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Review

The Role of Membrane Transporters in Plant Growth and Development, and Abiotic Stress Tolerance

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Abstract: Membrane transporters (MTs) are mainly localized at the plasma membrane (PM), tonoplast and vacuolar membrane (VM) of cells in all plant organs. Their work is to maintain the cellular homeostasis by controlling ionic movements across PM channels from roots to upper plant parts, xylem loading and remobilization of sugar molecules from photosynthesis tissues in the leaf (source) to roots, stem and seeds (sink) via phloem loading. The plant's whole source-to-sink relationship is regulated by multiple transporting proteins in a highly sophisticated manner and driven based on different stages of plant growth and development (PG&D), and environmental changes. The MTs play a pivotal role in PG&D in terms of increased plant height, branches/tiller numbers, enhanced numbers, length and filled panicles per plant, seed yield and grain quality. Dynamic climatic changes disturbed the ionic balance (salt, drought and heavy metals) and sugar supply (cold and heat stress). Due to poor selectivity, some of the MTs also uptake toxic elements in the roots that negatively impact on PG&D, later on also exported to upper parts and then deteriorate the grain quality. As an adaptive strategy, in response to salt and HMs plants activated PM and VM localized MTs that export toxic elements into vacuole, and also translocate in the root's tips and shoot. However, in case of drought, cold and heat stresses, MTs increased the water and sugar supply to all organs. In this review, we mainly reviewed recent literature from Arabidopsis, halophytes, and major field crops such as rice, wheat, maize and oilseed rape to argue on the global

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role of MTs in PG&D and abiotic stress tolerance. We also discussed the gene expression level changes and genomic variations within a species as well as within a family in response to developmental and environmental cues.

Keywords: abiotic stresses; gene-expression; genomics; ion homeostasis; plant growth and development; plasma membrane; sugar translocation

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1. Introduction

With the current pace, global population is expected to grow with minimum 25% and reach at "10 Billions" in 2050 [1]. Recent advances evidenced the role of membrane transporters (MTs) as they are the major shareholder in sugar transportation from leaf (source) to sink (root, stem and seed) (phloem loading) and nutrient transportation from roots to upper plants' parts (xylem loading) [2, 3]. So, efficient utilization of MTs not only helps researchers to increase grain yield directly but also expand arable land by improving tolerance mechanisms against various abiotic stresses i.e., salt, drought, cold, heat and heavy metals (HMs) [4-6].

Efficient translocation of mineral elements via MTs towards different upper plant's parts contribute to the improvement of plant growth and development (PG&D) in terms of increased number of branches or tiller numbers, plant height and grain yield. For example, Os*NPF7.3* MT is involved in the transportation of di-peptides (Gly-His) and tri-peptides (Gly-His-Gly) and its enhanced expression rate increased the growth of rice plants [7]. In another study, the above MT respond to organic nitrogen (N) supply and mediate N use efficiency (NUE) that resulted in the increased number and filled panicles per plant, N content in grain and grain yield [8]. Moreover, it also involved in long-distance translocation of methylated arsenic (As) to grains through its dimethyarsenate transporter activity [9]. For long-distance sugar transportation almost every plant species contains at least one member of a sucrose transporter (SUC) family that first uptake of sugar molecules, mainly sucrose in phloem and then transport to sink organs [10, 11]. For example, in Arabidopsis, *AtSUC6*, a high affinity H^+ symporter gene highly expressed in reproductive tissues translocate sucrose and maltose via PM to pollen tubes and synergid cells. Besides pollen tubes, AtSUC7 also expressed in roots and its expression is regulated sink-need-dependent manner [11]. To continue steady PG&D processes required necessary supply of photosynthesis assimilates. In rice, upregulation of *OsHAK1* play an important role in both vegetable growth and reproductive success in terms of root growth, pollen viability and fertility via its sucrose phosphate synthase activity [12]. Also, enhanced uptake of K^+ make plants less vulnerable to multiple abiotic-stresses induced osmotic stress. For detail overview on the role of MTs in PG&D, see section 2.

Both sugar and ionic homeostasis in the cells, tissues and organs is regulated by highly sophisticated membrane trafficking system.

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However, source-to-sink relationship can be disturbed mainly due to excessive uptake of salt and HMs, and other environmental factors such as drought, cold and heat. Uptake of hazardous elements is often due to poor selectability of MT/s as chemical properties of essential-minerals often matched with toxic elements as argued in [13]. Excess amount of sodium entering into plant cells often disturbed the Na^+/K^+ ratio in the cytosol. In response, plants activate Na^+/K^+ MTs to achieve the homeostasis. This tolerance mechanism is same both in glycophytes and halophytes but glycophytes are more susceptible as discussed in [14]. However, in rice, to tackle the salt and drought stress, two members of "Sugar Will Eventually be Exported Transporters (SWEETs)" family such as *OsSWEET 13* and *OsSWEET 15* are activated and enhanced the sugar supply across leaf and root organs in an abscisic acid (ABA)-dependent manner [15]. Over-expression of *TaZnF* in *A. thaliana* significantly increased tolerance to heat and cold stress as well as promotes early flowering [16]. Similarly, there are numerous studied in many plant species evidenced the pivotal role of MTs in uptake, sequestration, translocation and detoxification of toxic HMs including cadmium (Cd) [17], chromium (Cr) [18], lead (Pb) [19], As [20], cobalt (Co) and zinc (Zn) [21], for more detail see section 3.

We provided an overview of the roles of transporter proteins in PG&D in terms of their role in the improvement of plant architecture, seed yield, sugar transport and ionic balance for optimal plant growth by minimizing the impacts of external harsh environment. We also discussed the involvement of MTs in the uptake of toxic elements, and explored the diverse and genotypic translocation capability in root and shoot organs. Lastly, we try to provides the recent understandings how MTs respond to changing environments, for instance, strengthening of leaf sheath and increased sugar supply.

2. Role of Membrane Transporters in Plants Growth and Development

Biological role of proteins coding MTs in PG&D has recently been studied in various crop species including rice, maize, wheat and oilseed rape etc., and also their genetic verification in planta and in model plant Arabidopsis.

2.1. Plant architecture, seed yield and quality

Membrane transporters are involved in PG&D improvement through regulating plant architecture and NUE by enhancing tiller numbers and pattern, and plant height. Moreover, they are also involved in remobilization of mineral elements to grains that all-together improve the grain yield and quality. For example in rice, *OsNPF7.2* a nitrate transporter gene which is responsible in regulating the tiller number and grain yield [22]. In detail, their results delineated that in the over-expressed lines tiller number and seed yield were significantly higher compared to less tillers number and yield in mutant lines. Moreover, nitrate application found to enhance the activity of *OsNPF7.2* in terms of faster growth of tiller buds and

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improved root length and number, fresh and dry weight compared to mutant lines. Besides, they also noted that in tiller buds, the transcripts level of genes responsible for cytokinin pathway and cell cycle were enhanced in over-expressed lines. In another case, *OsNPF7.3*, a vacuolar membrane localized peptide transporter gene was found to involve in improving NUE and grain yield in rice as reported in Fang et al. [8]. Their results showed that expression level of *OsNPF7.3* was higher in vascular bundle of lateral roots and stem, and regulated by organic N sources. In reverse, reduction in plant growth, accumulation of amino acids in leaf sheaths and deterioration of leaf blades was recorded in the RNAi lines. However, in over expressed lines at later reproductive stage, N concentration was decreased in leaf but translocated towards grains. Thus, *OsNPF7.3* is involved the allocation of N and then improve the overall PG&D. In rice grain, concentration of mineral elements is higher compared with other staple crops such as maize and wheat. The movement of mineral elements to grain such as Zn and cadmium (Cd) is controlled by MT *OsZIP7*, a PM localized gene as investigated by Tan et al. [23]. Their results showed that expression level of above transporter gene was higher in parenchyma cells in both roots and nodes. Interestingly, in RNAi lines the retention of Zn and Cd content was recorded in above both organs, and Zn was distributed in whole root and away from leaves. As a whole OsZIP7 play a significant role in xylem loading and intravascular movement of Zn/Cd elements in nodes.

2.2. Ionic homeostasis, detoxification and transportation of mineral elements

To maintain the steady PG&D, there is need to maintain the ionic balance of important minerals. For instance, *OsHMA3*, a tonoplast localized MT gene maintain the Zn homeostasis during growth periods in rice [24]. They reported that root of rice accessions containing functional allele *OsHMA3* showed higher Zn tolerance compared to lines with loss-of-function. Moreover, in lines with functional allele also showed the higher expression of ZIP family MTs such as *OsZIP4*, *OsZIP5*, *OsZIP8*, and *OsZIP10*. These above findings suggested that *OsHMA3* play a central role in root of rice plants to maintain cellular Zn content at optimum level by its detoxification and storage into vacuoles. Although, mineral elements are necessary for optimum PG&D but above a certain level can be toxic. So, to adapt such hazardous condition, plant have evolved diverse NRAMP (Natural Resistance-Associated Macrophage Proteins) through process of alternative splicing (AS). For example, in rice, *OsNRAMP6* gene undergone AS and produced different NRAMP6 proteins such as full length (1- NRAMP6) and shortest (s- NRAMP6) [25]. These above variants are PM localized proteins coding MTs responsible for transportation of iron (Fe) and manganese (Mn). Interestingly, their expression level is induced when plants are under attacked by a fungal pathogen *Magnaporthe oryzae,* depends upon infection rate. Moreover, exogenous supplementation of Fe also enhanced the defense of rice plants against *M. oryzae*. Interestingly, they

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also highlighted that loss of function of *OsNramp6* resulted in increased resistance to above pathogen. This finding suggested the negative regulation of OsNramp6 occurred in response to rice immunity. However, in rice mutant plants (*nramp6*), significant deterioration of biomass was recoded that indicated its involvement in PG&D.

Essential mineral elements such as magnesium (Mg) are necessary for continuing optimum PG&D. To date, in total 12 Mg^{+2} transporters (MGTs) belong to a CorA-like gene family has been identified in maize that involved in transportation and maintaining of Mg^{+2} homeostasis [26]. They reported that *ZmMGT12* gene was very similar to a typical MGT in terms of two conserved TM domains and a GMN tri-peptide motif. The expression level of *ZmMGT12* were higher in roots, stems and leaves but was more prominent in leaves. Interestingly, higher expression rate in leaves was dependent on the rate of chlorophyll synthesis. Taken together, these findings suggested that *ZmMGT12* was responsible for transportation of Mg^{+2} ion and probably to the chloroplast. Similarly, in oilseed rape, a high affinity MT called *BnPHT1;4* belongs to a gene family PHT responsible in acquisition of phosphorus (P) to facilitate the seed germination and early seedling development [27]. Further, results also showed that expression level of *BnPHT1;4* was prominent in cotyledon at early seedling stage. In over-expressed plants higher rate of seed germination and seedling growth was recorded compared to WT. Moreover, changes in the accumulation of gibberellic acid (GA) and down-regulation of ABA were also noticed in the transgenic plants. Further, they also showed that exogenous application of GA and ABA were related to increment of seed germination and recovery of advanced seed germination related phenotype, respectively in the over-expressed seeds compared to WT. However, Pi deficiency and supplementation resulted in the deterioration of total P contents in cotyledons but increase in shoots and roots of over-expressed plants as compared to WT. These findings suggested that *BnPHT1;4* is involved in acquisition and mobilization of P elements to enhance the seed germination and seedling development at early stage in oilseed rape. In another study on *B. napus*, it has been found that in total of 49 PHT1 gene family members are involved in P acquisition and transportation. Among them, 27 PHT1 genes are located in A-subgenome and 22 in C-subgenome, and most of them are localized in the PM. Their results showed that variable expression pattern was recorded in response to P deficiency. Also, these above PHT1 genes expressed higher under various mineral elements such as N, potassium (K), Fe and sulfur (S), environmental stresses including salt and drought and phytohormones for instance, auxin and cytokinin. In a nutshell, *PHT1s* regulate the nutrient homeostasis and respond to various environmental stresses in combination with their own family members as well as with other genes in a sophisticated network [28]. For the thematic model about plant architecture and ion homeostasis see Figure 1.

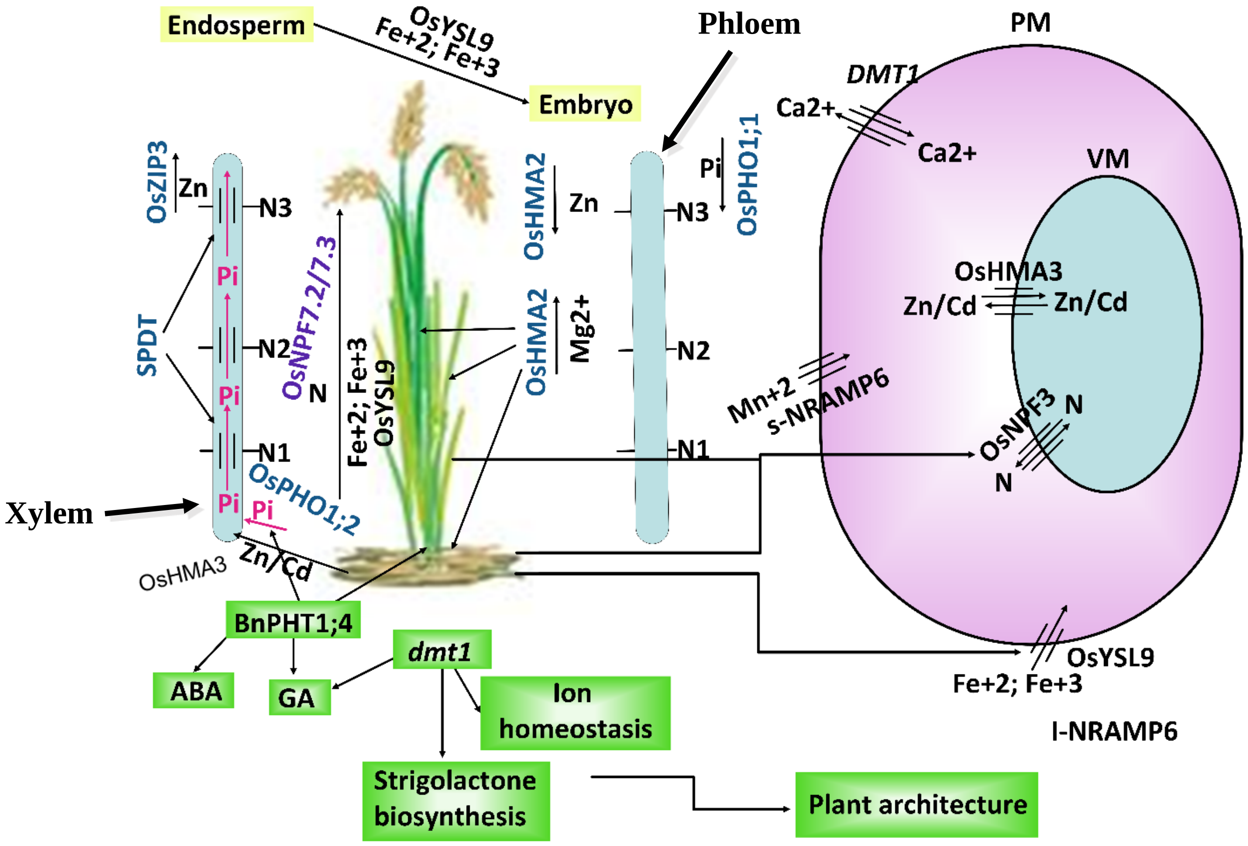

Figure 1. Thematic model represented the role of membrane transporters in improvement of plant architecture, seed yield and transportation of mineral elements and ion homeostasis.

Figure shows that OsYSL9 is localized at the plasma membrane (PM) translocate the Fe^{+2} and Fe^{+3} ions from roots to upper plants parts and also export these ions from endosperm to embryo resulted in the enhanced grain quality. OsNPF genes transport nitrogen towards leaf and involved in N use efficiency (NUE). OsPHO1;2 and BnPHT1;4 transport the available phosphorus (Pi) from the roots to xylem (xylem loading) and then SPDT export Pi across the nodes. Here, BnPHT1;4 also upregulate the genes involved in the pathways of ABA and GA resulted in the improvement of plant growth and regulation. Similarly, OsHMA3 localized at vacuolar membrane (VN), involved in the maintaining of Zn/Cd homeostasis and transport Zn/Cd from root to xylem and from onward OsZIP3 gene unload the Zn from xylem to grains. OsHMA2 is expressed in roots, stem and leaves export Mg^{+2} across xylem towards upper parts and translocate Zn in last node towards lower parts across phloem. Lastly, DMT1 gene localized at the PM and transport Ca^{+2}. However, in the mutant plants (dmt1) upregulation of GA, balancing of ion homeostasis and increased strigolactone biosynthesis process were observed that resulted in the overall improvement of plant architecture.

2.3. Remobilization of photosynthates

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In full daylight, plants produce plenty of carbohydrates including sucrose through the process of photosynthesis that for time being is stored in vacuoles. However, in the night to continue the normal PG&D, plants required its remobilization. For example, in maize, *SUCROSE TRANSPORTER2* (*ZmSUT2*), a sucrose /H^+ symporter gene localized on the vacuolar membrane and is responsible for export of stored sucrose to other parts as reported previously [29]. Their results depicted that, in field condition, reduction in plant growth, deterioration in the lengths of tassels and ears and a smaller number of kernels was recorded in mutant (zmsut2) plants compared to wild type (WT). Moreover, in zmsut2 mutants, accumulation of two-fold various sugars including sucrose, glucose, and fructose were noticed in leaves as compared with WT. These findings suggested that *ZmSUT2* is responsible for remobilization of sucrose out from vacuoles and then transportation towards growing tissues resulted in enhanced biomass and kernel yield.

Recently researchers discovered a novel MT family known as "SWEET" that majorly mediate the sugar translocation across cell-membranes. The SWEET family is unique in its structure as it contains seven transmembrane domains having two internal MtN3 motifs (possibly originated from prokaryotic gene duplication event) that are involved in sugar transportation [30]. To study the role of above MT family in sugar compartmentalization, Wang et al. (2018) isolated the CsSWEET16 transporter gene from *Camellia sinensis*, cloned and then transferred into Arabidopsis [31]. Sub-cellular localization analysis showed that CsSWEET16 was located at the vacuolar membrane. Moreover, their results stated that above transporter gene is involved in the sugar transport across vacuolar membrane and respond to cold-stress in transgenic Arabidopsis compared to WT. In another study, *DsSWEET12* MT, a member of SWEET family was identified in *Dianthus spiculifolius* and detailed its diverse role in PG&D, abiotic and biotic stresses [32]. Their qPCR data revealed that fold-change levels of *DsSWEET12* was enhanced in response to sucrose depletion, mannitol and H_2O_2. Further, co-localization analysis stated that it was located at the PM. In Arabidopsis, over-expressed *DsSWEET12* plants showed greater tolerance to osmotic and oxidative stress as well as have longer root length and increased fresh biomass compared to WT, which mainly dependent on continuous sucrose supply. Modification of "SWEET" members at transcriptional level can further enhanced its diverse roles in plants. For example, in rice, DNA Binding with One Finger 11 (OsDOF11) transcription factor (TF) mediate sugar translocation mechanism by promoting SWEET genes and also other sugar transporters [33]. In detail, T-DNA insertion mutant analysis showed *OsDOF11* was expressed higher in vascular cells of photosynthesis apparatus and other sink tissues such as root, young seedling and leaf. In the mutant *osdof11*lines, plants were semi-dwarf and having fewer tiller and smaller panicles compared to WT. Contrary, in the WT plants root length of young seedlings was significantly larger than mutant plants, which might be due to reduced sugar supply in mutant

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plants. Moreover, chromatin-immunoprecipitation analysis showed that OsDOF11 TF was directly attached with the promoter regions of other sugar transporter genes such as *OsSWEET11/14* and *OsSUT1* (whose expression levels are varied in different tissues), suggested that *OsDOF11* regulate the expression levels of these genes in a coordinated manner to mediate the sugar transport system through apoplastic loading.

Source-to-sink translocation of sugar molecules (i.e., sucrose, raffinose and polyols) mainly determined PG&D and depends on their sophisticated controlled transportation to all organs via phloem loading [10, 34]. Disturbance in phloem channels due to environmental cues can alter the sugar homeostasis in different plant tissues [2]. In Arabidopsis, to regulate source-sink relationships, two sugar MT families such as sucrose transporter (SUC) and SWEET play a specific role [35]. For example, in leaves, *AtSUC2* and *AtSWEET11/12* known to play a role in phloem loading and expressed higher when sucrose translocation was either increased or decreased due to osmotic stress. However, in roots, expression level of *AtSUC1* was upregulated, which indicated that it might be involved in sucrose unloading and roots growth. On the other hand, SWEET transporters such as *AtSWEET12/13/15* expressed higher in all tissue but *AtSWEET14* expressed differentially in root, stem and siliques and *AtSWEET9/10* only in stem and silique. Above findings suggested on-demand roles of these MTs in source-sink relationships. To sum up, in normal condition, root is always remained a main sink but under stressful environment carbohydrate allocation are decreased to rosettes and roots. For the thematic model about sugar transport see Figure 2 and Table 1 describes the detail on the role of MTs in PG&D.

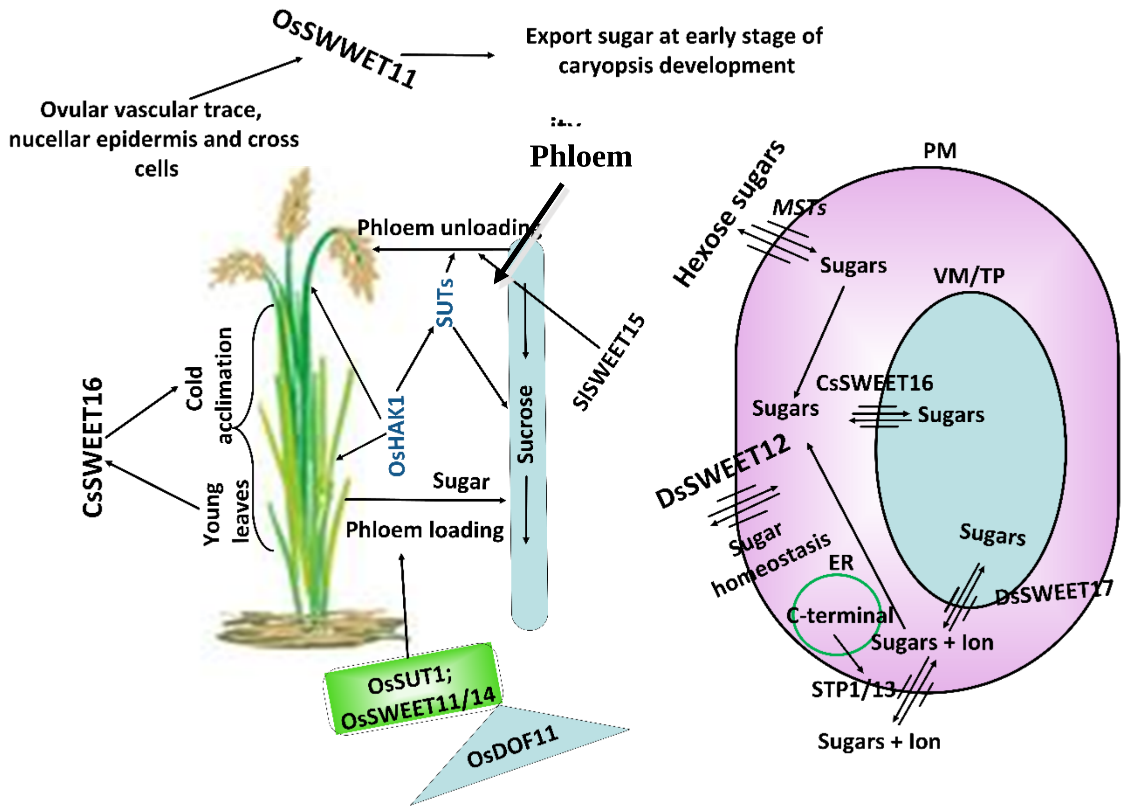

Figure 2. Sugar transport mechanism in plants.

The figure indicates the localization of STP1/13, DsSWEET12 and MST genes at the plasma membrane (PM). The C-terminal motif localized in endoplasmic reticulum (ER) involved in the PM localization of STP1/13 genes that transport the sugar and ions across the PM. Similarly, DsSWEET12 and MSTs transport only sugar molecules across the PM and DsSWEET17 and CsSWEET16 across the vacuolar membrane (VM)/tonoplast (TP). Here, OsDOF11 transcription factor binds with OsSUT1 and OsSWEET11/14 and upregulate their expressions levels that leads to phloem loading (transport sugars from leaf photosynthesis apparatus to phloem). OsHAK1 upregulate the downstream SUTs involved in phloem loading (leaf to phloem) and unloading (phloem to panicles) as well as upregulation of MSTs. CsSWEET16 highly expressed in young leaves and play a role on cold acclimation. OsSWEET11 expressed in reproductive tissues such as ovular vascular trace, nucellar epidermis and cross cells and export the sugar at early stage of caryopsis development (post-phloem loading) resulted in enhanced grain yield. For more details see text.

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Table 1. Role of membrane transports in plant growth and development.

Transporter protein	Plant species	Localization in tissue/organ/cell	Biological role	Ref.
OsNPF7.3	*O. sativa*	Lateral roots and stems	Contribute to NUE and grain yield	[8]
OsNPF7.2	*O. sativa*		Enhance tiller number and grain yield	[22]
OsZIP7	*O. sativa*	Parenchyma cells of vascular bundles in roots and nodes	Xylem loading in roots and transfer of Zn/Cd to grain	[23]
OsYSL9	*O. sativa*	Roots and non-juvenile leaves	Distribute iron to developing grains	[36]
OsHMA3	*O. sativa*	Tonoplast/roots	Zn detoxification in roots and storage in vacuoles	[24]
OsDMT1	*O. sativa*		Regulate plant architecture and ion homeostasis	[37]
NRAMP6	*O. sativa*	PM	Transport Fe and Mg and disease resistance	[25]
ZmMgT12	*Z. mays*	Root, stem and leaves	Maintain the Mg homeostasis in chloroplast	[26]
ZmSUT2	*Z. mays*	Tonoplast	It acts as sucrose/H+ symporter on the vacuolar membrane and remobilize stored sucrose for subsequent growing tissues	[29]
TaPTR2.1	*T. aestivum*	Tonoplast	Regulate the water status during seed germination at early stage	[38]
BnaPHT1	*B. napus*	PM	Pi acquisition and homeostasis and respond to various nutrient stresses including N, K, S and Fe	[28]
BnaPHT1;4	*B. napus*	Cotyledons of early developing seedlings	Pi homeostasis, seed germination and seedling growth through modification in biosynthesis of ABA and GA	[27]
OsZIP7	*A. thaliana*	PM	Increase Zn concentration by 25 % in the shoot of transgenic plants	[39]
DsSWEET12	*A. thaliana*	PM	Increased sugar supply and enhanced seedling growth (larger roots and fresh biomass)	[32]
DsSWEET17	*A. thaliana*	Tonoplast	It enhanced root length and fresh weight	[40]
OsDOF11	*O. sativa*	Photosynthetic cells	It upregulated OsSUT1 and OsSWEET11 and 14 genes expression and transport sucrose through apoplastic loading and enhanced resistance against Xanthomonas	[33]
OsSWEET11	*O. sativa*	Ovular vascular trace, nucellar epidermis and cross cells	It remobilizes the sugar from maternal tissues towards maternal-filial interface during early caryopsis developmental stage	[41]

ZmSWEETs	*Z. mays*	Leaf	Influence on the sugar dynamics from leaves towards developing ears	[42]
AtSUC6	*A. thaliana*	PM	Sugar accumulation in pollen tube and synergid cells	[11]
AtSTP1/13	*A. thaliana*	PM	Involved in sugar transport across cell membranes	[43]
OsHAK1	*O. sativa*	PM	It involved in controlling vegetative growth, panicle fertility and K+ mediated sugar homeostasis	[12]

3. Membrane transporters and abiotic stresses

3.1. Salt stress

Salt stress is an ongoing and potentially future risk, based on current and future research projects, which show the potential number of salt tolerance patents to be reached nearly up to18,000 [44]. It is a global challenge for agriculture farming, multi-component stress regulated by multiple genes and genetic networks [14]. High salinity induces sodium ionic (Na^+) stress in plants due to higher accumulation of Na^+ in the cytosol, prominently translocated towards upper parts of plants. After entering Na^+ into plant's body certainly disturbs the potassium ion (K^+) homeostasis that plays pivotal role in plenty of metabolic processes, especially balancing cytosolic Na^+/K^+ as reviewed earlier [14]. Disturbances in Na^+/K^+ ratio due to excess salt stress resulted in the deterioration of several physio-biochemical and developmental pathways [45]. Studies on MTs involved in the balancing of Na^+/K^+ ratio has been evidenced in several plant species [14, 45-49]. A possible strategy can be used to tackle the ionic influx, first to compartmentalize it in vacuoles, transport excessive Na+ from the affected cells and then distribute into root and other areal organs. For example, in Arabidopsis and halophytes such above sophisticated work is done by one of the MT called HKT (high-affinity K+ transporters) that involved in long-distance transportation [45]. Another strategy can also be adopted by plants to handle Na+ toxicity in the cell by maintaining the K^+ homeostasis as reviewed in [14, 46]. Whole genome sequencing technologies has explored that a large number of K^+ transporters of HAK/KUP/KT family that belongs to an APC (amino acid polyamine-organocation) super family spreads across the plant genome. They play a central role in K^+ uptake and then its translocation resulted in tolerance to salt induced osmotic stress, and involved in regulation of root morphology and shoot phenotyping. Moreover, in response to exogenous K^+ supplementation, so far six transcription factors (TFs) have been reported regulating the genes coding MTs of HAK/KUP/KT family. Further, to activate their proper functioning (in uptake of K^+) proteins of these above MTs are phosphorylated by complexes of calcineurin B-like (CBL)-interacting protein kinases (CIPKs). These findings indicated that how precisely MTs responsible for K^+ uptake and then their proper utilization in

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whole plant body are regulated by several layers of controllers in a network [46].

Variation in salt stress tolerance mechanism is largely dependent on the genotypic diversity within a species or genera. Ideally, to deep insight on the genetic makeup of different genotypes (to explore genetic diversity) of a plant species responsive to high salt stress, there is need to do thorough investigation under different salt concentrations. For instance, in rice, three genetically variable cultivars such as Koshihikari, Reiziq and Doongara (sensitive, moderately-tolerant, and tolerant, respectively) were investigated against their capability of maintaining ionic homeostasis in the cytosol, and export and transportation from roots towards shoots [50]. Their results based on electro-physiological and qPCR data showed that two times higher capacity of Na^+ efflux was recorded in tolerant cultivar in the elongation zone of root organ. Moreover, Na^+ efflux is partially controlled by the PM localized Na^+/H^+ antiporter salt overly sensitive (*OsSOS1*) MT, indicating that there are some other hidden mechanisms involved. Besides exclusion of Na^+ from root zone, there is another important hallmark involved in salinity stress tolerance, for example, the retention capability of K^+ in the root zone. Behind the K^+ holding, there are three complementary mechanisms such as activation of H^+-ATPase pump (at both transcriptional and functional levels), more tolerance of K^+ efflux channels against negative impact of reactive oxygen species (ROS) and lastly, should be higher up-regulation of *OsAKT1*s compared with *OsGORK* in tolerant genotypes. The genotypes that showed increased in above mechanisms (trait) can be incorporate in the salt stress breeding program for the development of commercial cultivars [50]. Similarly, in maize, Jiang et al. [51] explored the natural sequence variation in HKT MT using the population of 54 maize varieties were collected from diverse region in China. Their results showed that expression level of *ZmHKT1;5* gene was significantly involved in tolerant inbred lines under salt stress environment resulted in the forms of balancing Na+/K+ ratio and improved plant growth. Moreover, two SNPs (A134G and A511G) present in the coding region of above MT which significantly associated with salt stress phenotypic trait at different salt concentrations. Further, they produced transgenic tobacco lines using favorable allele of *ZmHKT1;5* from tolerant genotypes and displayed those over-expressed lines that were more tolerant to salt stress than WT. As a whole, above two SNPs might produce new amino acids resulted in higher expression of ZmHKT1;5 that may trigger the salinity stress mechanism by creating Na^+/K^+ homeostasis in the cytosol and enhance the activity of antioxidants to minimize ROS at low level.

Studies focusing on the over-expression of MTs shed a light to enhance the protective shield in plants against the high salt stress [47, 48, 52]. For example, in Arabidopsis, FERROCHELATASE 1 (FC1) a terminal enzyme of heme biosynthesis which is highly responsive to salt stress and involved in several metabolic and physiological processes [47]. Further, they reported that AtFC1 play a significant role

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in improving the Na^+/K^+ ratio by deterioration of Na^+ concentration and improving the K^+ accumulation and protecting cell membrane lysis by upregulation of SOS1 which encodes a PM localized Na+/H+ antiporter gene. Interestingly, in over-expressed AtFC1 plants, expression level of several high salt stress induced genes including NHX1 and AVP1 were reduced. This finding indicated that to regulate lower Na^+ stress, AtFC1 might follow other route rather than mechanism adopted for Na^+ sequestration. Similarly, in rice, Jadamba et al. [48] reported that over-expression of OsEXPA7, a key regulator of cell wall create a protective shield against salt stress induced oxidative stress. Their phenotypic data of over-expressed lines showed that OsEXPA7 significantly enhanced the salt tolerance and expression were more prominent in shoot apical meristem (SAM), root and leaf sheath. Furthermore, over-expression of OsEXPA7 statistically enhanced the K^+ accumulation and reduced Na^+ concentration in roots and leaves. Besides, they also recorded that antioxidant activities were enhanced that resulted in deterioration of ROS contents in over-expressed plants compared to WT. However, some stress-responsive proteins are highly conserved in both plants and animals that play a significant in tackling various posed environmental stresses. We have such an example in wheat [52], where a *TaSAP17-D* gene, a member of a multigene family so called stress associated protein (SAP) contain a conserved AN1/AN1 domain. Subcellular localization analysis showed that *TaSAP17-D* was localized to cytoplasm, nucleus, and cell membrane. Moreover, gene expression level of *TaSAP17-D* was higher in response to a wide range of stresses including salt, cold, polyethylene glycol (PEG) and exogenous ABA. Also, in transgenic lines of Arabidopsis upregulation of *TaSAP17-D* was noticed when plants subjected to salt stress that further confirmed its role in salt stress alleviation [52].

To look at the structural variations in the *cis-regulatory* elements of a MT can also open new avenues regarding the enhancement of salt stress response. For example, in halophyte *Aeluropus lagopoides, HKT subfamily II (AlHKT2) acts as a co-transporter of* Na^+ *and* K^+*, and helps in avoiding salinity stress* [53]*. Detail investigation showed that full-length promoter D1 of above MT has several cis-regulatory elements including MYB, W-box, MBS and ABRE etc. and are involved in salt and other stress response.* Moreover, a 760 bp of D1 promoter region was isolated and cloned. Analysis of GUS assay of transgenic plants (T2) confirmed the involvement of promoter region of *AlHKT2;1 MT in salt stress response*. Hence, more studies need to be foucused in direction of molecular and functional characterization of the K^+ and Na^+ MT systems.

3.2. Drought

Dynamic climate changes induced severe drought stress caused the decline in productivity of crops in terms of seed yield and quality [54]. So, the main objective of agricultural biotechnology program is to develop high yielding plants with better survival ability during water scarcity [55]. A combination of dry and wet lab approaches alone or in combination to explore new and well-utilize already reported MTs can brings solutions on a table to dealt with drought. For example, in wheat, ElBasyoni et al. [56] conducted a comprehensive study on the role of cell membrane stability (CMS) in response to heat and PEG 600 stress. Results based on association mapping of SNPs called from a panel of 2111 diverse spring accession with CMS trait. Interestingly, several significant SNPs were found to be highly associated with CMS. Moreover, candidate genes in the QTL regions were mainly related to solute transporters of CMS and other biochemicals responsive to abiotic stress. In the field trial data, they found that selected accessions (based on significant SNPs screening) produced more seed yield under above abiotic stresses environment. In a nutshell, genome-wide design breeding program of CMS can be used for the selection of parental genotypes for producing commercial cultivars that can grow well in drought affected soils. Comparative studies on the close relatives can be utilize for exploring crop stress tolerance behavior. For example, Hasan et al. [57] investigated the response of maize and sorghum plants under drought (10 d withholding water) in field condition. Their results showed that drought stress showed severe impact on maize as compared to sorghum in terms of physiological attributes such as shoot fresh and dry weight and leaf water content, gas exchange capacity and water use efficiency (WUE). Moreover, gene expression data regarding four PM intrinsic proteins (PIPs) of aquaporin gene family such as PIP1;5, PIP1;6, PIP2;3 and TIP1;2 showed that PIP1;5 and PIP2;3 in leaves and roots, respectively were highly regulated in response drought stress in sorghum but not expressed in maize. These above two proteins possibly play a central role in water transport to tackle drought severity. Further, their gene expression data suggested that in both species PIP1;6 probably contribute in CO_2 transportation and TIP1;2 in water transport under control condition but not in during drought stress.

Cloning of well-known MTs from tolerant and then genetic transformation to less tolerant plant can enhance coping capacity of drought stress. For example, Zheng et al. [58] recently cloned PIP2;3 from halophytes *Canavalia rosea* and then transformed in yeast and Arabidopsis to evaluated its heterologous expression systems under drought induced high osmotic stress. The expression level of *CrPIP2;3* was enhanced under osmotic stress. Its over-expression further relieves the drought induced impact by improving water homeostasis but not ROS scavenging in transgenic Arabidopsis plants. In plants, HAK MTs helps plants to cope with drought stress by improving K^+ acquisition and transportation. The question is how it is regulated in response to above stress? To answer this question, Chen et al. [59] investigated the regulatory role of *OsHAK1* in rice under osmotic and drought stress and found that its expression level was transiently increased in water deficit shoots and roots. Contrary, knockout mutants *oshak1* plants displayed reduction of growth at both vegetative and reproductive stages under drought stress environment. However, over-expressed plants showed better tolerance to

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drought stress in terms of reduced lipid peroxidation content, higher antioxidative activities (POX and CAT), positive regulation appeared in rice channel genes (*OsTPKb and OsAKT1*, involved in K^+ homeostasis and stress response) and recovery at reproductive stage that enhanced 35% grain yield compared with WT. In a nutshell, *OsHAK1* acts directly as well as a positive regulator of downstream genes to better cope with drought stress trait. So, this gene can be incorporated in molecular breeding program related to drought stress tolerance.

Optimization of photosynthesis related tissues, for instance, component of thylakoid membranes can be considered as an additional drought stress coping strategy. For this, rice is an excellent example [60], where a high yielding rice hybrid "HYP9" was investigated to explore changes in structure and composition of thylakoids, and photosynthesis inflorescence related parameters under PEG stress. They uncover the most affected components due to extreme drought such as ET0/RC, ET0/CS0 and φE0 were decreased. So, the question is how to avoid these above losses. The answer is also given in the above study. They applied the exogenous silicon (Si) and found that it significantly recovers the degradation of protein complexes such as PSI core binding LHCI, PSI core, mono- and tri-meric LHCII and F1-ATPase binding Cyt*b6/f* complex compare to drought stress alone. Further, results based on SDS-page analysis depicted those noticeable changes were recorded in terms of light harvesting complex, PSI stability, function and core content of PSII reaction centers and antenna content, electron transport chain, and ATP synthesis under PEG and PEG + Si treatments. As a whole, to minimize the drought induced alterations in thylakoid structures not only a good strategy to enhance the drought tolerance mechanism but also can be helpful in screening material for future breeding program.

3.3. Heat stress

To achieve the goal of "Zero Hunger" as proposed by FAO, heat stress is one of the major obstacles as it drastically disturbs the plants physio-biochemical processes, gene expression regulation networking, metabolome and proteome levels as review in [61]. As plants are sessile, so to withstand in dynamic confrontational environment they have evolved significant alterations in their adaptive behaviour. To date, plenty of genes coding MTs has been reported which play a pivotal role in diverse biological systems including plant metabolism and its production resulted in the increase of heat tolerance mechanism in plants [62, 63]. Regulatory proteins of MTs play a significant role in the regulation of PG&D as well as enhancement of coping potential against faced threat. For example, in plants, zinc finger (ZnF) protein belongs toa C3HC4-type TF family that play an important role in multiple developmental and environmental cues. In wheat, gene expression analysis of *TaZnF* showed that it is starts expression at the stage of post anthesis period 3-5DAA (a sensitive stage related to yield) and expressed higher in seed [16]. In transgenic Arabidopsis, over-expression of *TaZnF* resulted in the increased tolerance to both basal and high temperature stress compared to WT and also improved the PG&D. Moreover, over-expressed lines showed earliness in flowering, larger primary roots, more lateral branching, increased leaves number and size, enhanced fresh biomass, and overall improve in PG&D and ultimately yield. While continuous facing thermal stress, to maintain membrane integrity at optimum level is an important aspect in respective tolerance mechanism that should thoroughly be investigated. For example, in Arabidopsis, P4-type ATPases play an important role in stability of membrane system as they take part in transportation and homeostasis of phospholipids. In total, 12 P4-type ATPases are reported in Arabidopsis, so far. Among, aminophospholipid ATPase6 (ALA6) is one of the members of above family (P4-type ATPase). Researchers reported that loss of function mutation (ala6) resulted in the sensitivity to both low- and high-level temperature treatments. Contrary, in the over-expressed ALA6 lines, resistance to heat stress at seedling stage was recorded. Besides, they also uncovered that in point mutation (ALA6, one bp at conserved functional site) transgenic plants, still heat susceptibility was noticed just like loss-of-function ala6 mutant. Moreover, in ala6 mutant plants higher ion-leakage was observed indicated that lipid flipase activity is regulated by ALA6 in heat stress tolerance. So, ALA6 under heat stress play a vital role in membrane stability [64].

In response to heat stress, several different lipids accumulate in various cell organelles as an adaptive strategy. For example, in Arabidopsis, increases in galactolipids (containing linoleate, 18:2) in chloroplasts; phospholipids (containing palmitate, 16:0; stearate, 18:0; and oleate, 18:1) in the endoplasmic reticulum and PM, and triacylglycerol (containing α-linolenate, 18:3) and hexadecatrienoic acid (16:3) as lipid droplets were recorded in leaves in response to heat stress as reviewed in [65]. Response of cellular organelles at global network level can open new avenues contribute overall in heat stress mechanism in plants. As we know chloroplast is a central player for steady PG&D and its survival as well as performance of chloroplast is closely associated with cell general status while facing heat stress. To explain the vital role of chloroplast, in Arabidopsis, Paul et al. [66] examined chloroplast morphology, and proteome composition under single and repetitive heat stress periods, prolonged till two weeks. They depicted that in a single heat stress condition a significant alteration was observed compared to repetitive ones, where adaptive behavior was noticed. Moreover, changes in the chloroplast morphology in terms of adjustments and adaptation was dependent on protein translocation efficiency as evidenced in mutant plants of two chloroplast translocon units such as TOC64 and TOC33. Interesting, in adaptation period to repetitive heat stress, toc33 mutant displayed accumulation of HSP70 protein, while toc66 showed higher protein contents responsible for the determination of thylakoid membrane structure compared to WT plants. At reproductive stage, rice plants respond variably to heat stress in terms of panicle initiation, flowering time and grain filling. So, genotypic

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screening of rice accessions at grain filling stage can helps to assist in the development of future breeding plan to cope with yield loss due to heat stress [67].

Since plants located in regions where it is very hot and have to thrive, they need an intensive and continuous use of their cellular metabolism to control fluidity and preserve the structure. Previous research on fatty acid showed that the disparity between saturated and unsaturated fat levels found to be increased. Additionally, at elevated temperatures, the content of polyunsaturated and polyunsaturated fatty acids rises [68, 69]. Temperature is the most important contributing factor in regulating the degree of unsaturated fatty acid chains, and therefore has a significant impact on the properties of diaphragms [70, 71]. The numbers and locations of double bonds within fatty acid chains are modified by desaturation through an oxygen-based reaction [72]. In plants, the family of fatty acid desaturase (FAD), which plays a significant role in adapting to high-temperature stress, has been studied. Among the eight Arabidopsis FAD family members (FAD1 to FAD8), FAD2 and FAD3 were found on the ER membrane, while others are plasmid dictatorships [73].

3.4. Cold stress

Low temperature is a limiting factor for the PG&D, and productivity as well as geographical distribution of many crop species. So, plants must be capable to respond immediately to temperature changes to adapt well to growing environment and avoid metabolic losses. Plants have a variety of MTs that play a significant role for continuity of vital cellular processes, for instance, ionic homeostasis, maintenance of osmotic pressure, signal transduction and sequestration under cold stress condition [74]. For example, PIPs play a role in water uptake under both normal and stress conditions. An integrated approach based on physiological, cell biology and transcriptional profiling attributes stated that PIPs are involved in cold stress response [75]. Their results suggested that expression levels of two PIPs such as *PIP1;4* and *PIP2;5* were upregulated, and protein of *PIP2;5* and total PIPs contents were also increased to adapt under cold stress. These findings indicated that *PIP2;5* play a pivotal role in tackling impact of cold stress. Plants interaction with surrounding environment is due to it cuticle layer (composed of wax and cutin) that made of lipids and covers most of its areal surfaces. Thus, the protection of cuticle layer external harsh environment can be an important adaptive strategy of plants. For this purpose, plants have ABC subfamily G members (ABCG) are involved in the transportation of lipid molecules of cuticle to plant upper surface, outside. For example, *Thellungiella salsugineum* is an extreme stress tolerance plant that contain thick cuticle layer [76]. Cloning of *TsABCG11* from above plant and then transformation in its closed relative Arabidopsis stated that lower rate of water loss and chlorophyll content was recorded in over-expressed lines compared to WT under 4 °C. In the same transgenic lines significant changes were noticed in wax

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components (C31 and C33 alkanes) and cutin (C18:2) resulted in the improvement of wax and cutin contents in cuticle layer.

Besides, transgenic technique, there is need to find novel genes and protein using whole genome transcriptomics and proteomic approaches. Comparative transcriptome profiling can provide the millions of treatment-dependent reads that can be aligned with reference genomes (if available) and *de novo* (if not), and ultimate will provide 1000s of novel transcripts/genes. For example, in rice, mRNA sequencing of two contrasting cultivars i.e., Y12–4 (cold-tolerant) and 253 (cold-sensitive) showed that, a total of 42.44–68.71 million reads were obtained and later on aligned to nearly 30 and 29 thousand genes, respectively [77]. In the results, majority of cold tolerant of DEGs were found in tolerant genotype. Among all, they explored one cold responsive gene, low temperature growth 5 (LTG5) coding for UDP-glucosyltransferase was upregulated under cold stress, indicated that *LTG5* gene play a role in cold-stress mechanism in rice. Moreover, UDP-glucosyltransferase is located in a GSA1 QTL region that is responsible for grain size and abiotic stress response as recently reported in [78]. They sated that grain size is controlled by cell proliferation and expansion and regulated by glucosyltransferase activity towards flavonoid mediated auxin homeostasis and other related genes. However, in abiotic response, GSA1 remobilize the metabolic flux from lignin biosynthesis towards flavonoid biosynthesis resulted in the accumulation of glycosides which prevent rice plants from abiotic stress.

There are very few literatures available that answer the very basic question about the earliest response of plants to drop in temperature. Plant organelles, for instance, PM and its nearby extracellular and cytoplasmic cites are so called primary checkpoints for sensing the temperature fluctuations, and following events such as signal transduction and remobilization of solutes by MTs. To insight on cold sense and response mechanisms, Kamal et al. [79] investigated short-term changes in PM proteome using phosphor-proteomic method powered by mass spectrophotometry approach in Arabidopsis exposure to cold stress for 5-60 minutes. Their results revealed that rapid changes occurred in proteins responsible for ionic homeostasis, transportation of solutes and other proteins, protein modification, cytoskeleton organization, vesical trafficking, and signal transduction processes. Moreover, motif phosphorylation and protein kinases (PKs)-substrate network analysis suggested that plenty of PKs such as MAPKs, CDPKs, RLKs, and their substrates might also be played a role in cold response mechanism. In another study on PM proteome [80], Takahashi and his colleagues further insights on freezing tolerance by treated Arabidopsis suspension cultured cells with cold acclimation and ABA. To insight on the changes in the cellular response to above treatments, PM proteome was analysed using label-free protein quantification method. Data revealed that in total of 841 proteins were accumulated during transition of growth phase, cold acclimation and ABA environments. Among, 392 proteins were accumulated in respond to progression of growth phase and were divided to several functional groups, suggesting that PM physiology dependent on growth phase. Moreover, in response to cold acclimation, ABA play a role in signal transduction that resulted in the changes in PM proteome. As a whole, modifications in PM proteome in response to transition of growth phase impact on PM proteome related cold acclimation and ABA, which may influence the tolerance capability to freezing.

Exploration of cold response at genotypic/ species levels can further unveil the overall tolerance mechanism. For example, in maize [81], two widely used inbreds i.e., B73 and Mo17 and 37 other inbreds (grouped into stiff-stalk, non-stiff stalk and tropical clades) exhibits variable cold tolerance behaviour that were evaluated at seedling stage. Recombinant inbreds (B73 × Mo17) line (RIL) population were developed (total of 97 RILs) to identify the QTLs responsible for the cold response at seedling stage. Based on association with three phenotypic traits such as leaf colour, chlorophyll content, and tissue damage two QTLs on chromosome 1 and 5 were detected. The DEGs in these two QTLs responsive to cold stress are those genes with similar putative function like auxin and GA as well as general abiotic stress response. So, breeding with these QTLs can protect the maize plants from low temperate stress right after germination will suggest breeders would allow early sowing and provide prolonged growing period resulted in high crop yield.

3.5. HMs stress

With essential mineral elements, toxic HMs often co-exist in the arable soil that seriously threaten food productivity globally. Due to similar chemical properties of essential mineral elements and not essential HMs, some of the MTs such as Natural Resistance-associated Macrophage Proteins (NRAMPs) transport them to upper plant parts. In dwarf polish wheat (*T. polonicum*), TpNRAMP3, a member of NRAMPs family was identified that is responsive for PM localized protein and it is expressed higher in leaf blades, roots and first note at jointing, booting and grain filling stages, respectively [21]. In detail, *TpNRAMP3* was cloned and transformed into yeast and Arabidopsis. Results revealed that expression level of *TpNRAMP3* was increased in response to Cd, Co but not Zn in yeast, however in Arabidopsis expression was higher in response to Cd, Co and manganese (Mn) and but not for Zn and Fe in roots, shoot and over all whole plant. Moreover, their data stated that the above MT did not disturb transportation of above metals from roots to shoot. Thus, these results suggested that *TpNRAMP3* play a role in uptake of Cd, Co and Mn. After entering HMs into plant body must be degraded and detoxify, otherwise can deteriorate PG&D and ultimately yield. In rice, stress membrane proteins (SMPs) globally respond to abiotic stress including HMs. Recently, Zheng et al. [82] reported that *OsSMP1*, a member of SMPs family is localized on the cell membrane. Data stated that over-expression of *OsSMP1* significantly enhanced the tolerance mechanisms to multiple stresses such as salt, cold and HMs including Cd and copper (Cu). Overall, they suggested that *OsSMP1*

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positively regulate the abiotic stresses i.e., salt, cold and HMs through ABA mediated pathway and potentially can be utilized for rice stress breeding.

Among MTs, ABC transporters play a pivotal role in uptake and translocation of wide-range of metabolites and xenobiotic including HMs and have been studied in several plants' species including Arabidopsis [83, 84], rice [85] and *B. napus* [17]. Firstly, characterization of ABC transporters was done in popular species and later on transformed and studied their orthologs in other plants [83]. For example, *PtABCC1, a member of ABC transporter was cloned from Populus trichocarpa* and over-expressed in both *P. trichocarpa* and Arabidopsis [83]. Data revealed that transgenic plants were more tolerant to exogenously applied mercury (Hg) stress, and over-expressed plants accumulated 26-72% and 7-160% Hg in Arabidopsis and popular, respectively compared to WT. In a similar study, Wang et al. [84] cloned *PtoABCG36*, another member of ABC transporter family from *P. tomentosa* and reported that it is expressed almost in all plant organs including root, stem and leaves and play a significant role in Cd tolerance in Arabidopsis. In detail, its expression was increased more just after 12-hours exposure to Cd stress. Results revealed that, in over-expressed lines Cd accumulation was significantly decreased in terms of net Cd^{2+} efflux compared to WT. These findings suggested that *PtoABCG36*, acts Cd extrusion pump contributing Cd tolerance by decreasing its contents, indicating that transgenic plant with this MT can be a promising way to cultivate crop plants in Cd-affected soils. To broaden the scope of ABC transporters, in *B. napus*, Zhang et al. [17] reported that, a total of 314 ABC transporters were identified from high-throughput sequencing data. All 314 MTs further categorized into eight sub-families including ABCA-G and ABCI. Further analysis suggested that their expansion was due to allele duplication. Most of ABCs (233/314) were also verified by comparing with RNA-Seq data at seedling stage, and among them (233, BnaABCs), 132 were DEGs and 84 were significantly responsive to Cd stress. Lastly, the analysis of *cis-regulatory* elements suggested that eight Cd responsive DEGs showed significant variation, indicating their role in both environmental (abiotic stress and hormonal signaling) and developmental cues.

Like ABC transporters, HM ATpase (HMA) also play a significant role in metal ion transportation across membranes. For example, in wheat *TaHMA2* can transport Cd^{2+} and Zn^{2+} through cellular membranes [86]. Moreover, two motifs such as CCxxE and CPC in the N-MBD and N/C-terminal are mainly involved in the transportation of above metals. They produced four types of transgenic Arabidopsis plants, 1) over-expressed plant with normal *TaHMA2* functioning, 2) *TaHMA2 derivative* (substitution of glutamic with alanine in CCxxE motif) plants and 3) plants contain truncated N/C-terminal and mutants with cysteine in N-MBD region. Results stated that in first two types of plants there was increment in PG&D attributes such as root length and fresh biomass, and also enhancement in transportation in Cd and Mn ions from root to shoot. While in third type, tolerance and

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translocation activity of *TaHMA2* was impaired. In last type of plants, tolerance and transportation activity of *TaHMA2* was deteriorated compared to WT. These findings suggested that cysteine play a significant role in binding with Zn^{2+}/Cd^{2+} and then translocation from root to shoot. In another comparative study on rice, maize and sorghum, Zhiguo et al. [87] detailed the role of P_{1B} type HMA in metal ion transportation, and PG&D. A total of 31 P_{1B} type HMA genes were identified, among 11 each in maize and sorghum and nine in rice. Sequence composition and phylogenetic analysis suggested that above HM transporters were categorized in two sub-families and four of them were tandem duplicated. Members of HMA were diverse in terms of gene expression under Cd/Cu treatment and tissue specificity. Above finding suggested the utilization of HMAs (*TaHMA2 and* P_{1B} type HMA) in crop HM stress breeding program.

Exploration of genotypic variation at physio-chemical, molecular and genomics levels in coping the HMs can over all broaden the scope of HM tolerance mechanism [18, 88-92]. In maize, a group of researchers investigate the impact of four metals including Cd, Cu, Zn and Ni on roots young leaves (14 days after sowing, DAS) and mature leaves (21 DAS). Data represented that the concentration levels of all four metals were varied in all organs but more prominent in roots. Interestingly in older leaves metal transportation caused accumulation of ABA resulted in the closure of stomata and ultimately reduction in photosynthesis and fresh weight. Moreover, accumulation of three metabolites such as Tocopherols, polyphenols, and flavonoids were enhanced in shoots in response to Zn, Ni and Cu stress. Similarly, activities if antioxidants for instance SOD and DHAR were upregulated in the roots of Cu and Cd treated plants, however, APX was upregulated only in mature leaves. These findings suggested that organs response to metal ions is also associated with upregulation of antioxidants. In the last decade, tremendous amount of work has been done in terms of sequencing of many plant genomes that opening new avenues to look at i.e., PG&D and environmental cues. In *B. napus*, Gill et al. [18] explored the whole genome transporters (transportome) under Cr stress using high-throughput *de-novo* sequencing data. Results depicted that a total of 2867 and 2849 MTs were found in two contrasting cultivars, ZS758 (Cr-tolerant) and Zheda 622 (Cr-susceptible). In detail, under Cr stress condition, three novel MTs i.e., BnaA04g26560D, BnaA02g28130D, and BnaA02g01980D were found and their functions were similar to water transport through cell membranes. As a whole, a well-coordinated organ, antioxidant and MTs response is required for sequestration, translocation and detoxification of metal-ions to increase the plant stress resistance mechanism. Table 2 and Figure 3 describes the detailed role and mechanism of MTs in plants under abiotic stresses.

Table 2. Role of membrane transporters in abiotic stress tolerance.

Abiotic stress	Transporter protein	Plant species	Tissue/ organ	Biological role	Ref.

Salt	AlHKT2;1	*A. lagopoides*	Leaf/shoot /root	Na^+/K^+ co-transporter gene prevents plants from salinity stress	**[53]**
	OsCam1–1	*O. sativa*	Leaf	It involved in signaling, hormone-mediated regulation, transcription, lipid, carbohydrate and secondary metabolism, photosynthesis, glycolysis, TCA and glyoxylate cycle under salt stress	**[93]**
	AtFC1	*A. thaliana*	Roots, cotyledon, root, shoot, leaf and flower	It enhances K^+ accumulation and prevent cell membrane lysis, also upregulate the expression levels of NHX1 and AVP1	**[47]**
	ATG8	*A. thaliana*	Root/cortex cells	It plays a role in nutrient remobilization following salt induced autophagy	**[94]**
	PRE1/AAP1	*A. thaliana*	Root	It enhanced uptake and transportation of proline and prevent proline degradation	**[95]**
	OsAKT1	*O. sativa*	Root/elongation zone and shoot	Retain the K+ in root to balance Na+/K+ ratio	**[50]**
	ZmHKT1;5	*Z. mays*	Leaf	Balance Na+/K+ ratio and improve plant growth	**[51]**
Drought	CrPIP2;3	*A. thaliana*	Germinating seed, seedling and root	It plays pivotal roles in maintaining water and nutrition homeostasis	**[58]**
	PIP1;5/PIP2;3	*S. bicolor*	Root and leaf	Maintain WUE	**[57]**
	H+-ATPase	*C. sinensis*	Leaf	Maintenance of K+ homeostasis in mesophyll cells	**[96]**
	OsHAK1	*O. sativa*	Root and shoot	Involved in K acquisition, translocation, and homeostasis by upregulating *OsTPKb* and *OsAKT1*	**[59]**
	OsNAC5/6/9/10	*O. sativa*	Root	Target genes were involved in transmembrane/transporter activity, carbohydrate metabolism, vesicle and plant hormones	**[97]**
Cold	HSP70-16/VDAC3	*A. thaliana*	Seed, endosperm and embryo	Activation of the opening of VDAC3 ion channels, ABA transportation from endosperm to embryo and then inhibit the seed germination	**[98]**
	CsSWEET16	*A. thaliana*	Leaf and flower buds	Sugar transport across vacuoles and cold tolerance	**[31]**
	TsABCG11	*A. thaliana*	Root, stem leaf, rosette leaf, flower and silique	Thickening the leaf cuticle layer (wax and cutin) by exporting cuticle lipid molecules to prevent plants from cold stress	**[76]**
	AlTMP2	*A. littoralis*	Root and leaf	Improve membrane stability	**[99]**
	AtPIP1;4/AtPIP2;5	*A. thaliana*	Root and shoot	Play a role in cold acclimation and freezing tolerance	**[75]**

	VAB3/NHX2/NHX5	*E. botschantzevii*	Shoot	Cold acclimation	**[100]**
	SOS1/VP2/HA3	*E. salsugineum*	Shoot	Cold acclimation	**[100]**
Heat	TaZnF	*A. thaliana*	14-day seedling	Larger primary roots, lore lateral branches, increased in leaf size and numbers, promote early flowering and enhanced fresh biomass	**[16]**
	P4-type ATPase	*A. thaliana*	14-day seedling, rosette leaf, flower (stamen and pistil) and silique	It involved in flipping of lipids that cope with heat stress	**[64]**
	OsSUS	*O. sativa*	Flag leaf, stem-sheath and spikelet	It acts as a signalling molecule to mediate source and sink relationship under heat stress	**[101]**
HMs	TpNRAMP3	*T. polonicum, Polish wheat*	Leaf and root	Transport Cd, Co and Mn but not transport Fe or Zn that induced HMs toxicity	**[21]**
	PtABCC1	*P. trichocarpa/A. thaliana*	Root	It enhances the accumulation and tolerance to Hg	**[83]**
	PtoABCG36	*A. thaliana*	Leaf/stem/root	It acts as an extrusion pump to decrease Cd uptake and enhance tolerance to Cd stress	**[84]**
	PtoABCG36	*O. sativa*	Root and shoot	Export Cd from root and enhance Cd tolerance	**[85]**
	OsSMP1	*O. sativa*	Leaf	Acts as a positive regulator of Cd and Cu tolerance via ABA-dependent pathway	**[82]**
	LmSAP	*Tobacco*	Leaf and root	Enhance accumulation of Cu, Cd and Mn, decrease H2O2 content, upregulated SOD, POD and CAT activities and stress related metallothioneins i.e., Met1-5	**[102]**
	AtCNGC1/10/13/19	*A. thaliana*	Primary root and seedling	Play a role Pb toxicity by reducing its uptake	**[103]**
	AtCNGC11/13/16/20	*A. thaliana*	Primary root and seedling	Play a role Cd toxicity by reducing its uptake	**[103]**
	SaNramp6	*A. thaliana*	Root, stem and leaf	Improve Cd accumulation	**[104]**
	OsLCT1/OsHMA2/OsZIP3	*O. sativa*	Root and shoot	Co-expression of HM transporters improve the root and shoot lengths under Zn and Cd stress	**[105]**

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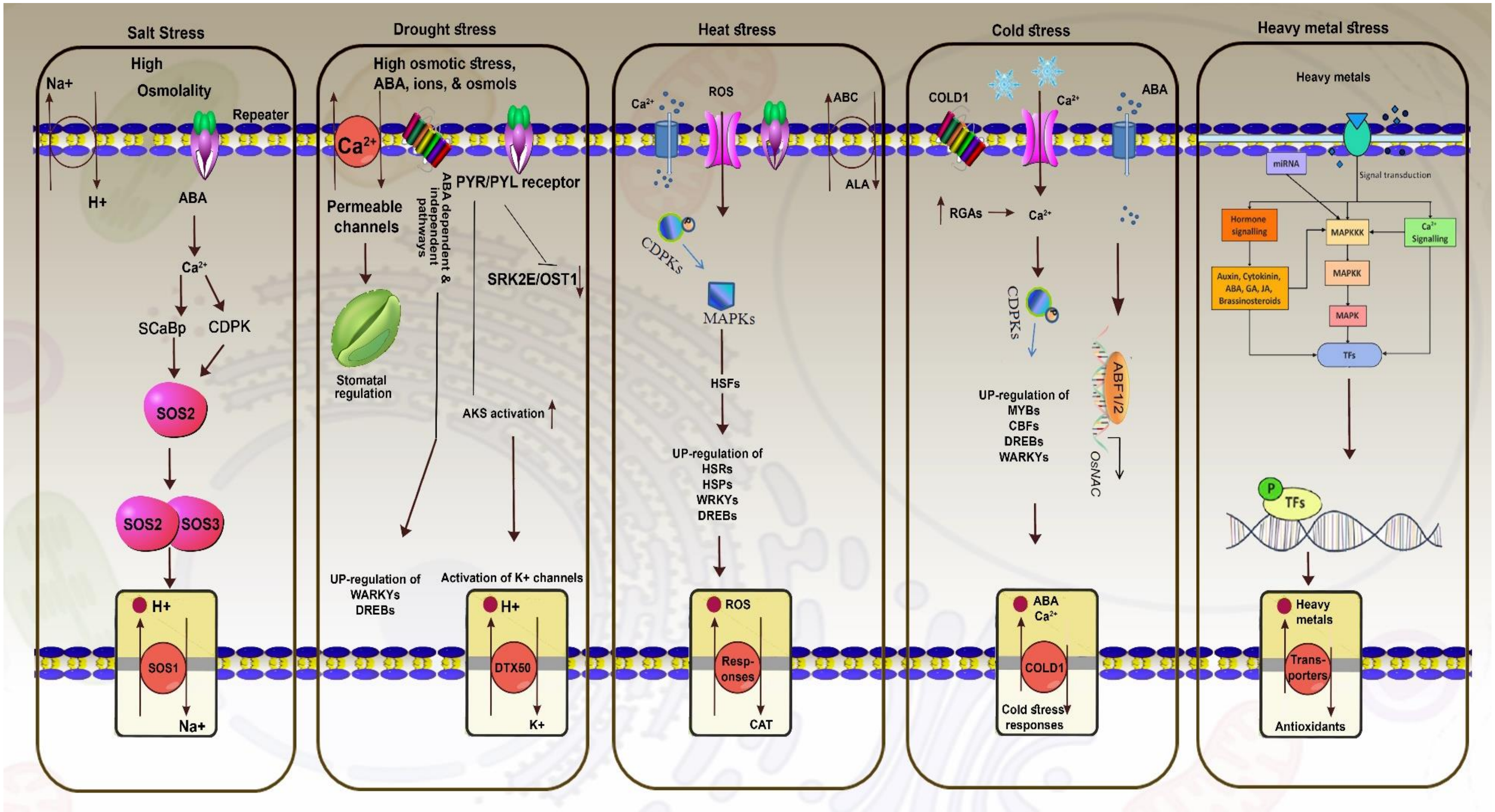

Figure 3. Role of MTs in response to abiotic stresses: Illustration of each stress with their transporter proteins showing that up-regulation and down-regulation of gene in response to stressed plants. For more details see text. .

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4. Closing remarks

The global role of MTs in the improvement of PG&D and abiotic stress tolerance is recently been appreciated extensively. As the MTs networking spreads in all plant's parts. So, they efficiently regulate cellular and long-distance trafficking that is usually plant-stage and environment driven. In the PG&D, *OsNPF7.2*, *OsNPF7.3* and *OsZIP7* genes in rice improved the plant architecture by enhancing the root length, tiller numbers and grain yield as well as involved in the translocation of N and other mineral elements such as Zn and Cd towards grain. For continuous normal functioning, plants need regular supply of sugar molecules including sucrose, fructose and glucose etc. and water, and mineral elements. For this job, plants have plenty of MTs (almost 18% of Arabidopsis genome are associated with cellular membranes [106] and ~ 2850 putative unigenes in *B. napus* acts as MTs under Cr stress **[18]**). They remobilize photosynthesis assimilates with the help of SUT, SUC and newly discovered SWEET genes families. Moreover, MTs are specialized to transport sugar molecules through VM and PM from the leaf to growing tissue such as roots, rosette leaves and seeds. However, under the stressed conditions (i.e., salt, drought, cold, heat and HMs) source-to-sink coordination disturbed. Under the unfavorable conditions, plants activated stress specialized MT genes families such as HKT, FC, EXPA and SAP (salt); PIP and ATPase (drought); ZnF and P4-type ATPase (heat); PIP and ABC (cold); and NRAMP, SMP, ABC and HMA (HMs). Mainly, these MTs maintain the Na^+/K^+ ratio by uptalking more K^+ and controlling of Na^+ efflux at VM and PM, homeostasis of mineral elements in the cell as well as export to upper parts like grain to enhance the grain quality and water trans-membrane activity under salt and drought stress. Under extreme temperature stress, MTs protect membrane integrity and also helps plants to adopt multiple strategies such as enhance the number of leaves, side branches, larger primary roots and earliness in flowering. The MTs response to cold stress is slightly similar to drought and heat as they are involved in minimizing the osmotic stress and ion homeostasis but additionally, they export lipid molecules to strengthen leaf cuticle layer. In case of toxic elements, MTs play a role in uptake of HMs such as Cd, Co, Cr and Mn and translocate them into root tips and shoot and even to leaves or other upper parts. Moreover, the MTs could enhance the phytoremediation capacity of also enhanced the activities of antioxidants to minimize the ROS levels. As a whole, engineering of MTs could enhance sugar and water supply as well as better ionic homeostasis leads to increase the PG&D and withstand capacity of plants against dynamic climate changes. As now more near-to-complete genomes have already been assembled in >400 of plant species and still continuing even with better version/s. So, researchers need to explore whole genome MTs as "Transportome" in a sophisticated network related to developmental and environmental cues.

Conflicts of Interest: All authors declare that they do not have any conflict of interest related to this study.

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