Analysis on The Role of Arbuscular Mycorrhizal (AM) Symbiosis in Enhancing Tolerance to Different Abiotic Stresses: Through A Proteomic Approach

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This review paper analyzes the contribution of Arbuscular Mycorrhizal (AM) symbiosis in enhancing endurance to different abiotic stresses including drought, salinity, heavy metal, and heat stress. AM symbiosis, a mutualism between AM fungi and plants, induces protein cascades, phytohormone biosynthesis, glycopeptide secretions, and morphological changes that help plants to change appearances, regulate osmotic homeostasis and remove reactive oxygen species (ROS) from the external stress. The paper focuses on complex interactions of specific protein networks to protect the cell, with diagnosis on the possible direction of future studies. It emphasizes the interdisciplinary approach based on engagement of local society to achieve sustainable agriculture.

Keywords: Arbuscular mycorrhizal symbiosis, Abiotic stress, Drought, Heat, Heavy metal, Homeostasis, Protein cascade, Salinity, Sustainable Agriculture

Introduction

The importance of preserving plant resources and improving the agricultural industry has dramatically increased due to exacerbation of climate change. Anthropogenic activities since the Industrial Revolution have left a tremendous carbon footprint and environmental contamination on the Earth, and their adverse effects are threatening the sustenance of food resources. Increase in greenhouse gas concentration not only hinders photosynthesis capabilities, but it aggravates land degradation and global warming, fostering a more barren environment for the plants to grow. For instance, the representative concentration pathway (RCP) 8.5 climate model, assuming the carbon emission will be constant until 2050, suggested that the production of major crops in the United States would drop by 31% by 2020[1]. The socioeconomic aspect of the issues should also be emphasized, since it not only threatens the food sustenance of countries with low food security levels, but also aggravates the chronic poverty of low-developed countries. An interdisciplinary approach among sociology, ecology, botanics, and molecular biology is currently imperative to achieve sustainable development.

The increase in the global mean temperature triggers drought, floodings, extreme temperature, higher salinity, and subsequent land degradation, which destroy the farms and foster a favorable environment for plant pathogens to disseminate. These factors provide abiotic stress to crops by rendering the habitats inhospitable to plants. Globally, the economic loss from increasing plant diseases recorded at least $220 billion, which takes 40% of economic revenue from agricultural activities (Food and Agriculture Organization of United Nations, 2021). Also, it is expected that 69% of the total capability of plant production is lost due to the effect of abiotic stresses[2]. Therefore, obtaining organic and eco-friendly solutions from nature has been crucial for human society to solve the food production crisis. With increasing attention on the role of microbacteria in the ecosystem as carbon absorber and nutrient benefactor against climate change, utilizing arbuscular mycorrhizal (AM) symbiosis between mycorrhizae and plants has emerged as one of the novel solutions that utilizes existing beneficial relationships between microbiome and plants. It is known to provide trace nutrients and induce a change in genetic expression, which activates the signal transduction pathway promoting growth and immunity[3]. As the most detailed and fundamental comprehension of the biological process comes from the transcription of proteins, taking a proteomic and molecular view would be important in this research to define the impact of AM symbiosis on the plants. This research focuses on analyzing the role of AM symbiosis in enhancing tolerance to different abiotic pressures through proteomic approaches.

Arbuscular Mycorrhizal Symbiosis

To define the key terms, AM symbiosis is a type of mutualism between plant roots and Arbuscular Mycorrhizae such as Glomeromycota. The fungus provides nutrients for the plant (N, P, S, Zn, Cu^{2+}) and accumulates CO2 in the soil, whereas the plant

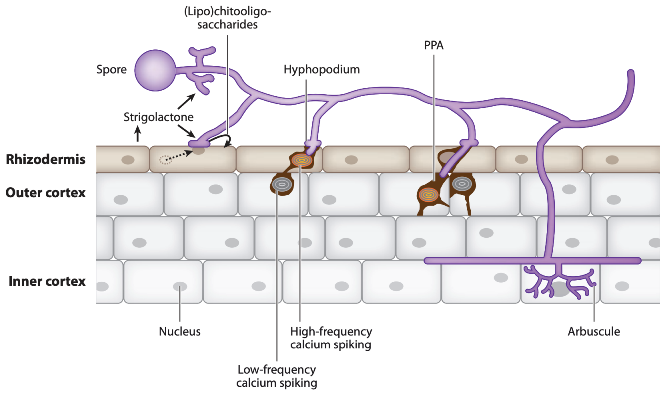

Fig. 1 The accommodation of AM fungi, taken from Gutjar and Parniske(2013)[6]

provides the places for fungal habitation[4]. In an evolutionary perspective, discovery of AM symbiosis in ancestor plants such as ferns and lichen implies that the relationship assisted plants to settle down in the harsh land environment[5].

Gutjahr and Parniske(2013)[6] defined the process of vesicular AM accommodation inside the cortical cell of the plants. The plants develop a prepenetration apparatus (PPA) with stretched hyphae in order to exchange materials, forming an arbuscule in the inner cortex. It is followed by the high-frequency calcium spiking as a secondary messenger of the activating signal pathways of discerning the AM fungi.

The recent studies focus on the role of AM symbioses in enhancing the expression of immunity related genes, activation of resistance genes, and pathways such as Induced Systemic Resistance (ISR) and Microbiome Associated Molecular Pattern (MAMP). The two pathways refer to enhancement of chemical barriers to protect plant cells from invasion of specific pathogens. Hao's study(2019)[7] summarized the interactions between pathogens and AM: (1) ameliorated plant growth, (2) enhanced ISR through secretion of jasmonic acid(JA) and ethylene, which allows transcription of defense genes, (3) modified vector pressure that suppresses reproduction of nematodes in the plant roots[7,8]. A study also revealed transcriptional changes in genes encoding proteins specialized for AM symbiosis, using RT-PCR on a sample RNA of M.truncatula infected with G. intraradices[3]. However, several researchers suggested that increased transfer of phosphates promoted the growth of yellow dwarf virus in barley and cereal, not providing protection for the plants[9]. Therefore, the function of AM symbiosis remains as both friends and foe to the plants, despite their clear benefits. Reviewing prior research regarding immunity is necessary, since they suggest that AM symbiosis might cause any proteomic changes to defend the plants from external threats.

Mechanisms against Abiotic Stresses

It is also crucial to focus on the ability of AM symbiosis to mitigate impacts from abiotic stress, not confined to pathogenic infections. Abiotic stress refers to inorganic external stimuli that impede the survival and growth of the plants, including drought, high salinity, extreme temperature, and heavy metal[10]. This section will concisely explain the effect of the abiotic stresses and existing proteomic mechanisms to deal with them. Comprehension on proteomic response of the plants to abiotic stress is highly important as they inspire the core question of this discussion: how can symbiosis with other microbiomes influence this protein network?

1. Drought can be described as a prolonged lack of precipitation. It was addressed that drought lowers the water potential of plant cells and impedes photosynthesis or transpiration by adjusting the enzyme and hormone activities (Figure 2)[11]. As a response, various proteins related with water movement such as aquaporins and membrane-stabilizing proteins are expressed to facilitate water supply. Phytohormones(plant-induced hormones) and signal molecules(Ca^{2+}) are released to induce Mitogen-Activated Protein (MAP) cascades, which are a series of protein responses that produce multiple transcription factors regulating drought resistance. For instance, an experiment reported that the pathway induced adjustment in transcription of more than 770 genes of Arabidopsis thaliana[12]. Also, the pathways can order expression of antioxidant activation to remove toxic components from the stress, amino acids proline synthesis to balance the osmotic concentration, and proteins for stomatal closure, to prevent water loss through transpiration. In addition, it is remarkable that plants undergo rhizogenesis, which is formation of short, tuberized, and hairless roots against osmotic change, since it leaves the question of whether microbiomes can interact with newly formed roots. In summary, change in protein activities and superficial appearance assisted plant's adaptation to droughts.

2. Salinity stress can be defined as excessive influx of sodium and calcium ions from the root cells due to land degradation. It can be summarized that it causes high osmoscity and nutrition toxicity, leading to the denaturation of cell membrane and protein[13]. This additionally disrupts photosynthesis and seed germination, with toxic spare oxygen radicals called reactive oxygen species (ROS). A study identified the molecular mechanisms of plant response to salinity stress[14]. The salinity overly sensitive (SOS) pathway composed of a family of proteins that detect salinity, capturing the Ca^{2+} release from the root and pumps out the excess Na^{+} in the cytoplasm. MAP cascades composed of MPK proteins control the signal transduction and

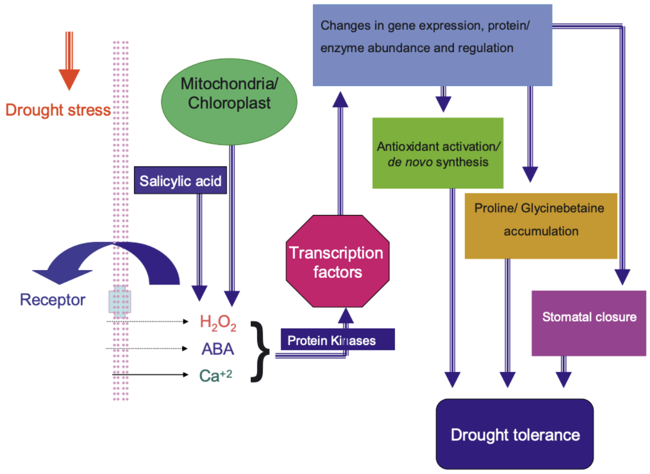

Fig. 2 Proposed cellular events against drought stress, taken from Farooq, et al.(2009)[11]

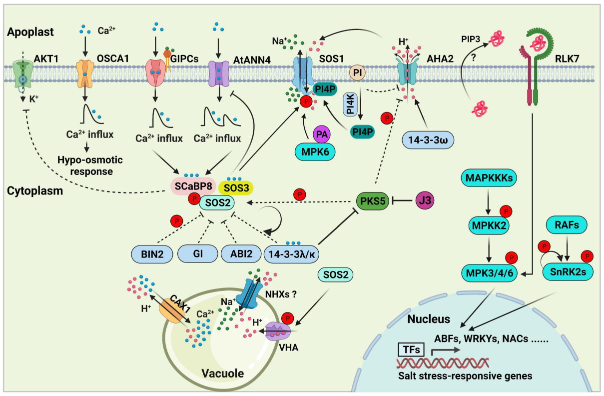

Fig. 3 Signal transduction pathway of maintaining ion homeostasis from salt stress, taken from Liang Ma, et al.(2022)[17]

produce the precursor of the transcription factor for expressing self-stress responsive genes[15]. HTK proteins act as K^+ transporters to remove excessive Na^+ in the root cells, helping to maintain the balance between Na^+ and K^+[16]. In addition to the protein cascades above, diverse Ca^{2+} dependent proteins detect messengers such as Ca^{2+} and phytohormones, triggering protein phosphorylation and change in gene expression. Therefore, plants have evolved interactions between proteins to increase salinity resistance.

3. Moreover, extreme temperature due to global warming can also be an abiotic stress, which negatively affects blooming, fruit production, strength, or habitat of plant communities. High temperature denatures proteins or nucleic acids, decreasing the photosynthesis rate of chloroplast and vital gene expressions[18]. Heat Stress Response-Heat Shock Protein (HSR-HSP) pathway enhances tolerance to high temperature, with Heat Shock transcription factor A1s (HsfA1s) as the master regulator of the heat-stress response. Then epigenetic modifications and action of small RNAs assist the expression of HSP. The study showed that Phytochrome Interacting Factor 4 (PIF4) is operated by thermal signals to cause the thermomorphogenesis of plants, a change in appearance such as the elongation of hypocotyl to improve transpiration. The pathway amplifies production of phytohormones not limited to auxin, brassinosteroids (BR), and gibberellin (GA), which develop hypocotyl, produce enzymes and transcription factors for biosynthesis, and decompose inhibitor proteins. This metabolism can be adjusted by different sunlight wavelengths, temperatures, and circadian clocks[18,19]. Thus, it can be concluded that plants developed mitigation of heat shock and thermomorphogenesis by signal pathways.

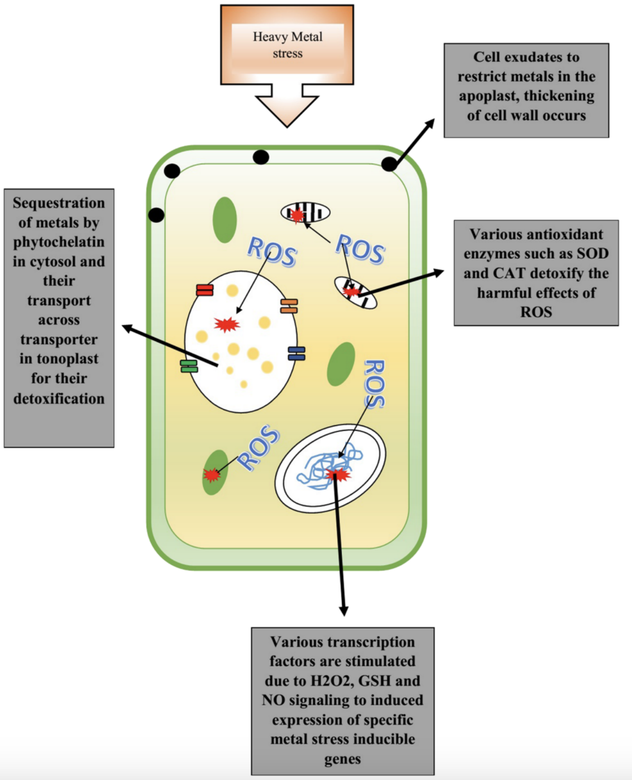

Fig. 4 Signal transduction pathway of maintaining ion homeostasis from salt stress, taken from Ghori, et al.(2018)[20]

4. Lastly, accumulation of heavy metal can be a significant abiotic stress in modern society. Toxic metal elements such as Cd, Mn, Zn, Cu, Fe, Co, Ni, As, have increased due to

rapid industrial growth and waste contamination. Though limited amounts of metal elements are required as trace nutrients and cofactors in plant metabolism, excessive influx impacts the homeostasis of an organism. According to the study of Ghori, et al.(2019)[20], primary response to the stress contains inflammation of the cell, thickening of the cell wall, and change in pH level of the rhizosphere in order to prevent uptake of heavy metal. In similar cases of other abiotic stresses, phytohormones(especially jasmonic acid, ethylene, and salicylic acid) and phenolic compounds lessen lethal impact from ROS. Also, factors such as H2O2 and N2 are found to be linked with pathways such as the calcium-calmodulin pathway(cellular target for metal particles) and MPK cascade mentioned earlier, producing transcription factors for the expression of metal stress-resistant genes and antioxidant enzymes. A study also revealed that certain Quantitative Trait Loci (QTL), a unique genetic region that causes variations in phenotype, is related with expression of heavy metal stress resistance in Ze, Fn, and Cd.(Courbot et al, 2007)[21]. The bioremediation of heavy metals, which is alleviation of toxicity using organic sources, through assistance of Plant-Growth-Promoting (PGP) bacteria has been observed, which will be discussed later.

These mechanisms address sophisticated proteomic interactions regarding how plant species respond to abiotic stresses. The following review would proceed on to how AM symbiosis can impact these proteomic order and functions to enhance tolerance against four abiotic stresses in a molecular scale. It also suggests how the relationship between microbiome and plants should be further studied.

Results

Drought Stress

The hyphae of AM symbiosis improve the efficiency in water absorption by increasing the surface-to-volume ratio in the root hair. In addition, it functions to regulate absorption of water resources, osmotic balance, and cascades through causing change in protein interactions related with drought endurance. It was identified that specific combinations of AM fungi based on their inocular identity can provide different effects to the soybean culturing, implying that AM fungi and plants share mechanisms to selectively recognize each other[22]. The studies of Al Karaki, et al.(2004)[23] and Gholamhoseini, et al.(2013)[24], AM plants showed greater leaf size, root length and strength, and nutrient fertilization. Several studies have observed overall benefits provided to the plants by AM symbiosis. For instance, Boutasknit, et al.(2020)[25] addressed that AM symbiosis improved physiological and biochemical parameters including stomatal conductance, the maximum photochemical efficiency of PSII (Fv/Fm), and plant uptake of mineral nutrients (P, K, Na, and Ca), which were apparently higher in AM shoots than non-mycorrhizal (NM) shoots. It also reported a decrease in accumulation of H2O2 and malondialdehyde, which cause oxidative damages in Ceratonia siliqua. Examining how AM symbiosis orders particular molecular change can be specified into the context of osmoregulation, biosynthesis, proteomic and genetic alteration, and providing defense to antioxidants.

1. AM symbiosis assists osmoregulation, achieving balance in water potential inside and outside of the cell. AM fungi convey ion electrolytes that are vital for survival and metabolism of plants; for instance, K^+ is related to causing stomatal closure and maintaining stem strength. According to the study of Yooyongwech, et al.(2016)[26] using sweet potatoes, osmoregulation occurs through increased absorption of solutes called osmolytes, such as sucrose and proline. These components have various functions for adjusting water potential in that they act as osmoprotectants: lowering increased osmotic pressure inside the plant cells, maintaining cell turgor during the drought, and stabilizing macromolecules and initiating signals and pathways for plant growth. However, the osmotic effect of sugar and proline is still controversial in spite of its benefit to mitigate drought injuries. Ruiz-Sánchez, et al.(2010)[27] presented low proline levels in AM plants compared with non-mycorrhizal plants, implying the formation of proline in AM plants. This might be due to the fact that AM plants are originally less susceptible to the drought and enhanced proline degradation suppressed the glutamate synthesis pathway for producing proline (Zou et al., 2013)[28]. Therefore, further research incorporating differences in environment and plant species among the prior studies is required.

   Moreover, there has been continual research on the post-transcriptional and post-translational regulation of aquaporin expression by AM symbiosis. Aquaporins are membrane-intrinsic proteins that control the movement of water across the cell membrane. Ruiz-Lozano, (2003)[29], Javot and Maurel (2002)[30] proposed that improved water absorption by AM fungi must indicate greater activation of aquaporins to facilitate the water supply. Uehlein, et al.(2007)[31] suggested that increased MtPIP2;1 and MtNIP1 protein expression in AM plants are results of physiological changes in order to assist the water transport. The study of Li, et al.(2013)[32] observed reinforced expression of aquaporin genes GintAQPF1 and GintAQPF2 in G. intraradices under drought situations. The experiment of Barzana, et al.(2014)[22] using maize plants revealed that a certain group of ZmPIP2;2 and ZmPIP2;6 genes related with aquaporin formation was upregulated by AM symbiosis during sustained drought, although the specific relationship was unclear during short-term droughts. Furthermore, the study of Quiroga, et al.(2019)[33] noticed increased phosphorylation of PIP2 aquaporins and prominently high water permeability coefficient in only AM plants. However, since it is still difficult to clearly determine the mechanism that AM symbiosis activates aquaporin expression, extra research would be required to identify the specific pathways of aquaporin formation.

2. In addition, AM symbiosis contributes to the biosynthesis of phytohormones and glycopeptides, which offer enhanced endurance to external stresses. It is important to find out the role of phytohormones since they are synthesized and functioned under expression of certain genes and initiation of protein-mediated signal pathways, while phytohormones can also influence protein expression and networks, vice versa. It is reported that secretion of hormones such as ABA, Indoleace-tic Acid (IAA), and Indole butyric acid (IBA) and gibberellin (GA) was prominently greater in AM plants when compared with non-mycorrhizal plants[34]. Abscisic Acid (ABA) is a hormone essential for AM colonization and drought resistance. AM symbiosis enhancing ABA secretion has been observed in various plant species including tomato , maize, and lettuce, et cetera[16,35–38]. Not only host plants but also AM fungi produces ABA itself, supplementing ABA level in plants therefore leading to stomata closure, reduced transpiration, and expression of drought tolerance genes[36]. Not limited to ABA, the table below contrasts summarized functions of different hormone pathways that are activated or produced from AM fungi during the drought stress. Secretion of glycopeptide glomalin supported improved water and nutrient uptake from the topsoil. The review emphasizes the importance of focusing on the remaining questions on the ambivalent effects of AM-derived phytohormones. Recent studies show that several hormones such as IAA and gibberellin are found to interact with auxin to maintain proper hormone level. Since auxin has contrasting effects in low level and high level, this property appears to be concerned with controversial effects of phytohormones. Also, gibberellin shows opposite influences on Arum-type and Paris-type AM plants, divided based on morphological differences[44]. Some hormones make crosstalk with each other or show antagonistic effects. The researches still suggest that secretion of hormones from AM fungi can be both beneficial or harmful to the plant, requiring more delicate research and genetic techniques to maximize benefits from augmented endo-hormone production. Also, there are possibilities that different plants possess different signal transduction systems, showing distinguished impacts from phytohormones.

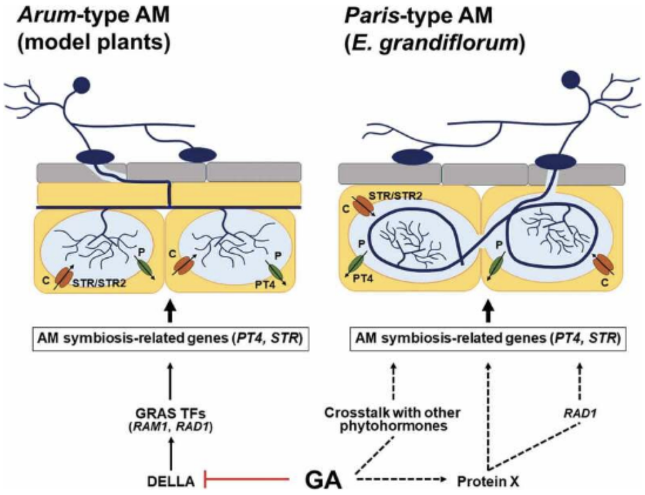

Fig. 5 Gibberellin response in different type of AM plants, taken from Takaya, et al.(2020)

3. The change in protein interactions and gene expressions caused by AM symbiosis is also remarkable under drought stress. From aquaporins to precursors of phytohormone synthesis, AM symbiosis can order specific expression of certain genes that can augment endurance to abiotic stresses. Zou, et al.(2019)[49] showed expression of several Plasma-membrane intrinsic proteins (PIPs) and Nodulin-26 like intrinsic proteins (NIPs) leading to better endurance to drought stress, again highlighting the aid of AM fungi on water transport. One of the controversial proteins to focus on is Late Embryogenesis Abundant (LEA) proteins, which are known to protect internal proteins from osmotic imbalances or desiccations[50]. However, it was reported an unmeaningful change in expression level of LEA-D11 in AM-inoculated lettuces, suggesting that LEAs are not concerned with drought endurance. As addressed, complicated interactions among proteins have contributed to efficient symbiosis. With the development of DNA sequencing technologies and softwares such as BlastX, genome studies on population-level would help comprehension on the roles of protein in AM symbiosis. Also, studying the relationship with mRNA splicing mechanisms and genetic recombination of preferred proteins would open a novel way for mass plant production.

4. Lastly, the mechanisms of AM symbiosis that eliminate ROS is crucial to minimize plant damage from the abiotic stress. It is reported that AM plants produce antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GPX), as well as some antioxidants including ascorbic acid (ASC) and glutathione (GSH)[51]. In addition, Zhumei, et al.(2022)[52] identified reduction in

Table 1: Comparison of different phytohormones that react drought stress

Name	Function	Details
Abscisic Acid (ABA)	Induction of arbuscule formation[35], Stomata closure, Increase in hydraulic conductivity[39]	Expression of gene NCED(essential enzyme of carotenoid-based ABA synthesis pathway)[37], Require PP2A Phosphatase genes to secrete ABA[40]
Indoleace-tic Acid (IAA)	Induction of arbuscule formation at low level and repression at high level[41]	AM induced gene SIGH 3.4 producing IAA-amido synthase, negatively regulating mycorrhization[42]; Drought stress causing IAA oxidase, reducing IAA capability[41]
Indolebutyric Acid (IBA)	Lateral root growth, AM response imitation in non-AM plants	Secretion of IBA from AM colonies in maize[43]
Gibberellin (GA)	Leaf and stem elongation; Inhibition of arbuscules formation in Arum-type AM plants[44]; Reported promotion of arbuscules formation in Paris-type AM plants[45]	Degradation of DELLA protein, impeding AM responses in Arum-type AM plants; Hormone crosstalks and unknown protein promoting AM responses in Paris-type AM plants[44]
Jasmonic Acid (JA)	Providing drought resistance by osmotic adjustment and antioxidant properties, Forming arbuscules; Decrease of colonization in cases[45]	Expression of allene oxide synthase and jasmonate-induced JIP23, necessary for JA synthesis[46]; Expression of PR4 defense gene reducing AM activations[45]
Salicylic Acid (SA)	Induction of pathogenic defense (ISR and MAMP), ion regulation and carbohydrate metabolism[47], aquaporin regulation[48]	Decrease in production of Lpr and Lo with possible correlationship of increased PIP2 aquaporin formation, Alteration of NO pathways[48]

O2-, H2O2, and malondialdehyde(MDA) production in AM plants, which are harmful byproducts of metabolism. More specifically, heat shock proteins (HSP) appear to be concerned with removal of ROS. Wen-Ya, et al.(2022)[53] further elaborated that components of the heat shock transcription factors family such as SPL7, HsfA1b, HsfA4a, and HsfA8 showed increased expression in AM plants and improved endurance to ROS. These antioxidants assist to protect proteins and nucleic acids from oxidation, which is a shared mechanism in all four abiotic stresses covered in this review paper.

Salinity Stress

Recent studies have revealed that AM symbiosis contributes to mitigation of salinity stress. The symbiosis helps osmoregulation of water, nutrient, inorganic ions, and organic acids against the stress.

1. AM fungi evoke morphological changes to the plants to minimize osmotic imbalances and photosynthetic losses. Zhu, et al.(2018)[54] reported reinforced root epidermis and biomass, greater leaf size, and increased stomatal conductance and maximum rate of gas to water vapor in AM plants. Since salinity stress restrains photosynthesis capability and ceases cell division of the root system, it is imperative for plants to secure methods to continue photosynthesis. These changes in appearances clearly address historical adaptations of the plants under extreme conditions for survival.

2. Several studies have proven that AM symbiosis assists osmoregulation of water and ions(especially Na^+, Cl-, and K^+). For instance, Hao, et al.(2021[7])'s experiment compared AM and non-mycorrhizal maizes in 0 or 100mM NaCl, and AM plants showed dense roots, better Na^+-K^+ level homeostasis, and differential regulation of ion transporting genes such as ZmSOS1. In addition, Zhu, et al.(2015)[55] showed improved ability in P and Ca storage, carbon assimilation, and nitrogen fixation in the roots of the plants to increase efficiency of metabolism. Meanwhile, it is crucial to comprehend the protein network that helps osmoregulation, as depicted in the subsequent illustration.

In order to avoid mass influx of NaCl into the cytosol, plants maintain a low level of Na^+ inside the cell; in other words, relatively high K^+:Na^+ proportion. NHX, the Na^+-H^+ antiporter(transporting two molecules in opposite directions simultaneously), exchanges both elements, pumping out the sodium ions outside the cell. SOS protein family consists of a pathway that removes excess Na^+ inside the cell from the NaCl stimuli. HKT protein(High Potassium transporters), AKT protein, and SKOR protein emit Na^+

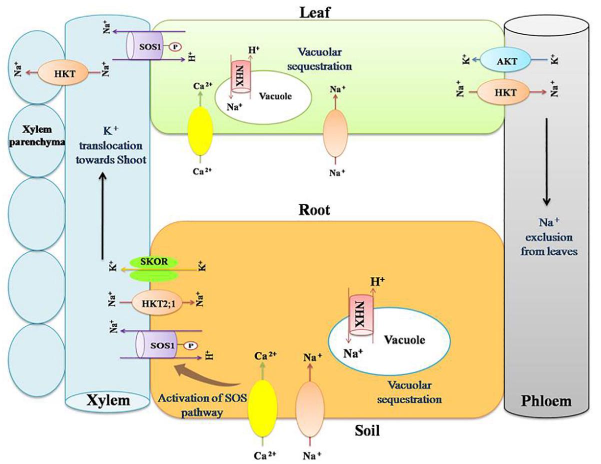

Fig. 6 Protein network of osmotic regulation, taken from Heikham, et al.(2019)

Table 2: Different osmolytes related with salinity regulation[56]

Name	Function
Proline	Stress marker and osmoprotectant ro maintain cell turgor; Improved nutrition supply; Recovery of salinity-derived denaturations
Polyamines	Solutes maintaining osmotic balance; Include putrescine, spermine, and spermidine as major polyamines
Sugars	Hydrolysis of polysaccharides and disaccharides to glucose to keep uniform osmotic level
Trehalose	Reducing ROS, assisting osmolyte formation, keeping Na^+-K^+ ratios

outside the cell and draw K^+ inside the cell, keeping homeostasis. These multiple systems of proteins show intricate mechanisms of adjusting Na^+, K^+, and Ca^{2+} level.

On the other hand, maintenance of enzymes and micronutrients are also important. The ability of AM to regulate the influx and release of ions and water improves photosynthesis by expressing the proteins composing the photosystem II, carotenoids(act as pigments and antioxidants), and chloroplasts. It is also known to increase the activities of RubisCO, photosynthesis apparatus, and other enzymes.(Evelin et al., 2019)[56] Parcel, et al.(2011)[57] added that AM fungi is also related with the expression of aquaporin, LEAs, Na^+-H^+ antiporters, and cyclic nucleotide gateways in the plasma membrane, which are the specific gateways for inorganic ions in the plants. The AM symbiosis enhances the activation of proteins, facilitating water and ion transport against the increased salinity.

3. Moreover, the AM fungi lessen the negative impact from ROS through a similar mechanism as with drought stress. Not confined to the increased expression of ROS-related enzymes listed above(SOD, CAT, APX, et cetera), diverse osmolytes help to reduce NaCl-derived damages and eliminate ROS accumulated in the cytosol. Table 2 organizes different functions of osmolytes[56]. Not only osmolytes mentioned in Table 2 but some amino acids and fatty acids also can function as osmolytes. It is important to remember osmoregulation is the collaboration of interaction of protein families and synthesized osmolytes. Since salinity stress and land degradation is a serious ongoing issue, utilizing AM symbiosis with the ability to lessen negative impact from salinity stress would greatly improve crop production in barren areas such as the Sahel region. This paper expects that research on the salinity-tolerant genes of plants originally living under marine ecosystems, such as seaweeds and Gelidium amansii could lead to genetic recombination that could create a totipotent plant with maximized salinity resistance.

Heat Stress

AM symbiosis is known to mitigate harmful effects from heat stress as reported in several studies. For instance, Nguyen, et al.(2018)[47] compared the biomass of AM and non-mycorrhizal maizes under drought stress, and then identified that AM symbiosis alleviated negative impact on plant growth from the high temperature. Also, the experiment of M. Reva, et. al(2021)[58] supplemented that greater nutrition absorption and production ability and fruit qualities were observed in the AM plants. The benefits of AM symbiosis on providing endurance to heat stress can be specified into evoking metabolism change with thermomorphogenesis, regulating diverse protein expression, and elimination of ROS oxidants.

1. AM fungi can cause change in growth and pattern formation of the plants under high temperature, known as thermomorphogenesis. The process includes elongation of hypocotyl, seed germination and root growth, and reinforcement of body structure which improve transpiration, water use, and nutrition(C, P, N) absorption. In molecular perspective, the study of Xio-Jie, et al. (2022)[17] using Arabidopsis and endophytic fungi showed an increase in expression of DNA replication-related genes, secretion of ethylene and jasmonic acid, and regulation of PIF4 protein, which evokes thermosensory growth responses. The PIF4 pathway also augments production of auxin, brassinosteroids (BR), and gibberellin (GA), which helps plants to strengthen body structure and adjust it to minimize heat damage.

Phytochrome B is a thermosensory receptor that senses change in temperature and daytime length, even changing the Pf-Pfr conversion that adjusts plant adaptation to time and temperature, affecting speed of blooming and organ formation. Evelin, et al.(2019)[56] identified that AM plants contain higher levels of chlorophylls and light-harvesting ability, therefore improving photosynthesis capability and showing higher fv/fm ratio especially focused on photosystem II. Sonal, et al.(2021)[59] contrasted levels of photosynthetic parameters including activation of photosystem and electron transport, which were found to be lower in non-mycorrhizal plants. Also, it discovered that AM plants had a significantly higher proportion of neutral lipid fatty acid (NLFA) with lower lipid peroxidation than non-mycorrhizal plants, allowing better root growth, sturdiness, and photosynthesis. The result indicates that NLFA and prevention of lipid peroxidation prevented plant body structures from high heat stress. These mechanisms would function as the primary defense to the heat.

2. Expression of several proteins related to heat stress are also regulated by AM fungi. Following studies are important in comprehension of the role of AM fungi in adjusting proteomic components. Viktor, et al.(2023)[53] found that AM symbiosis increased expression of phosphate transporter proteins that facilitated nutrition movement and maintenance of the solute potential. Hongjian, et al.(2023)[60] suggested a new experiment regarding heat stress using melatonin application on AM plants, shown to be connected with ABA, GA, and cytokine pathways affecting plant strength. Also, the treatment increased the expression of chlorophyll-catabolic genes(CCGs), senescence-linked genes, and transcriptional factors of melatonin and phytohormone biosynthesis. Consequently, Perennial Ryegrass showed increased photosynthesis, turf quality, strength, and decreased membrane lipid peroxidation. This research is important in that it attempted to find out diverse combinations of external chemical treatments on AM symbiosis to maximize expression of heat endurance. However, this paper could not find a meaningful relationship between AM inoculation and HSP activation, which is one of the principal mechanisms in reaction to the heat stress. Also, some research showed variable results, with certain genes downregulating or upregulating the metabolism process. These differences might originate from lack of comprehension on the function of unique genes and proteins in individual scales. Therefore, future studies should focus on investigating genotypic compatibility between AM fungi and plant species, as well as comparing the amino acid sequence among specific gene families that provide stress tolerance to the plants, in order to reduce discrepancies in the research results.

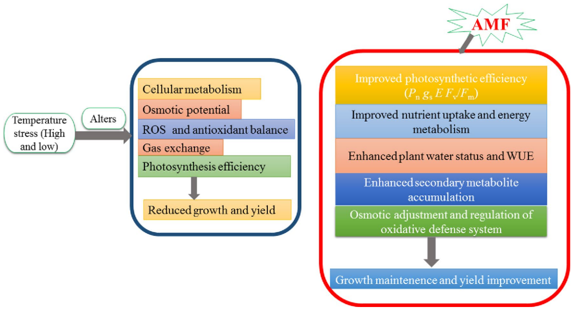

Fig. 7 Impact of AM fungi on plant response to heat stress, cited from Naheeda, et al.(2019)[61]

3. Removal of ROS and oxidants is also important under heat stress, but the elimination occurs in a similar process as that of drought stress and salinity stress. AM fungi increase the activation of ROS scavenger enzymes and reduce production of oxygen byproducts and malondialdehyde (MDA). Figure 7 summarizes response to heat stress of AM plants. Since the heat stress is most directly correlated with drought issues of climate change, it is crucial to utilize the benefits of AM fungi in alleviating heat and drought stress. In the history of evolution, C4 and CAM have been the main drought-resistant metabolism of the plants, by separating the time or place where light reaction and dark reaction occurs. It can be expected that combination between AM symbiosis and introduction of C4 or CAM traits could maximize stress endurance.

Heavy Metal Stress

Recent studies have revealed the potential of AM symbiosis to remediate heavy metal stress from industrial wastes and soil contaminations. As some heavy metal(Cu, Fe, et cetera) is necessary for plant survival in small amounts, plants have developed specific gateway proteins to selectively allow influx of heavy metal. However, this resulted in paradoxical effects of AM fungi both promoting and restricting metal absorption.(Göhre and Paszkowski, 2006)[62] This section will analyze absorption regulation brought by AM fungi and proteomic mechanisms, and suggest advanced utilization of AM fungi as a bioremediator.

1. AM symbiosis induces adjustments in structures and nutrition absorption, causing thicker cell walls and regulating influx of heavy metals. The experiment of Nurudeen, et al.(2021)[63] focused on mitigation of heavy metal toxicity in Glycine Max(L.) based on the assumption that increased phosphorus absorption and plant growth would be concerned with toxicity remediation. The result showed that plant leaf size and height increased, the absorption of phosphorus improved, as well as translocation and bioaccumulation capability regarding Pb, Cu, and Zn were changed

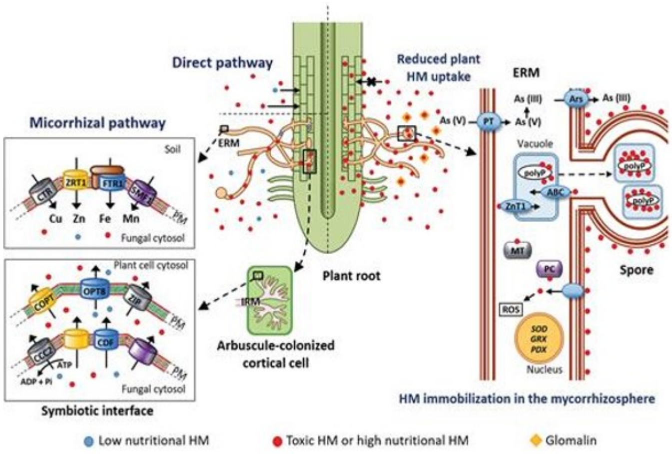

Fig. 8 Heavy metal absorption mechanisms under AM symbiosis, taken from Nuria, et al.(2016)[64]

under AM inoculation. This study supported the remediating ability of AM fungi, which reduced doubts on the contradictory observations on the ameliorative effects of AM fungi. Also, according to Yuxuan, et al.(2023)[53] using AM fungi and E.Grandis at different levels of Cadmium, AM fungi retained Cadmium in fungal structures that protected the cell from excessive influx of heavy metal. This study is important in that it defined the mechanism of heavy metal stress alleviation.

2. AM symbiosis also can cause proteomic and genetic changes to activate enzymes and proteins necessary for toxicity remediation. Metal transporter complexes in plant and AM fungi assist to maintain metal homeostasis between soil and plant root system, as addressed in the figure 8. According to Nuria, et al.(2016)[36], in conditions lacking heavy metal, plants take up heavy metal directly through the mycorrhizal pathway composed of transporter proteins with great affinity to metal, such as Cu transporter, Cu-ATPase, and cation diffusion facilitator that ease metal ion influx. On the contrary, in conditions abundant of heavy metal, increased influx of phosphorus reject introduction of heavy metal to plant cells, which is a process named phytoextraction. Meanwhile, in the phytostabilization process, heavy metals are fixed in the mycorrhizosphere of the fungal structure, reducing the ultimate heavy metal uptake of the plants. The pathway consists of proteins including P and Zn transporter, and SOD to eliminate ROS. Therefore, the study has clearly identified the mechanisms how AM fungi can control the influx and efflux of heavy metal through a complicated protein network along the pathways. AM fungi such as Glomus have shown possibilities in alleviating toxicity of heavy metal in the soil.

   Phytoremediation, introduction of plants and microorganisms to reduce detrimental components in the soil, has emerged as a new solution to deal with soil contamination in industrial and agricultural areas. Capabilities of AM fungi to absorb heavy metal is anticipated to be able to purify the polluted soil. Still, more studies are required to foster the soil environment that is appropriate for AM fungi to form AM symbiosis with the plants, which would make phytoremediation effective.

Analysis

Protein is the core macromolecule of every living organism, necessitating proteomic approach to analyze the complex phenomenon in the environment In particular, this review focused on identifying role of the protein networks in providing endurance to four different types of abiotic stresses under AM symbiosis: drought, salinity, heat, and heavy metal stress. First, in case of drought stress, AM fungi contribute to water osmoregulation, secretion of phytohormones, expression of transcription factors, and activation of antioxidants. However, some proteins such as LEA and aquaporins require further experiments to demonstrate their relationship with AM fungi. Second, in case of salinity stress, AM fungi enhance water and ion(Ca^{2+}, K^{+}, Na^{+}) osmoregulation using transporter proteins and osmolytes, as well as evoking reinforcement of the plant organs and activating antioxidants. Third, in case of heat stress, AM fungi triggered thermomorphogenesis, antioxidants activation, and expression of PIF4 protein that controlled thermosensory growth and initiated biosynthesis of plant hormones. However, it was unexpected that the paper could not find a meaningful connection between AM symbiosis and HSP proteins. Fourth, in case of heavy metal stress, this review focused on the role of AM fungi in phytoextraction and phytostabilization at the different levels of heavy metal. Figure 9 summarizes the mechanisms and examples of AM symbiosis providing tolerance to abiotic stresses.

Overall, it was remarkable that various proteins were working together in the signal pathways to protect plants, activated by AM fungi inoculation. Also, AM symbiosis could cause macroscopic change in appearance, photosynthesis capability(fv/fm ratio), and Pr-Pfr system, indicating AM fungi have a huge impact on the function of the plants. Although other bacterias such as Rhizobium form mutualistic relationships with the plants, Rhizobium and AM symbiosis are different in that Rhizobium is restricted to leguminous plants and its main function is nitrogen fixation. Therefore, AM symbiosis could assist a broader range of plants to live on the land, enhance nutrition, and overcome multiple abiotic stresses. Further suggestions on the topic will be elaborated in detail in the subsequent section.

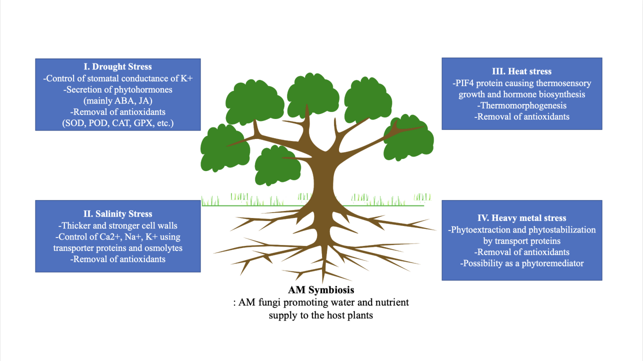

Fig. 9 Summary of the review (self-drawn)

Discussions

Limits

In this section, the paper first addresses main limitations on the current study methods. Most of studies are confined to the experiment with a specific plant (i.e. Tomato, rice) and model plant Arabidopsis. Though collecting individual cases is essential to directly observe cause and effect, it includes the hazardness of generalization and does not reflect physiological differences among the plants. Also, while AM fungi brings palpable benefits to plants, it still has a hazard to act as a foe to plants. Therefore, it would be necessary to categorize and classify the plant groups based on their properties and responses with AM fungi that can integrate former studies with various plants, allowing efficient and accurate analysis on the effect of AM symbiosis and phytohormones showing ambivalent effects. For instance, in study on the impact of gibberellin in AM symbiosis, plants were divided into Arum-type and Paris-type that could clearly convey different mechanisms of accepting gibberellin, facilitating the process of identifying contrasting effects of phytohormones. Also, it should be verified whether the impact of AM symbiosis might vary according to factors such as the root length, climate(desert, alpine, temperate, tundra, et cetera), and taxonomical plant type(gymnosperm, angiosperm, et cetera). These reasons show why experiments should be performed to diverse groups of plant species in diverse environments. Next, regarding the question of whether AM symbiosis can be beneficial or detrimental, there are clarified the conditions that the impact of AM symbiosis can differ according to genetic combination between plant and fungi, robustness of plant roots, and phosphate level of the surrounding (Berger and Gutjahr, 2021)[65]. Therefore, with great advancement in bioinformatics, analysis and classification based on the genome sequence using digital softwares would enable detailed research to provide a balanced view on whether AM symbiosis brings positive or negative impact on plant productivity. Lastly, it has been experimentally difficult to standardize the degree of AM symbiosis, since the experimenters can merely check whether the plant is inoculated by the bacteria or not. Hence, experimenters need further examination on the relationship between amount of bacteria inoculation and magnificence of the molecular responses.

Suggestions

This paper suggests that future research has to focus on (1) identifying the role of AM symbiosis against multiple combinations of abiotic stresses, and (2) searching for the methods to apply the concepts of AM symbiosis in actual farming fields. Multiple occurrences of stresses such as drought, salinity, and heavy metal stress have been a threat to agriculture. Therefore, as the review of Zandelinas, et al.(2017)[66] emphasizes, comparing the response of AM plants under different combinations of abiotic stresses is necessary in order to clearly define the function of AM symbiosis. Also, there should be contemplations on the methods to utilize AM symbiosis in farming fields, with reasonable price and high accessibility for socially alienated farmers, especially where suffering from inferior agriculture facilities and low self-supply rate. For instance, there has been increased sales of VAM fertilizers that include mycorrhizal fungi to improve nutrition supply and nitrogen fixation. According to Mordor Intelligence(2022)[14], the VAM market is expected to grow 114% from 2023 to 2028. While AM-based bioremediators and biofertilizers have high cost in the short-run due to use of organic materials, it is expected that their cost would decline in the long-run as they spread widely and many people raise recognition about them. This process would require coordination of technological development and policymaking, considering various stakeholders.

In addition, as multiple research studies above revealed, AM has high potential to be used as a phytoremediator that alleviates heavy metal concentration in industrial areas. Phytoremediation using AM or genetic transduction of phytoremediating traits would greatly help to solve heavy metal issues, promoting improved human health and crop production, achieving sustainable development goals against climate change. Especially, Glomus mosseae, Glomus claroideum, Acaulospora longula, Gigaspora gigantea have proven to be effective as phytoremediators. They supplement the plant's function to absorb the heavy metal from the rhizosphere, therefore detoxifying the contaminated soil. However, in the long term, it might have side effects such as transfer of contaminants through fallen leaves or other predators, requiring cautious utilization. In general, AM symbiosis is still meaningful to research from theories to actual applications, considering its versatility in crop production and phytoremediation. This paper highlights again that utilizing AM symbiosis on ameliorating food crises necessitates an interdisciplinary approach that connects biological concepts, farmers, and sustenance of human society. Also, despite the prosperity of technologies, eco-friendly change in economic structure, introduction of social policies, and fair distribution of limited resources would have to be put in a priority to combat global warming and food issues.

Methods

Data was accessed mainly from professional websites such as NCBI, Google Scholar, Springer, and other credible organizations for thesis papers and statistics. The reviewed studies generally adopted similar research methodologies, by injecting Growth-promoting arbuscular mycorrhizae to the plant species and observing how the response was different with a negative control. Since the only variable was abiotic stressor, other variables are assumed to have remained constant in both experimental group and control group. Then, proper genetic analysis techniques such as RT-PCR or RNA assays were utilized to supplement the observation , according to the purpose of an experiment.

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