Advances and the Application Value of Plant Copper Transporters

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Abstract Copper pollution in soil leads to poor crop quality and low yield, which is a major problem in agricultural production. Plant copper transporters have functions of copper absorption, chelation, compartmentation and metabolic utilization, and participate in the regulation of copper homeostasis in plant. This review presumptively summarizes the gene expression characteristics, subcellular localization and the effecting of knockout or overexpression genes of plant copper transporters; emphatically illuminates the roles of plant copper transporters in copper uptake and root retention, long-distance transport, intracellular compartmentalization and accumulation in reproductive organs; and put forwards the research direction and prospect. The aim is to provide scientific theoretical basis for the screening and cultivation of copper-tolerant crops in order to prevent the potential threat of food safety in agricultural land with slight copper contamination, and help to establish efficient crop breeding mechanism and reasonable planting measures.

Keywords Plant; Copper contamination; Copper transporter

With the extensive use of pesticides, fungicides and wastewater irrigation in agriculture, copper contamination has become a major problem in sustainable agricultural production (Muhammad et al., 2015; Zhu et al., 2018). A survey in 2014 showed that the exceeding standard rate of copper contamination in China reached 2.1% (Ministry of Environmental Protection, 2014). Copper pollution has high persistence and inhibits plant growth, resulting in crop yield reduction (Huang et al., 2016). Plants have a complex copper steady-state network, regulating the absorption and distribution of copper (Boutigny et al., 2014), and maintaining the normal growth of plants in a certain range of copper concentration soil. Plant accumulation and interaction with growth environment can be used to improve soil copper pollution. Studies have shown that plants tend to retain excessive copper in roots and stems, but not accumulate too much in seeds. Therefore, using crops to improve slight copper-contaminated soil is a feasible way to improve the harmful effects of copper pollution and meet the food demands of the intensively increasing population (Muhammad et al., 2015). However, high copper accumulation in food and feed edible parts poses a threat to human and animal health (Kulhari et al., 2013). Food safety must be guaranteed. Therefore, it is necessary to understand the absorption mechanism of copper by plants, as well as to understand the distribution characteristics of copper in plants, and to achieve the dual purpose of reasonable improvement of copper-contaminated soil and food production safety.

In recent years, there have been a lot of reports on the toxicity level, physiological and biochemical effects, and damage mechanism of copper to plants. The tolerance of plants to copper toxicity is closely related to the absorption and transfer of copper. As an important part of plant copper homeostasis regulation system, plant copper transporters have functions of copper absorption, chelation, compartmentation, and metabolic utilization, and are key factors affecting copper stress tolerance, copper removal efficiency and copper distribution in plants. Plant copper transporters have been paid more and more attention, but there is a lack of systematic research reports. This review summarizes latest research progress of plant copper transporters, which provides a theoretical basis for deepening the understanding of plant copper homeostasis and improving crop breeding of copper-contaminated soil.

1 Characteristics of Plant Copper Transporters

Plant transporters with the ability of copper transporter include plant copper transporter (COPT), heavy metal transporting ATPase (HMA), yellow stripe like (YSL), Zinc/Iron regulated transporters (ZIP) and other family proteins. COPT has abundant functions, such as participating in copper uptake, copper distribution in overground tissues, re-circulation of intracellular copper storage and pollen development (Sancenón et al., 2004; Dumay et al., 2006; Carrio-Segui et al., 2015). The COPT characteristics related to copper transport have been proved as follows. Studies have shown that the expression of copper transporter gene has tissue specificity and copper induction characteristics. In general, these copper transporters are expressed in different plant tissues, such as *AtCOPT1/2/5* and *AtYSL2* in *Arabidopsis thaliana*. Some transporters have high tissue specificity. Among the studied copper transporters, most of the COPT family proteins are expressed in roots and reproductive tissues. For example, *AtCOPT1/5*, *MtCOPT1/4/5/*, *VvCTr1*, *TaCT1* are expressed in roots. And *MtCOPT3* has nodule specificity. *AtCOPT1/3/5/6* are expressed in reproductive tissues, *AtCOPT1/5/6* are particularly abundant in pollen, *AtCOPT5* is expressed in nuts, *TaCT1* is expressed in spikelets. And a small amount of *COPT* such as *AtCOPT6*, *MtCOPT8* and *VvCTr1* are expressed in stems and leaves (Table 1; Table 2; Table 3).

Table 1 The characteristics of known COPT associated with copper transport in plant

Species	Gene	Express parts	Induced expression	Subcellular localization	Reference
Arabidopisis thaliana	*AtCOPT1*	Most tissues, roots, pollen, reproductive tissue	High copper down	Plasma membrane	Amparo et al., 2018 Senovilla et al., 2018
	AtCOPT2	Most tissues	High copper down	Plasma membrane	Amparo et al., 2018 Senovilla et al., 2018 Garcia-Molina et al., 2013
	AtCOPT3	Reproductive tissue	-	Chamber of secretory pathway	Amparo et al., 2018
	AtCOPT5	Most tissues, roots, reproductive tissues, pollen, and nuts	-	Vacuole	Amparo et al., 2018 Garcia-Molina et al., 2013 Klaumann et al., 2011
	AtCOPT6	Reproductive tissue, xylem and phloem vascular tissue	High copper up	Plasma membrane	Amparo et al., 2018; Garcia-Molina et al., 2013 Jung et al., 2012
Brassica napus	*BnCOPT2*	Roots	Up in copper deficient roots	-	Billard et al., 2014
Medicago truncatula	*MtCOPT1*	Roots	-	Plasma membrane	Senovilla et al., 2018
	MtCOPT3	Nodules	-	-	Senovilla et al., 2018
	MtCOPT4/5	Roots	-	-	Senovilla et al., 2018
	MtCOPT8	Roots, xylem and phloem vascular tissue	-	-	Senovilla et al., 2018
Triticum aestivum	*TaCT1*	Xylem and phloem vascular tissue, roots, spikelets	High copper down	Golgi apparatus	Li et al., 2013
Vitis vinifera	*VvCTr1*	Xylem and phloem vascular tissue, leaf, roots	Deficient copper up	Tonoplast	Leng et al., 2015
	VvCTr2/8	-	High copper up	-	Martins et al., 2014

HMA drives the transmembrane transport of heavy metal ions by hydrolyzing ATP, which is responsible for copper efflux, transport (Mills et al., 2005). *HvHMA1*, *GmHMA8*, and *AtHMA5* are expressed in roots and flower, *HvHMA1* is expressed in seeds (Table 2).

Table 2 The characteristics of known HMA associated with copper transport in plant

Species	Gene	Express parts	Induced expression	Subcellular localization	Reference
Arabidopisis thaliana	*AtHMA1*	Green organization	-	Chloroplast envelope	Boutigny et al., 2014
	AtHMA5	Roots, flower	High copper up	Plasma membrane	Patterson et al., 2006
	AtHMA6	Roots, shoots	-	Chloroplast	Catty et al., 2011
	AtHMA8	Overground part	High copper degradation	Thylakoid membrane	Gayomba et al., 2013 Zhang et al., 2018 Tapken et al.,2012
Oryza sativa	*OsHMA5*	Root column sheath cell, the xylem area of the vascular bundle at the node, pedicel, petiole, vascular tissue of fruit shell	High copper up	Plasma membrane	Patterson et al., 2006 Deng et al., 2013
	OsHMA9	Xylem and phloem vascular tissue	High copper up	Plasma membrane	Lee et al., 2007 Patterson et al., 2006 Deng et al., 2013
Brassica napus	*BnHMA1*	Leaf	Up in copper deficient leaf	-	Billard et al., 2014
Glycine max	*GmHMA8*	Leaf	-	Thylakoid membrane	Bernal et al., 2007
Hordeum vulgare	*HvHMA1*	Leaf, seeds	High copper down	Chloroplast envelope, the cell cavity of grain aleurone	Mikkelsen et al., 2012
Vitis vinifera	*VvPAA1*	-	High copper down	-	Martins et al., 2014

YSL transports copper ions chelated by nicotinamide (NA) and plays a role in long-distance transport of copper (Waters et al., 2006). *AtYSL2/3* and *HvYSL2* are expressed in roots, stems and leaves (Table 3).

Table 3 The characteristics of known YSL associated with copper transport in plant

Species	Gene	Express parts	Induced expression	Subcellular localization	Reference
Arabidopisis thaliana	*AtYSL1*	Most tissues, roots	High copper down	Plasma membrane	Waters et al., 2006 Chu et al., 2010
	AtYSL2	Most tissues, roots, stems	High copper down	Plasma membrane, periphery of vessel parenchyma cell	Didonato et al., 2004 Garcia-Molina et al., 2013
	AtYSL3	Young leaves, stems, roots	High copper down	Plasma membrane	Waters et al., 2006 Chu et al., 2010 Amparo et al., 2018
Oryza sativa	*OsYSL16*	Roots, stems, phloem and vascular tissue of leaves	Up in copper deficient stems	Plasma membrane	Zheng et al., 2012
Arachis hypogaea	*AhYSL3.1*	Roots, stems, young leaves, and old leaf	High copper up; up in copper deficient roots	Plasma membrane	Dai et al., 2018
	AhYSL 3.2	Roots, stems, young leaves, old leaf, lateral root, taproot	Up in copper deficient roots	—	Dai et al., 2018
Hordeum vulgare	*HvYSL2*	Stems, young leaves, root endoderm	Up in copper deficient leaf	—	Araki et al., 2011

Tissue expression of copper transporters reflects their functional regions. COPT family proteins expressed in roots are closely related to copper absorption. YSL family proteins play a role in copper absorption and long-distance transport. COPT and HMA family proteins play an important role in reproductive tissues. And ZIP also participates in the copper homeostasis and transport of roots (Stephens et al., 2011).

The expression of these family proteins is significantly affected by copper status in plants. Under copper deficiency, the expression of *AhYSL3.1/3.2* and *BnCOPT2* in roots, *OsZIP12/4* and *VvZIP4* in roots and stems, and *BnHMA1* and *HvYSL2* in leaves is up-regulated. While the expression of *AtCOPT1/2*, *TaCT1*, *AtYSL2*, *VvZIP2*, *VvPAA1*, *MtZIP1/5/6* and *HvHMA1* is down-regulated, and *AtHMA8* is degraded under high copper. High copper induced the expression of *AtHMA5*, *VvCTr1/2/8*, *VvZIP4*, *AhYSL3.1*, *OsHMA5/9* up. The up-regulated genes in roots under copper deficiency can improve the ability of copper absorption, and the up-regulated genes in stems and leaves may mobilize copper in plants to transport copper from the storage part to the parts where life activities are more needed, to compensate for the possible loss of activity and reduce the effects of copper deficiency. When copper is high, most of the copper transport genes are down-regulated, indicating that the feedback inhibition of copper absorption, while the up-regulated transport genes such as *HMA* may transfer and store excess copper to achieve the purpose of detoxification. The expression of copper transporter gene is regulated by regulatory network. Studies have shown that plant *SPL7* transcription factor is a key regulator of copper response genes. In Arabidopsis *spl7* mutants, transporter genes such as *COPT1*, *COPT2*, *ZIP2*, YSL2, FRO3, as well as some transcription factors are mistakenly regulated. The *spl7* mutant showed strong growth phenotype in low copper environment (Gayomba et al., 2013). OsATX1 interacts with heavy metals P1B-ATPases HMA4, HMA5, HMA6 and HMA9 in rice (*Oryza sativa*). OsATX1 may transport and distribute copper by transferring copper to HMA, so as to maintain copper homeostasis in different rice tissues (Zhang et al., 2018).

1.1 Effects of knockout or overexpression of copper transporters on plants

The overexpression of heterologous copper transporters can improve the ability of the copper absorption in plants (Singh et al., 2011), and affect the copper homeostasis of plants. Plants overexpressing *COPT1* or *COPT3* showed increased endogenous copper levels, but the expression and survival of circadian-related genes is compromised (Andrés-Colás et al., 2010). The roots of rice overexpressing *AtCOPT1* were shortened under high copper and iron deficiency conditions, and the iron homeostasis was significantly affected (Andrés-Bordería et al., 2017). The root copper concentration of *OsATX1* overexpression rice decreased, but the copper accumulation in reproductive tissues and old leaves increased (Singleton and Brun, 2007). Under the condition of copper deficiency, the root growth damage of *Atcopt5* mutant seedlings stably transformed by *VvCTr1* was completely repaired, which supported the view that VvCTr1 was involved in intracellular copper transport (Martins et al., 2014). COPT5 played a certain role in the transfer of copper from roots to other tissues, and the root copper concentration of *AtCOPT5* insertion mutant decreased (Carrio-Segui et al., 2015). *OsHMA9-1* and *OsHMA9-2* mutants showed increased sensitivity to elevated copper levels, supporting the view that *OsHMA9* plays a role in cell copper efflux (Lee et al., 2007). Above results confirm that these transporters are of great significance for copper homeostasis and other metal homeostasis in plants.

1.2 Subcellular localization of copper transporter

As transporters, COPT, HMA, YSL, ZIP and other copper transporters are mostly located in the membrane system of cells. Some COPT proteins are active in the plasma membrane, others are active in the intracellular membrane, such as the tonoplast, which is involved in copper entry and exit. AtCOPT2/6, MtCOPT1, OsHMA5, AtYSL2, AhYSL3.1, OsZIP1 are in plasma membrane. AtHMA6 is in chloroplast, AtHMA1 and HvHMA1 are in chloroplast envelope, AtHMA8 and GmHMA8 are in thylakoid membrane with small clusters and uneven distribution. AtCOPT5 is in copper storage organelle-vacuole, VvCTr1 trans-Golgi network is in the tonoplast through the anterior vacuole cavity, TaCT1 is in the Golgi apparatus. OsZIP1 is in endoplasmic reticulum. HvHMA1 is in the cell cavity of grain aleurone. AtCOPT3 is in a chamber of secretory pathway, and AtYSL2 is in the periphery of the highly vacuolar vessel parenchyma cell, which is difficult to distinguish the specific location on the two layers of the membrane. Subcellular localization is an important verification part of protein function. According to these localizations, copper transporters are in the plasma membrane or important organelle membrane and play an important role in intracellular copper exchange.

2 Plant Copper Transporter and Copper Absorption and Transfer

2.1 Role of copper transporters in root copper absorption and retention

Plants rely on their roots to get copper from the soil. Sunflower (*Helianthus annuus*) (Yeh et al., 2015), radish (*Raphanus sativus*) (Hladun et al., 2015) and perennial peanut (*Arachis hypogaea*) have high copper bioaccumulation capacity. High affinity COPT could mediate copper absorption in roots. And AtCOPT1/2 and ZmCOPT1/2 could absorb Cu^+. While *Atcopt1* inhibited copper absorption (Sancenón et al., 2004). COPT2 played a role in obtaining and distributing copper in the case of copper deficiency. Rice OsCOPT2/3/4 mediated high affinity copper uptake with OsCOPT6, while OsCOPT7 exerted copper uptake function alone (Yuan et al., 2011). ZmCOPT3 had low affinity copper (Wang et al., 2018). COPT protein transported Cu^+, but the most available form of copper in soil was Cu^{2+}. Therefore, copper reduction may promote the absorption of root cells. FRO protein in *Arabidopsis thaliana* absorbs reduced soil iron in plant roots. And copper deficiency also induced the expression of FRO3. FRO may also participate in the reduction and absorption of copper. It was also found that AtYSL2 in *Arabidopsis thalian* uptake copper chelated by NA in roots (Didonato et al., 2004). The copper absorption efficiency of plants depends on soil characteristics and copper bioavailability, as well as these transporters on the plasma membrane of plants.

After plants were given high copper, the copper content in roots increased significantly, but the copper content in stems did not increase at the early stage of stress, which indicates that copper is mainly accumulated underground after absorption (Desjardins et al., 2016). The results showed that maize (*Zea mays*) (Kacálková et al., 2009), rice (*Oryza sativa*) (Thounaojam et al., 2012), wheat (*Triticum aestivum*) (Mahmud et al., 2013), perennial ryegrass (*Lolium perenne* L.) (Chen et al., 2017), alfalfa (*Medicago sativa*) (Wang et al., 2006), ramie (*Boehmeria nivea* L.). (Rehman et al., 2019) and mustard (*Brassica juncea*) (Motior et al., 2013) had the ability of Cu accumulation in roots. Leaf is a photosynthetic tissue with high metabolic activity, which prevents the transfer of copper to leaves. It is an effective copper tolerance mechanism, which is related to gene expression and root secretion. Apple (*Malus pumila*) with different copper fixation ability showed transcriptional specificity of copper absorption and transport genes. Apple rootstocks with strong copper fixation ability retained more copper in the root cell wall and subcellular partition (Wan et al., 2019) by regulating the expression of copper transport genes. The copper tolerance of alfalfa was related to the secretion of organic acids and cell wall structure. The increase of copper concentration had little effect on the biomass of roots, stems and leaves of alfalfa. The increase of organic acid content in roots and cation exchange capacity of cell wall prevented the transport of copper to the overground part, and effectively protected the overground tissue (Wang et al., 2006).

2.2 Plant copper transporters participate in long-distance copper transport *in vivo*

The plant stem plays a central role in transferring and distributing minerals, water and other solutes (Printz et al., 2013). In order to maintain the copper content required for participating in life activities, copper should be transferred from roots to stems. Before entering the xylem, copper should be discharged from the root symbionts, transported to the xylem through parenchyma and endodermal cells, and brought to the mature leaves through transpiration, loaded to the phloem, and reached the new leaves, flowers and seeds. Studies have shown that the copper concentration in the overground part of some plants is higher than that in the underground part, for example, the copper concentration in sunflower seedlings is 2~4 times higher than that in roots (Rahman et al., 2013). The copper content and accumulation in the overground of rice seedlings increased with the increase of copper supply, but the that in root did not increase with exogenous copper (Si et al., 2007). *Helianthus annuus*, *Raphanus sativus* and *Apium graveolens* have higher ability to transfer copper from roots to stems (Yeh et al., 2015; Hladun et al., 2015; Li et al., 2019). There are great differences in rhizome transfer ability of copper in different species or in the same species. The potential of copper absorption and copper transport in lettuce (*Lactuca sativa* L.) is higher than that in spinach (*Spinacia oleracea* L.). And the copper of lettuce root is higher than that of stem, 60%~80% of copper is stored in roots, but there is no significant difference in copper in spinach roots to stems (Ayari et al., 2010). In another study, the Cu^{2+} accumulation in roots of spinach seedlings was lower than that in overground parts (Gong et al., 2019). The results showed that there was more copper accumulation in the roots of *Boehmeria nivea*, followed by leaves and stems, but the copper accumulation in stems was the highest in *Diyarbakır* (Cevher-Keskin et al., 2018).

Consistent with the role of xylem loading, the derivation of copper from cells is necessary for high copper tolerance and long-distance transport. HMA5 plays an important role in copper compartmentation and copper detoxification in roots. AtHMA5, OsHMA5 and OsHMA9 may be involved in copper efflux. Mutant studies verified its copper transport capacity from roots to stems. *AtHMA5* knockout or conserved region mutations resulted in increased sensitivity to high copper and copper accumulation in rhizomes, while mutants showed copper sensitivity and decreased copper transport capacity (Kobayashi et al., 2008). Knockout of *OsHMA5* resulted in the decrease of copper content in xylem sap of rice mutant, while the increase of copper concentration in root (Tapken et al., 2012). The root tip of *OsHMA9* mutant accumulated copper (Lee et al., 2007). Despite the above experimental evidence, the specific mechanism of copper ion transport in xylem and phloem is not clear, and there is a lack of direct evidence. YSL transports copper ions chelated by nicotinamide (NA) with high affinity in xylem and phloem. Tomato NA accumulated too much copper due to lack of mutants, and the copper transport in xylem was greatly reduced. The addition of exogenous NA could restore the copper level in xylem and support the role of NA in xylem copper transport. AtYSL1/2/3 (Didonato et al., 2004; Amparo et al., 2018), HvYSL2 (Araki et al., 2011) could transport Cu^{2+}-NA. OsYSL16 transported Cu-NA to young tissue through phloem and knocked out *OsYSL16*. The copper concentration in old leaves was higher than that in young leaves (Zheng et al., 2012). When copper was excessive, the high expression of peanut *AhYSL3.1* gene led to the decrease of copper level in young leaves of tobacco and rice (Dai et al., 2018). *Medicago sativa* has a potential remediation effect on copper-contaminated soil (Zhu et al., 2018). In this study, *MsYSL6* gene was cloned from *Medicago sativa.* After soaking seeds for 7 d under 6 μM copper stress, it was found that the average germination rate of tobacco seeds of *MsYSL6* overexpression was 44% higher than that of wild type and showed better growth state. After 15 d, it was found that the average root length was 43% longer than that of wild type, and the average fresh weight was more than 39%. It is suggested that *MsYSL6* may be involved in copper transport in plants and can improve the tolerance of plants to copper stress, and its specific transport mechanism needs to be further studied.

Copper is not easily redistributed from old leaves to young leaves and meristem. Therefore, young leaves and stems are more susceptible to nutritional deficiency than mature leaves. The copper accumulation in the old leaves of *Brassica napus* was higher than that in young leaves (Khurana et al., 2006). However, studies also showed that copper deficiency reduced the growth rate of *Brassica napus* by less than 19%, while the copper content in the old leaves decreased by 61.4%, indicating that there was a copper reflow process between leaves.

2.3 Copper transporters and intracellular compartmentalization
After entering the leaf cells, copper ions are distributed in different organelles compartmentally. Copper is mainly distributed in the cell wall and cytosol in maize cells, and the copper content in the organelles such as nucleus, chloroplast and mitochondria are low. With the increase of copper concentration, the distribution of copper to cell wall decreased and to cytosol increased. The copper content was the highest in the cytosol of stem, followed by the cell wall (Si et al., 2008), and the copper content in the cell wall of leaf was the highest. Black dots distributed in vacuoles and cell walls of spinach leaves under high copper stress, which might be Cu^{2+} accumulation (Cevher-Keskin et al., 2018). Copper is distributed in vacuoles and cytoplasm containing a large number of chelates, which can reduce toxicity. On the other hand, vacuoles may be a transport pathway in cells. After metal ions are transported to the cytoplasm through vacuoles, they may encounter copper transporters only at a short distance of diffusion.

At high copper concentration, part of copper is transferred to the stroma Cu/Zn superoxide dismutase mediated by AtHMA6 and copper chaperone protein CCS, and part of copper is transferred to AtHMA6(PAA1) on the inner membrane of chloroplast through the copper chaperone protein AtPCH1. AtHMA6 transports copper into the chloroplast stroma through the chloroplast envelope (Catty et al., 2011; Boutigny et al., 2014), and then AtHMA8(PAA2) transports copper to plastocyanin in the thylakoid lumen for normal photosynthesis (Tapken et al., 2012). Arabidopsis AtHMA1 participates in copper homeostasis in chloroplasts (Boutigny et al., 2014). When cells are copper deficient, HvHMA1 mobilizes copper in chloroplasts to be excreted to the cytoplasm (Mikkelsen

et al., 2012). The interaction between AtHMA5 and copper chaperone ATX1-like plays a role in the compartmentation and detoxification of copper (Zhang et al., 2018). AtHMA5 may obtain copper from cytoplasmic copper chaperone. AtHMA7 transports copper to the Golgi apparatus and transmits copper as a co-factor through the endoplasmic reticulum to ethylene receptor located in the endoplasmic reticulum, which participates in the biological process of ethylene receptor (Chen et al., 2002). AtCOPT5 promotes the efflux of copper from vacuoles when copper is deficient. The copper content of *Atcopt5* knockout vacuole is 1.5～1.8 times higher than that of normal, which showed a strong defect of photosynthetic electron transport of plastocyanin (Klaumann et al., 2011). Under high copper stress, rice *OsZIP1* knockout inhibited rice growth, while overexpression promoted rice growth. The dual localization of endoplasmic reticulum and plasma membrane also suggested that OsZIP1, as a metal efflux protein under excessive copper stress, may play a role in changing environmental conditions (Liu et al., 2019).

2.4 Plant copper transporters and copper accumulation in reproductive organs

Copper is required for plant flowering and seed maturation. Most of the copper in the reproductive tissue of *Arabidopsis thaliana* enters directly from the root through the vessel system, while others may be mobilized by the nutrient tissue vessel system through the phloem. Selective transfer of copper in seeds or grains may be the result of selective absorption of copper by vascular transport cells in plant reproductive tissues. Due to low transport efficiency, crop grains usually maintain normal copper levels (Kubota et al., 1992). Even if copper was added, the increase of copper in maize grains was still lower than that in leaves and stems. However, copper treatment increased the absorption of copper in various parts of radish, copper was evenly distributed and accumulated in roots, stems, leaves, flowers and seeds of radish (Hladun et al., 2015). The concentration of copper in soybean (*Glycine max*) seeds was higher than that in leaves and stems (Reddy et al., 1989). The copper accumulation in edible parts of crops grown on high copper soil needs to be closely monitored. Even for forage crops or ornamental plants, leaves and flowers may be foraging by herbivores and pollinators, so there is still a potential threat.

The copper content in flowers and seeds of *AtYSL2* and *AtYSL3* mutants was lower, but higher in rosette leaves and stems (Didonato et al., 2004). COPT5 transferred Cu from root to reproductive tissue (Klaumann et al., 2011), the pollen of *Atcopt1* inhibition line was dysplastic, and the content of copper in pod and seed decreased (Sancenón et al., 2004). Under the condition of copper deficiency, the copper content in rosette leaves of Atcopt6 mutant increased, and the copper content in seeds decreased. Reintroduction of *AtCOPT6* could save this phenotype (Garcia-Molina et al., 2013; Martins et al., 2014). OsHMA5 is a copper transporter in the xylem at the reproductive stage. The grain copper concentration of *OsHMA5* knockout mutant is significantly lower than that of wild type, and the yield is also very low (Tapken et al., 2012). OsYSL16 transports copper to seeds through the phloem. Knockout of *OsYSL16* resulted in higher copper concentrations in boot leaves and rice husks and lower copper concentrations in grains (Zheng et al., 2012). HvHMA1 in *Hordeum vulgare* might be involved in mobilizing copper from the aleurone cells during grain filling. The down-regulation of *HvHMA1* by RNA interference significantly increased the Cu content in grains (Mikkelsen et al., 2012).

3 Progress

Copper contamination control is a worldwide problem. Cultivation of copper-tolerant plants is one of the methods to alleviate copper contaminated soil. Planting crops in agricultural land with slight copper contamination should pay attention to the potential threat of food safety. The research on plant copper transporters, especially the understanding of their copper distribution mechanism in different parts, can provide scientific theoretical basis and help to establish efficient breeding mechanism and reasonable planting measures. Previous studies have used mutants and overexpression to clarify the physiological functions of some copper transporters, which proved that copper transporters transport copper to specific target organs. Nevertheless, further research is needed to deepen understanding of copper transporters. The following aspects can be carried out. Firstly, the biological characteristics of copper transporters including transporter substrates, expression patterns, and functional sites can be further clarified. Secondly, clarify the functional division of copper transporters and the synergistic overlap

redundancy relationship. Thirdly, clarify the regulatory effect of transcription factors on copper transporters. It is worth noting that copper transport is inseparable from copper chaperone proteins in plants, and transporters such as ABC and MATE may also be involved in copper transport. Cu interacts with other mineral elements, which may seriously affect the copper homeostasis of plants. Referring to the research results of copper transporters in model plants, the mechanism of copper detoxification and tolerance of crop copper transporters was studied by broadening plant research types, making full use of mutant analysis, heterologous expression and omics, and the suitable varieties were screened by copper transporters as indicators. On the basis of in-depth understanding of the mechanism of copper transporters, the use of genetic engineering methods, such as the use of tissue-specific promoters, to achieve the expression of copper transporters in specific parts, but also can inhibit gene expression or gene editing, so as to improve specific traits, change the copper status of plants, block the transfer and accumulation of copper to edible parts, cultivate new crops with high copper tolerance and food safety, which has important application value for the improvement of copper-contaminated farmland.

Authors' contributions

Acknowledgments

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