basel* 8:542
- Haberer G, Kieber JJ (2002) Cytokinins. new insights into a classic phytohormone. *Plant Physiol* 128:354–362
- Hernandes C, Miguita L, de Sales RO, Silva ED, de Mendonca POR, da Silva BL, Klingbeil MDG, Mathor MB, Rangel EB, Marti LC, Coppede JD, Nunes FD, Pereira AMS, Severino P (2020) Anticancer activities of the quinone-methide triterpenes maytenin and 22-beta-hydroxymaytenin obtained from cultivated maytenus ilicifolia roots associated with down-regulation of mirna-27a and mir-20a/mir-17-5p. *Molecules* 25:760
- Higuchi M, Pischke MS, Mahonen AP, Miyawaki K, Hashimoto Y, Seki M, Kobayashi M, Shinozaki K, Kato T, Tabata S, Helaruttu Y, Sussman MR, Kakimoto T (2004) In planta functions of the *Arabidopsis* cytokinin receptor family. *P Natl Acad Sci USA* 101:8821–8826
- Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. *Plant Soil* 321:153–187
- Horak J, Grefen C, Berendzen KW, Hahn A, Stierhof YD, Stadelhofer B, Stahl M, Koncz C, Harter K (2008) The *Arabidopsis thaliana* response regulator arr22 is a putative ahp phospho-histidine phosphatase expressed in the chalaza of developing seeds. *Bmc Plant Biol* 8:77
- Hussain A, Shah ST, Rahman H, Irshad M, Iqbal A (2015) Effect of IAA on in vitro growth and colonization of nostoc in plant roots. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2015.00046>
- Ishikawa K, Terada K, Suzuki T, Yamada H, Yamashino T, Mizuno T (2002) His-to-asp phosphorelay and cytokinin signaling: [i] ahk2/3/4 his-kinase are cytokinin-binding receptors. *Plant Cell Physiol* 43:S107–S107
- Jeon J, Kim J (2013) *Arabidopsis* response regulator1 and *Arabidopsis* histidine phosphotransfer protein2 (ahp2), ahp3, and ahp5 function in cold signaling. *Plant Physiol* 161:408–424
- Jeon J, Kim NY, Kim S, Kang NY, Novak O, Ku SJ, Cho C, Lee DJ, Lee EJ, Strnad M, Kim J (2010) A subset of cytokinin two-component signaling system plays a role in cold temperature stress response in *Arabidopsis*. *J Biol Chem* 285:23369–23384
- Jeon J, Cho C, Lee MR, Binh NV, Kim J (2016) Cytokinin response factor2 (crf2) and crf3 regulate lateral root development in response to cold stress in *Arabidopsis*. *Plant Cell* 28:1828–1843
- Jones RJ, Schreiber BMN (1997) Role and function of cytokinin oxidase in plants. *Plant Growth Regul* 23:123–134
- Kakimoto T (2003) Biosynthesis of cytokinins. *J Plant Res* 116:233–239
- Kurepa J, Shull TE, Smalle JA (2018) Cytokinin-induced growth in the duckweeds *Lemna gibba* and *Spirodela polyrhiza*. *Plant Growth Regul* 86:477–486
- Lakehal A, Chaabouni S, Cavel E, Le Hir R, Ranjan A, Raneshan Z, Novak O, Pacurar DI, Perrone I, Jobert F, Gutierrez L, Bako L, Bellini C (2019) A molecular framework for the control of adventitious rooting by tir1/afb2-aux/iaa-dependent auxin signaling in *Arabidopsis*. *Mol Plant* 12:1499–1514
- Li X, Mo XR, Shou HX, Wu P (2006) Cytokinin-mediated cell cycling arrest of pericycle founder cells in lateral root initiation of *Arabidopsis*. *Plant Cell Physiol* 47:1112–1123
- Li PX, Yu QZ, Gu X, Xu CM, Qi SL, Wang H, Zhong FL, Baskin TI, Rahman A, Wu S (2018) Construction of a functional casparian strip in non-endodermal lineages is orchestrated by two parallel signaling systems in *Arabidopsis thaliana*. *Curr Biol* 28:2777–2786
- Li TT, Kang XK, Lei W, Yao XH, Zou LJ, Zhang DW, Lin HH (2020) Shy2 as a node in the regulation of root meristem development by auxin, brassinosteroids, and cytokinin. *J Integr Plant Biol* 62(10):1500–1517
- Lin C, Sauter M (2020) Control of root system architecture by phytohormones and environmental signals in rice. *Isr J Plant Sci* 67:98–109
- Lv XM, Zhang YX, Hu L, Zhang Y, Zhang B, Xia HY, Du WY, Fan SJ, Kong LA (2020) Low-nitrogen stress stimulates lateral root initiation and nitrogen assimilation in wheat: Roles of phytohormone signaling. *J Plant Growth Regul* 40(1):436–450
- Ma Y, Cao J, He J, Chen Q, Li X, Yang Y (2018) Molecular mechanism for the regulation of aba homeostasis during plant development and stress responses. *Int J Mol Sci* 19:3643
- Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124:33–44
- Martinez-Arias C, Sobrino-Plata J, Macaya-Sanz D, Aguirre NM, Collada C, Gil L, Martin JA, Rodriguez-Calcerrada J (2020) Changes in plant function and root microbiome caused by flood and drought in a riparian tree. *Tree Physiol* 40:886–903

- Miyawaki K, Tarkowski P, Matsumoto-Kitano M, Kato T, Sato S, Tarkowska D, Tabata S, Sandberg G, Kakimoto T (2006) Roles of *Arabidopsis* atp/adp isopentenyl transferases and trna isopentenyl transferases in cytokinin biosynthesis. *PNAS* 103:16598–16603
- Mok DWS, Mok MC (2001) Cytokinin metabolism and action. *Annu Rev Plant Phys* 52:89–118
- Naulin PA, Armijo GI, Vega AS, Tamayo KP, Gras DE, de la Cruz J, Gutierrez RA (2020) Nitrate induction of primary root growth requires cytokinin signaling in *Arabidopsis thaliana*. *Plant Cell Physiol* 61:342–352
- Nguyen TN, Tuan PA, Mukherjee S, Son SH, Ayele BT (2018) Hormonal regulation in adventitious roots and during their emergence under waterlogged conditions in wheat. *J Exp Bot* 69:4065–4082
- Nishimura C, Ohashi Y, Sato S, Kato T, Tabata S, Ueguchi C (2004) Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in *Arabidopsis*. *Plant Cell* 16:1365–1377
- Pas J, von Grotthuss M, Wyrwicz LS, Rychlewski L, Barcizewski J (2004) Structure prediction, evolution and ligand interaction of chase domain. *FEBS Lett* 576:287–290
- Ramireddy E, Hosseini SA, Eggert K, Gillandt S, Gnad H, von Wiren N, Schmulling T (2018) Root engineering in barley: Increasing cytokinin degradation produces a larger root system, mineral enrichment in the shoot and improved drought tolerance. *Plant Physiol* 177:1078–1095
- Riefler M, Novak O, Strnad M, Schmulling T (2006) *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell* 18:40–54
- Rowe JH, Topping JF, Liu J, Lindsey K (2016) Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *New Phytol* 211:225–239
- Ryu H, Cho YG (2015) Plant hormones in salt stress tolerance. *J Plant Biol* 58:147–155
- Sakakibara H, Takei K (2002) Identification of cytokinin biosynthesis genes in *Arabidopsis*: A breakthrough for understanding the metabolic pathway and the regulation in higher plants. *J Plant Growth Regul* 21:17–23
- Sakamoto T, Sotta N, Suzuki T, Fujiwara T, Matsunaga S (2019) The 26S proteasome is required for the maintenance of root apical meristem by modulating auxin and cytokinin responses under high-boron stress. *Front Plant Sci* 10:590
- Shkolnik-Inbar D, Bar-Zvi D (2010) *Abi4* mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in *Arabidopsis*. *Plant Cell* 22:3560–3573
- Soares A, Niedermaier S, Faro R, Loos A, Manadas B, Faro C, Huesgen PF, Cheung AY, Simoes I (2019) An atypical aspartic protease modulates lateral root development in *Arabidopsis thaliana*. *J Exp Bot* 70:2157–2171
- Sun BB, Chen LQ, Liu JC, Zhang XN, Yang ZN, Liu W, Xu L (2016) Taa family contributes to auxin production during de novo regeneration of adventitious roots from *Arabidopsis* leaf explants. *Sci Bull* 61:1728–1731
- Sun XC, Chen FJ, Yuan LX, Mi GH (2020) The physiological mechanism underlying root elongation in response to nitrogen deficiency in crop plants. *Planta* 251:84
- Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T, Sakakibara H (2004) *Atipt3* is a key determinant of nitrate-dependent cytokinin biosynthesis in *Arabidopsis*. *Plant Cell Physiol* 45:1053–1062
- Tokunaga H, Kojima M, Kuroha T, Ishida T, Sugimoto K, Kiba T, Sakakibara H (2012) *Arabidopsis* lonely guy (*log*) multiple mutants reveal a central role of the *log*-dependent pathway in cytokinin activation. *Plant J* 69:355–365
- Trifunović-Momčilov M, Paunović D, Milošević S, Marković M, Jevremović S, Dragičević IČ, Subotić A (2020) Salinity stress response of non-transformed and *AtCKX* transgenic centaury (*Centaurea erythraea* Rafn.) shoots and roots grown *in vitro*. *Ann Appl Biol* 177:74–89
- Waidmann S, Rosquete MR, Scholler M, Sarkel E, Lindner H, LaRue T, Petrik I, Dunser K, Martopawiro S, Sasidharan R, Novak O, Wabnik K, Dinnyen JR, Kleine-Vehn J (2019) Cytokinin functions as an asymmetric and anti-gravitropic signal in lateral roots. *Nat Commun* 10:1–14
- Wang XL, Wang JJ, Sun RH, Hou XG, Zhao W, Shi J, Zhang YF, Qi L, Li XL, Dong PH, Zhang LX, Xu GW, Gan HB (2016) Correlation of the corn compensatory growth mechanism after post-drought rewatering with cytokinin induced by root nitrate absorption. *Agr Water Manage* 166:77–85
- Wang XL, Qin RR, Sun RH, Hou XG, Qi L, Shi J (2018a) Effects of plant population density and root-induced cytokinin on the corn compensatory growth during post-drought rewatering. *PLoS ONE* 13:e0198878
- Wang XL, Qin RR, Sun RH, Wang JJ, Hou XG, Qi L, Shi J, Li XL, Zhang YF, Dong PH, Zhang LX, Qin DH (2018b) No post-drought compensatory growth of corns with root cutting based on cytokinin induced by roots. *Agr Water Manage* 205:9–20
- Wang HZ, Leng X, Xu XM, Li CH (2020a) Comprehensive analysis of the *tify* gene family and its expression profiles under phytohormone treatment and abiotic stresses in roots of *Populus trichocarpa*. *Forests* 11:315
- Wang Y, Lu JW, Ren T, Li PF, Liu QX, Li XK (2020b) Effects of exogenous cytokinin on photosynthesis, senescence, and yield performance of inferior rice tillers grown under different nitrogen regimes. *Photosynthetica* 58:137–145
- Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H, Schmulling T (2003) Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* 15:2532–2550
- Wu J, Zhang N, Liu ZG, Liu SY, Liu CX, Lin JH, Yang H, Li S, Yukawa Y (2020) The *atgstu7* gene influences glutathione-dependent seed germination under *aba* and osmotic stress in *Arabidopsis*. *Biochem Biophys Res Commun* 528:538–544
- Xia JC, Zhao H, Liu WZ, Li LG, He YK (2009) Role of cytokinin and salicylic acid in plant growth at low temperatures. *Plant Growth Regul* 57:211–221
- Xu Y, Burgess P, Zhang X, Huang B (2016) Enhancing cytokinin synthesis by overexpressing *ipt* alleviated drought inhibition of root growth through activating *ros*-scavenging systems in *Agrostis stolonifera*. *J Exp Bot* 67:1979–1992
- Xun QQ, Wu YZ, Li H, Chang JK, Ou Y, He K, Gou XP, Tax FE, Li J (2020) Two receptor-like protein kinases, *mustaches* and *mustaches-like*, regulate lateral root development in *Arabidopsis thaliana*. *New Phytol* 227:1157–1173
- Ye BB, Shang GD, Pan Y, Xu ZG, Zhou CM, Mao YB, Bao N, Sun LJ, Xu TD, Wang JW (2020) *Ap2/erf* transcription factors integrate age and wound signals for root regeneration. *Plant Cell* 32:226–241
- Yokoyama A, Yamashino T, Amano YI, Tajima Y, Imamura A, Sakakibara H, Mizuno T (2007) Type-b *arr* transcription factors, *arr10* and *arr12*, are implicated in cytokinin-mediated regulation of protoxylem differentiation in roots of *Arabidopsis thaliana*. *Plant Cell Physiol* 48:84–96
- Yu YH, Wang P, Bai YC, Wang Y, Wan HN, Liu C, Ni ZY (2020) The soybean *f-box* protein *gmfbx176* regulates *aba*-mediated responses to drought and salt stress. *Environ Exp Bot* 176
- Zhang KR, Diederich L, John PCL (2005) The cytokinin requirement for cell division in cultured *Nicotiana plumbaginifolia* cells can be satisfied by yeast *cdc25* protein tyrosine phosphatase. Implications

- for mechanisms of cytokinin response and plant development. *Plant Physiol* 137:308–316
- Zhang W, Swarup R, Bennett M, Schaller GE, Kieber JJ (2013) Cytokinin induces cell division in the quiescent center of the *Arabidopsis* root apical meristem. *Curr Biol* 23:1979–1989
- Zhou J, Li Z, Xiao G, Zhai M, Pan X, Huang R, Zhang H (2020a) Cyp71d81 is a key regulator involved in growth and stress responses by mediating gibberellin homeostasis in rice. *J Exp Bot* 71:1160–1170
- Zhou YY, Zhang Y, Wang XW, Han X, An Y, Lin SW, Shen C, Wen JL, Liu C, Yin WL, Xia XL (2020b) Root-specific nf-y family transcription factor, pdnf-yb21, positively regulates root growth and drought resistance by abscisic acid-mediated indoleacetic acid transport in *Populus*. *New Phytol* 227:407–426
- Zurcher E, Muller B (2016) Cytokinin synthesis, signaling, and function—advances and new insights. *Int Rev Cel Mol Bio* 324:1–38

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