

## REVIEW ARTICLE

# Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress

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## Keywords

Abiotic stress; fatty acids; H<sub>2</sub>O<sub>2</sub>; mycorrhiza; polyamines; water relations.

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## ABSTRACT

With continued climate changes, soil drought stress has become the main limiting factor for crop growth in arid and semi-arid regions. A typical characteristic of drought stress is the burst of reactive oxygen species (ROS), causing oxidative damage. Plant-associated microbes, such as arbuscular mycorrhizal fungi (AMF), can regulate physiological and molecular responses to tolerate drought stress, and they have a strong ability to cope with drought-induced oxidative damage *via* enhanced antioxidant defence systems. AMF produce a limited oxidative burst in the arbuscule-containing root cortical cells. Similar to plants, AMF modulate a fungal network in enzymatic (e.g. *GmarCuZnSOD* and *GintSOD1*) and non-enzymatic (e.g. *GintMT1*, *GinPDX1* and *GintGRX1*) antioxidant defence systems to scavenge ROS. Plants also respond to mycorrhization to enhance stress tolerance *via* metabolites and the induction of genes. The present review provides an overview of the network of plant – arbuscular mycorrhizal fungus dialogue in mitigating oxidative stress. Future studies should involve identifying genes and transcription factors from both AMF and host plants in response to drought stress, and utilize transcriptomics, proteomics and metabolomics to clarify a clear dialogue mechanism between plants and AMF in mitigating oxidative burst.

## INTRODUCTION

Plants are continuously exposed to the external environment, including abiotic and biotic stresses (Nath *et al.* 2016). Abiotic stress produces adverse effects on crop growth and production (Millar & Bennett 2016). Among the different forms of abiotic stress, drought stress is one of most devastating abiotic factors threatening crop growth and productivity worldwide (Guo *et al.* 2020). With climate change and the expansion of human activities, drought stress is becoming more severe and frequent in arid and semi-arid regions. Drought stress triggers several destructive effects in plants, including osmotic imbalance, membrane system damage, cellular metabolic disturbance and abnormal water distribution (Guo *et al.* 2020; Yang *et al.* 2020b).

In plants, drought stress triggers the generation of toxic reactive oxygen species (ROS), such as the superoxide anion free radical (O<sub>2</sub><sup>•−</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the hydroxyl radical (OH•) because of the overflow of electrons from chloroplasts, mitochondria, peroxisomes and plasma membranes (Tiwari *et al.* 2018; Bhattacharjee 2019). Accumulation of ROS induces an oxidative burst in plants subjected to drought stress, and thus causes oxidative damage, including lipid peroxidation, nucleic acid damage, protein oxidation and programmed cell death (Choudhary *et al.* 2018). Therefore, the adverse effects of drought on plant growth are partly attributed to ROS accumulation (Yang *et al.* 2020a). Plants also develop enzymatic and non-enzymatic antioxidant defence systems to reduce their oxidative stress (Choudhary *et al.* 2018). An ascorbic acid-glutathione (AsA-GSH) cycle in plants is utilized to detoxify H<sub>2</sub>O<sub>2</sub> (Bashri & Prasad 2016). It is

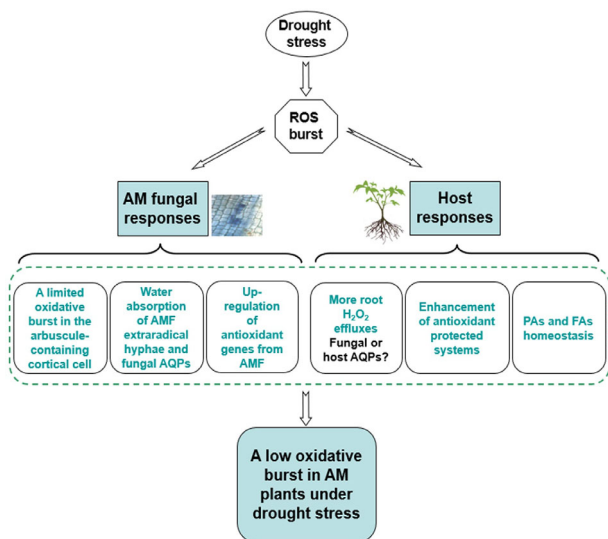
already recognized that ROS levels generate two distinct physiological responses: high concentrations of ROS trigger cell death while low levels of ROS act as a signal to initiate the protective responses of G-proteins and redox-sensitive proteins (Das & Roychoudhury 2014).

Symbiotic arbuscular mycorrhizal fungi (AMF) are involved in many plant processes, such as nutrient acquisition, photosynthesis, root formation and stress resistance (Humphreys *et al.* 2010; Chiu *et al.* 2018; Lanfranco *et al.* 2018; Liu *et al.* 2018; Rivero *et al.* 2018; Gavito *et al.* 2019; Qiao *et al.* 2019). AMF from the phylum Mucoromycota, subphylum Glomeromycotina, establish the most common arbuscular mycorrhiza (AM) with the roots of 72% of vascular plants (Bonfante 2018). AM is characterized by the presence of arbuscules inside root cortical cells (Strullu-Derrien *et al.* 2017). Arbuscules are the site of nutrient exchange from the AMF to the host. Symbiotic associations are characterized by the stimulation of host nutrient uptake by AMF in return for photosynthetic products and lipids form the plants for AM fungal growth. AMF act as protection for host plants against drought stress *via* a series of underlying mechanisms, including (i) direct water absorption of the mycorrhizal extraradical mycelium; (ii) physiological improvements in nutrient acquisition, osmotic adjustment, hormone balance, antioxidant protective systems, fatty acids (FAs) and polyamines (PAs); (iii) host morphological adaptation; (iv) soil structure improvement by the mycorrhizal extraradical mycelium; and (v) molecular changes (Blee & Anderson, 2000; Ruiz-Lozano *et al.* 2012; Wu *et al.* 2013, 2019; Millar & Bennett 2016; Nath *et al.* 2016; Wu & Zou 2017; Liu *et al.* 2018; Zhang *et al.* 2020).

Earlier studies have shown that AM roots are associated with ROS generation and scavenging; on the other hand, AMF also trigger ROS generation. Moreover, H<sub>2</sub>O<sub>2</sub> accumulation in arbuscule-containing cortical cells of roots implies the arbuscule degradation during their life cycle (Wu *et al.* 2014). AMF equipped with antioxidant enzymes and non-enzymatic antioxidants respond to ROS accumulation, thus mitigating the oxidative burst (Lanfranco *et al.* 2005; González-Guerrero *et al.* 2007, 2010; Benabdellah *et al.* 2009a, 2009b). In addition, AMF activate the protective systems of host plants to alleviate oxidative damage under abiotic stress (Wu *et al.* 2014; Kapoor & Singh 2017). The network of plant–AM fungus dialogue for mitigating a ROS burst under drought stress is extremely complex (Fig. 1).

### ACCUMULATION OF ROS IS ASSOCIATED WITH THE ESTABLISHMENT OF ARBUSCULAR MYCORRHIZAS

Earlier studies paid great attention to the ROS burst of plants, whereas there is limited information regarding ROS accumulation of mycorrhizal plants, especially in mycorrhiza-containing



**Fig. 1.** Schematic diagram of the role of arbuscular mycorrhizal fungi (AMF) in mitigating drought-induced oxidative burst in host plants. Drought stress seriously induces the accumulation of reactive oxygen species (ROS) in plants, thus resulting in an oxidative burst. Mycorrhizal plants have two response patterns to deal with the ROS burst. The first pathway is arbuscular mycorrhizal (AM) fungal responses. AMF produce a limited oxidative burst in the arbuscule-containing root cortical cell. In addition, expression of antioxidant genes from AMF are induced, and AM fungal extraradical hyphae and fungal aquaporins (AQPs) participate in water absorption and transport to hosts, collectively alleviating the drought-induced oxidative burst. The second pathway is host responses, in which antioxidant protective systems, especially antioxidant enzyme gene expression patterns, are enhanced; polyamine (PAs) and fatty acid (FAs) homeostasis is optimized; more root H<sub>2</sub>O<sub>2</sub> is effluxed to the rhizosphere. The two approaches sometimes act together. For example, PAs in AMF can be transferred to the host, thus affecting PA homeostasis in hosts, fungal AQPs may participate in root H<sub>2</sub>O<sub>2</sub> effluxes, fungal antioxidant genes interact with host antioxidant enzyme genes, collectively enhancing the antioxidant protective system. Thus, the dialogue between both AMF and host plants in mitigating a drought-induced oxidative burst involves a complex network.

root cells. ROS production by AMF is a common phenomenon (Fester & Hause 2005) and is central to the fungal colonization process. ROS levels are dynamic during fungal colonization, symbiosis formation and degradation (Segal & Wilson 2017). Hydrogen peroxide generation in mycorrhiza-containing cortical cells ensures the initial AM fungal colonization in roots, although the generation is transient and quickly eliminated by superoxide dismutase (SOD), catalase (CAT) and carotenoids (Kapoor & Singh 2017). In *Medicago truncatula* plants, H<sub>2</sub>O<sub>2</sub> has been found to accumulate in the arbuscule-containing root cortical cells, where the arbuscules are clumped and less branched, corresponding to the later stage of the arbuscule life cycle (Salzer *et al.* 1999). When H<sub>2</sub>O<sub>2</sub> is diffused across the hyphal wall of the arbuscule branches, initiation of the fungal programme for senescence occurs (Fester & Hause 2005). Hence, ROS accumulation precedes the degradation of arbuscules. The accumulation of H<sub>2</sub>O<sub>2</sub> may be derived from the activation of carotenoid biosynthesis or plant stress responses. In addition to arbuscules, H<sub>2</sub>O<sub>2</sub> has also been found in AMF extraradical hyphal tips, whereas arbuscules were not observed in vesicles and hyphopodia (Salzer *et al.* 1999). The fungal hyphal cytosol and surface of the intercellular hyphae also accumulated H<sub>2</sub>O<sub>2</sub> (Fester & Hause 2005), implying a mycorrhizal protection system in response to stress. Therefore, the oxidative burst would be partly restricted to arbuscule-containing root cells, thereby mitigating the oxidative damage to non-AM cells.

In addition, H<sub>2</sub>O<sub>2</sub> was found to be mainly accumulated during the later stage of the development of arbuscules; therefore, H<sub>2</sub>O<sub>2</sub> might be a molecule that signals programmed cell death in arbuscules of AM and acts as an indicator of arbuscule degradation (Lanfranco *et al.* 2005; Wu *et al.* 2014). Belmondo *et al.* (2016b) analysed the expression of five respiratory burst oxidase homologue (*Rboh*, a NADPH oxidase involved in the generation of H<sub>2</sub>O<sub>2</sub>) genes in the arbuscule-containing root cells of *M. truncatula* colonized by *Gigaspora margarita*. Among these five *MtRboh* genes, only *MtRbohE* expression was induced in the cell, which was related to an event in the growth of arbuscules. RNAi silencing of *MtRbohE* further indicated plentiful intercellular hyphae and infrequent arbuscules in the mycorrhizal plants (Belmondo *et al.* 2016b). In bean (*Phaseolus vulgaris*), *RbohB* silencing had a positive effect during the early stages of AM, and *PvRbohB* was involved in ROS scavenging and hormone homeostasis (Fonseca-García *et al.* 2019). Under Pb stress, the *MtRbohC-G* gene of *M. truncatula* roots was down-regulated by inoculation with *Rhizophagus irregularis*, which was accompanied by lower H<sub>2</sub>O<sub>2</sub> accumulation (Zhang *et al.* 2019b). These authors conclude that the *Rboh* gene could mediate ROS homeostasis and thus is associated with arbuscule formation and development (Belmondo *et al.* 2016a).

### ANTIOXIDANT GENES OF AMF PARTICIPATE IN MITIGATING ROS ACCUMULATION

The antioxidant systems in plants can be optimized to remove ROS under drought stress conditions (Ruiz-Lozano *et al.* 2012; Wu & Zou 2017; Wu *et al.* 2019). There is a small amount of evidence that the antioxidant system of AMF contributes to the mitigation of oxidative stress. Several studies have cloned antioxidant genes from AMF, which participate in mitigating ROS accumulation (Table 1). In *Glomus intraradices*, a

**Table 1.** Antioxidant genes identified from arbuscular mycorrhizal fungi (AMF) and its roles.

AM fungal species	antioxidant genes from AMF	location of AMF	mycorrhizal fungal gene roles	references
<i>Gigaspora margarita</i>	<i>GmarCuZnSOD</i>	Germinated spores and fungal structure in root cells	ROS-inactivating system	Lanfranco <i>et al.</i> (2005)
<i>Glomus intraradices</i>	<i>GintGRX1</i>	Extraradical mycelium	Keeping cytoplasmic proteins in a reduced state, in which H <sub>2</sub> O <sub>2</sub> and/or O <sub>2</sub> <sup>•-</sup> are signal molecules	Benabdellah <i>et al.</i> (2009b)
	<i>GintMT1</i>	Extraradical mycelium	Regulation of redox status and protection from ROS	González-Guerrero <i>et al.</i> (2007)
	<i>GintPDX1</i>	Extraradical mycelium	Stimulating vitamin B6 biosynthesis and thus acting as antioxidant of ROS	Benabdellah <i>et al.</i> (2009a)
	<i>GintSOD1</i>	Extraradical mycelium and mycorrhizal roots of Ri-T DNA transformed carrot	Scavenging ROS and a factor for mycorrhizal establishment	González-Guerrero <i>et al.</i> (2010)

metallothionein (MT) gene, *GintMT1*, was isolated from the extraradical mycelium (González-Guerrero *et al.* 2007). The expression levels of *GintMT1* in response to 500 µM paraquat (an intracellular superoxide generator) was up-regulated nine-fold after 7 days of exposure, indicating that *GintMT1* has a role as a protectant from oxidative damage. Therefore, *GintMT1* may be involved in regulating the redox status of the AMF extraradical hyphae via its metal chelation or thiol groups, resulting in the mitigation of ROS accumulation in AMF and their hosts.

In *G. margarita*, a Cu/Zn SOD gene, named *GmarCuZnSOD*, has been identified from germinated spores (Lanfranco *et al.* 2005). This gene was highly expressed in mycorrhizal root cells, and thus was responsible for localized H<sub>2</sub>O<sub>2</sub> accumulation in the intracellular fungal structure, such as collapsing arbuscules. Hence, the fungal SOD possibly controlled plant defence responses to maintain the function of the arbuscules. According to the transcriptome data for *G. margarita*, ROS scavenger genes in the fungus were identified as associated with the detoxification of ROS, these included glutathione peroxidase, Cu/Zn-SOD, thioredoxin reductase, peroxiredoxins and selenocompound metabolism (Salvioli *et al.* 2016). Based on proteomic and transcriptomic data, *G. margarita* with an endobacterium had lower levels of both protein carbonylation and H<sub>2</sub>O<sub>2</sub> and higher levels of fungal respiration and total glutathione than those without an endobacterium, indicating that endobacteria elicit mycorrhizal fungal antioxidative activity (Vannini *et al.* 2016). Further, the enhancement of antioxidant activity in AMF by endobacteria is subsequently transmitted to the host plant, according to analysis of protein carbonylation profiles in the fungal partner. However, it is not clear how AMF transmit this cellular signalling to the host plant.

In the model AM fungus *G. intraradices*, a *GintSOD1* encoding Cu/Zn-SOD maintained this function in scavenging ROS because up-regulation of *GintSOD1* was found after ROS-inducing agents were included (González-Guerrero *et al.* 2010). According to Corradi *et al.* (2009), fungal SOD possesses directional selection for diversity and provides evolutionary advantages in response to stress. Root exudates of *Lotus japonicus*

have been found to slightly induce the expression of *GmarCuZnSOD* (Lanfranco *et al.* 2005). In brief, fungal SOD acts as an antioxidant enzyme to scavenge ROS, as well as being a component in AMF – plant dialogue for mycorrhizal establishment and plant defence responses.

Benabdellah *et al.* (2009a, 2009b) isolated a *GintPDX1* that encodes a protein involved in vitamin B6 biosynthesis, and that a *GintGRX1* encodes the glutaredoxin from *G. intraradices*. Moreover, *GintPDX1* is expressed in the fungal life cycle, with the highest expression levels observed in intraradical fungal structure, and is induced by exogenous H<sub>2</sub>O<sub>2</sub> and ROS-inducing agents (Benabdellah *et al.* 2009a); hence, *GintGRX1* plays a role in alleviating ROS (Benabdellah *et al.* 2009b). Both *GintGRX1* and *GintPDX1* act as antioxidants in protecting the AMF and/or the plants against oxidative damage.

In short, AMF, similar to plants, possess a regulated network of antioxidant enzyme systems (*e.g.* *GmarCuZnSOD* and *GintSOD1*) and antioxidant systems (*e.g.* *GintMT1*, *GintPDX1* and *GintGRX1*) for scavenging ROS.

## PLANT RESPONSE MECHANISMS ON AMF-MITIGATING ROS BURST UNDER DROUGHT STRESS

Drought stress can induce adverse effects in plants, while plants trigger response mechanisms at morphological (escape and avoidance), physiological (compatible solute accumulation, antioxidant defence systems, phytohormonal homeostasis, etc.) and molecular levels to cope with drought stress (Jangra *et al.* 2019). Drought escape is a primary adaptation mechanism to avoid stress by improving water uptake and reducing water loss (Nadeem *et al.* 2019). Plants also accumulate compatible solutes, including soluble sugars, proline, glycine betaine, organic acids, potassium, calcium, etc., to facilitate water uptake by decreasing cytoplasmic osmotic potential (Tani *et al.* 2019). Antioxidant defence systems in plants are activated to remove further ROS accumulation, thus mitigating the oxidative burst induced by drought stress (Choudhury *et al.* 2017). As an endophytic fungus, AMF colonize the root system of their hosts and further enhance plant response mechanisms to drought. Here, we only review recent advances in mitigating the ROS burst of host plants under drought stress.

## Antioxidant defence systems

A low oxidative burst in AMF-colonized host plants is associated with enhancement of antioxidant enzymes (SOD, CAT, guaiacol peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase and ascorbate peroxidase) and non-enzymatic antioxidants (AsA, carotenoids, GSH and  $\alpha$ -tocopherol) in the defence system activated by AMF, as reviewed in detail by Bahadur *et al.* (2019), Lenoir *et al.* (2016), Kapoor & Singh (2017), Wu *et al.* (2013, 2014) and Wu & Zou (2017). It has been estimated that AMF increase production of antioxidant enzymes in plants by approximate 16% (Lokhandwala & Hoeksema 2019). In sesame plants exposed to severe drought stress, *Funneliformis mosseae* and *Rhizophagus irregularis* collectively enhanced ascorbate peroxidase, polyphenol oxidase, guaiacol peroxidase (G-POD), CAT and phenylalanine ammonia lyase (PAL) activity, followed by an increase in total soluble sugar content (Gholinezhad *et al.* 2020). Similarly, Langeroodi *et al.* (2020) showed an enhanced activity of antioxidant defence systems in chicory with *R. irregularis*, such as SOD, POD, AsA and GSH, accompanied by lower H<sub>2</sub>O<sub>2</sub> accumulation and reduced oxidative damage. Baslam & Giocochea (2012) further found that AMF improved the accumulation of antioxidant compounds in lettuce (mainly carotenoids and anthocyanins) and that the improvement was larger under drought stress than under well-watered conditions. Elevation of antioxidant defence systems in mycorrhizal plants under drought stress removes further ROS accumulation, thus mitigating toxicity effects on lipids, proteins and DNA, with significant consequences for plant functioning under stress (Acuña-rodríguez *et al.* 2020). Thus, mycorrhizal plants have higher drought tolerance than non-mycorrhizal plants through the activation of antioxidant defence systems.

Mycorrhizal *Triticum aestivum* plants colonized by *Glomus mosseae* had a higher content of antioxidant secondary metabolites, such as anthocyanins, flavonoids and total phenolic compounds than control plants not inoculated with mycorrhiza when exposed to soil water deficit (Bitaraf *et al.* 2020). The increase in flavonoids under mycorrhization is due to an increase in PAL activity (Mirjani *et al.* 2019) and the up-regulated expression of genes involved in the flavonoid biosynthetic pathway (Aseel *et al.* 2019). The phenylpropanoid pathway was also elicited in mycorrhizal tomato plants (Chialva *et al.* 2018). It is documented that flavonoids, as antioxidants, directly scavenge ROS, and that flavonoids also stimulate spore germination, mycorrhizal hyphal growth and arbuscule formation within the host roots to establish mycorrhizal fungal activity (Salloum *et al.* 2018; Mirjani *et al.* 2019). Total content of phenols was increased by *Glomus versiforme* in maize under two drought regimes, indicating that AMF-induced phenols, as active scavengers of ROS, protect cellular structures and the functioning of their hosts (Begum *et al.* 2019). Numerous studies have confirmed that AMF inoculation enhances concentrations of carotenoids in *Aloe barbadensis*, *Citrus aurantifolia*, *Ricinus communis* and *Zinnia elegans* under drought stress (Zhang *et al.* 2018b; Bagheri *et al.* 2019; Khajeeyan *et al.* 2019; Nejad *et al.* 2019). An increase in carotenoids in drought-stressed host plants by AMF inoculation could quench more <sup>1</sup>O<sub>2</sub> from excess light energy, thereby protecting the photosynthetic machinery from photo-oxidative damage (Shi *et al.*

2015). Baslam *et al.* (2013) reported that inoculation with *Glomus fasciculatum* and a commercially mixed mycorrhizal fungal inoculum increased concentrations of major carotenoids, chlorophylls and tocopherols in lettuce, which further eliminated ROS. Under arsenic toxicity, AMF also increased antioxidant (carotenoids and  $\alpha$ -tocopherol) concentrations in wheat in response to oxidative stress (Sharma *et al.* 2017). Based on RNA sequence analysis, the AMF cope with oxidative stress *via* induction of multiple metabolic processes, including antioxidant enzyme genes, increased methionine content, sulphur uptake and the pentose phosphate pathway (Venice *et al.* 2017).

## Water absorption of mycorrhizal extraradical hyphae and aquaporins

The degree of oxidative burst is closely related to the water status of plants under drought (Bhattacharjee 2005). Under drought stress, the water content in plants decreases, indicating the occurrence of oxidative stress (Arora *et al.* 2002). AM can form an extraradical mycelium of >100 cm in the plant rhizosphere to enlarge the root surface area for better water acquisition and allow the plant to survive at extremely low soil moisture content (Allen 2006). However, the AMF extraradical mycelium directly takes up water from the soil matrix through the hyphal tips and then transfers this water through the fungal cytoplasm or inner wall layers into root cortical cells, without any obstruction from the plant membrane system (Allen 2007). The water within the hyphae is not lost because of the hydrophobic cell walls. Such mycorrhizal mycelium provides a 'highway' for water extraction from arid soils, and thus plays a critical role in the uptake and transfer of unavailable plant water sources under adverse conditions (Allen 2007). Zhang *et al.* (2018a) calculated that the water absorption rate of the AMF extraradical hyphae ranged from 0.13 to 1.97 mg H<sub>2</sub>O·h<sup>-1</sup>, depending on the AM fungal species, and that the hyphal water absorption rate was dramatically higher under a soil water deficit than in saturated soils. A reduction in hyphal water redistribution (water flows out of the hyphae into the rhizosphere) in the mycorrhizosphere *versus* the non-mycorrhizosphere was found under a soil water deficit, indicating more water is maintained by mycorrhizal plants (Zou *et al.* 2018).

Six aquaporin (AQP) genes were identified in *G. intraradices* and *Rhizophagus clarus*, where *GintAQP2* and *RcAQP3*, respectively, were involved in water absorption (Aroca *et al.* 2009; Li *et al.* 2013; Kikuchi *et al.* 2016). Host AQP gene expression can be down-regulated, up-regulated or unchanged by AMF under drought stress, implying a diverse response pattern (He *et al.* 2019; Zou *et al.* 2019; Cheng *et al.* 2020). In *Lotus japonicus*, Giovannetti *et al.* (2012) identified two putative AQP genes, *LjNIPs* and *LjXIP1*; *LiNIP1* was expressed in arbuscule-containing cortical cells of roots, accumulated in the inner membrane system of arbusculated plant cells and could transport water. However, fungal AQP expression patterns are a compensatory method for host AQP expression under drought stress (Aroca *et al.* 2009; Ding *et al.* 2020). Therefore, plant and fungal AQP synergistically help roots to absorb water under drought stress, and AM plants are subject to a lower degree of soil drought than non-AM plants, thus having a lower oxidative burst. Future work will revolve around the

functional role of host AQP induced by AMF under drought stress in arbusculated plant root cells.

### Root H<sub>2</sub>O<sub>2</sub> efflux

In plants, root H<sub>2</sub>O<sub>2</sub> efflux is an indicator of the degree of oxidative stress (Sousa-Lopes *et al.* 2004; Huang *et al.* 2017). In trifoliolate orange, inoculation with *Funneliformis mosseae* caused an increase in H<sub>2</sub>O<sub>2</sub> efflux in plant roots under drought stress, which was dependent on root class and region (Zou *et al.* 2015; Huang *et al.* 2017). The negative correlation between root net H<sub>2</sub>O<sub>2</sub> efflux and root H<sub>2</sub>O<sub>2</sub> or malondialdehyde concentration suggests a low oxidative burst in mycorrhizal plants exposed to drought stress (Fig. 1) (Huang *et al.* 2017). AMF inoculation under soil drought induced an increase in root calmodulin (CaM) and Ca<sup>2+</sup> influx (Huang *et al.* 2014; Zou *et al.* 2015), and Ca<sup>2+</sup> influx was positively correlated with H<sub>2</sub>O<sub>2</sub> efflux (Zou *et al.* 2015), indicating that Ca<sup>2+</sup>/CaM may initiate H<sub>2</sub>O<sub>2</sub> signal transduction to respond to H<sub>2</sub>O<sub>2</sub> efflux. Because of the similar electrochemical properties of H<sub>2</sub>O and H<sub>2</sub>O<sub>2</sub>, some AQP proteins, *e.g.* an aquaporin gene (*So-aqpA*) isolated from *Streptococcus oligofermentans*, was a bidirectional H<sub>2</sub>O<sub>2</sub>-transferring AQP, and thus AQP-modulated H<sub>2</sub>O<sub>2</sub> efflux is an important mechanism in ROS detoxification (Tong *et al.* 2019). It is not clear whether the AQP genes of both the AMF and host are involved in root H<sub>2</sub>O<sub>2</sub> efflux.

### Up-regulation of antioxidant enzyme gene expression

Antioxidant enzyme gene (*e.g.* SOD, POD and CAT) expression in lettuce, trifoliolate orange and wheat is activated under a combination of the presence of AMF and plant drought stress (Ruiz-Lozano *et al.* 2001; Mustafa *et al.* 2017; Zhang *et al.* 2019a; He *et al.* 2020). The expression of CAT and POD in arbuscule-containing cells colonized by *G. intraradices* was up-regulated (Blee & Anderson 2000), suggesting that mycorrhizas stimulated localized defence responses in mycorrhizal plant roots. Under drought stress, *Mn-sod II* expression in lettuce plants was induced by *G. mosseae* and *G. intraradices*, which protected mycorrhizal plants against oxidative stress (Ruiz-Lozano *et al.* 2001). Similarly, AMF up-regulated expression levels of *PtFe-SOD*, *PtMn-SOD*, *PtPOD* and *PtCAT1* in trifoliolate orange exposed to drought stress, thus maintaining low ROS levels (*e.g.* H<sub>2</sub>O<sub>2</sub>) (Zhang *et al.* 2019a; He *et al.* 2020). Relative expression of the phenylalanine ammonia-lyase gene was higher in roots of micropropagated plantlets of *Satureja khuzistanica* inoculated with *Rhizoglyphus fasciculatum* than that in non-AMF inoculated control plantlets on days 7 and 42; thus monitoring flavonoid biosynthesis for low oxidative damage (Mirjani *et al.* 2019). The improved tolerance of AMF-inoculated sunflower under high temperatures was due to up-regulated expression of the glutathione S-transferase gene (Mayer *et al.* 2017). Hence, the above results suggest that AMF trigger the overexpression of host antioxidant enzyme genes in response to drought stress (Fig. 1), thereby leading to reduced accumulation of ROS in host plants (He *et al.*, 2020).

### Polyamines and fatty acids

Polyamines (PAs) are low-molecular weight aliphatic polycations, and three main types are found in plants: spermine

(Spm), spermidine (Spd) and putrescine (Put) (Liu *et al.* 2015). PAs can bind anionic macromolecules (*e.g.* nucleic acids and proteins) to regulate transcription and translation, as well as maintain membrane stability and modulate antioxidant systems (Liu *et al.* 2015). PA degradation can induce H<sub>2</sub>O<sub>2</sub> generation (Pottosin *et al.* 2014); hence, the relationship between PAs and ROS is complex. Zhang *et al.* (2020) found higher Put concentrations and lower Spd and Spm concentrations in drought-stressed trifoliolate orange after inoculation with *F. mosseae*, accompanied by the up-regulation of PA synthase genes and PA catabolic enzyme genes. Changes in PAs through mycorrhization trigger ROS signals for stress responses *via* low H<sub>2</sub>O<sub>2</sub> accumulation by up-regulating *PtMn-SOD*, *PtCu/Zn-SOD* and *PtCAT1* (Fig. 1). In addition, Put catabolism, together with an improvement in N assimilation and the accumulation of  $\gamma$ -aminobutyric acid, was found in mycorrhizal maize plants under drought stress (Hu & Chen 2020). AM plants contain higher PA levels under stress conditions, which further improve root growth, thereby better responding to stress (Sannazzaro *et al.* 2007). Exogenous Put in combination with AMF is an effective strategy for coping with nodule senescence in leguminous plants under stress (Garg & Sharma 2019) and cadmium toxicity in sorghum (Kumar & Pathak 2019) because Put is an important stimulus for arbuscule formation and mycorrhizal colonization (Salloum *et al.* 2018). Therefore, PAs, especially Put, may inhibit the auto-oxidation of metals, impairing the supply of electrons for ROS generation, and directly acting as ROS scavengers or inducing the initiation of antioxidant systems (Liu *et al.* 2015).

In addition, fatty acids (FAs) located in the plant cell membrane are a major determinant of membrane integrity and function (Wu *et al.* 2018). Lipoxygenase located in the cell walls can lead to hydroperoxidation of polyunsaturated FAs for ROS generation. FAs play an important role in maintaining AM growth (Luginbuehl *et al.* 2017). After 4 days of AMF inoculation, FA desaturation of host plants was found in response to AMF colonization (Vangelisti *et al.* 2020). Wu *et al.* (2019) reported that under drought stress, FA components in the roots of trifoliolate orange had a decreased content of saturated FAs and an increased content of unsaturated FAs with *F. mosseae* colonization, accompanied by up-regulation of FA desaturase genes (*e.g.* *PtFAD2*, *PtFAD6* and *PtA9*). Therefore, mycorrhizal plants had a relatively higher unsaturation index for FAs than non-mycorrhizal plants grown under soil water deficit (Wu *et al.* 2019). RNA sequencing further revealed that the most differentially expressed genes in mycorrhizal plants were associated with lipid metabolic processes under drought stress (Tarnabi *et al.* 2020). AMF colonization induced high cell membrane lipid fluidity, improved membrane integrity and cell wall rigidity of host plants (Essahibi *et al.* 2018), which could then cope with an oxidative burst. Mycorrhiza-mediated FA changes in components and their unsaturation levels are a precondition for reducing the oxidative burst in host plants under drought stress (Fig. 1).

## CONCLUSIONS AND OUTLOOKS

Arbuscular mycorrhizal fungi can potentially mitigate effects of drought stress on their host plants, and the proposed mechanisms involved have been reviewed previously (Wu *et al.* 2013; Lenoir *et al.* 2016). Understanding these mechanisms will

enable better use of AMF to protect crops against drought stress. Previous studies have revealed the role of antioxidant defence systems in physiological and molecular responses to AM under drought stress, whereas the regulative network for ROS in antioxidant defence systems still needs to be elucidated based on 'omics' techniques. For example, an endobacterium of *G. margarita* elicited fungal antioxidant activity, and then the fungus transmitted this to the host plant through signalling pathways in the system plant–AM and fungus–endobacterium (Vannini *et al.* 2016). It is not clear whether ROS scavenging enzyme genes from both AMF and hosts synergistically enhance drought tolerance of host plants. However, arbuscule-containing plant cells modulate the synthesis of pathogenesis-related protein PR-b1 and the distribution of hydroxyproline-rich glycoprotein, and thus the existence of arbuscules in root cortical cells is associated with plant defence responses (Song *et al.* 2011). Future in-depth studies on changes in ROS production and antioxidant defence systems in arbuscule-containing plant root cortical cells will help to understand the regulatory network of mycorrhizas in tolerating drought stress.

In AMF, *GintMT1*, *GintSOD1* and *GmarCuZnSOD* genes were identified and their functions partly clarified in AMF and/or plants. In scavenging ROS, SOD are the first line of defence for removing  $O_2^{\bullet-}$  in  $H_2O_2$  generation. Therefore, more antioxidant enzyme genes must be identified from AMF

and analysed to reveal their functions. The gene *RbohE* from *M. truncatula* is involved in AM development, whereas the role of *RbohE* in AMF is unknown. Further studies should be undertaken on the role of *RbohE* from AMF and fungal partners to clarify the function of this gene in AM.

The  $H_2O_2$  derived from polyphenol oxidase-mediated oxidation is a signal for stomatal closure, and thus polyphenol oxidase must be part of the signalling mechanism that induces plant defence responses (Sequera-Mutiozabal *et al.* 2016). It is necessary to clarify the relationships among polyphenol oxidase,  $H_2O_2$  and stomatal closure.

The AMF enhance plant tolerance to drought stress, depending on the ecotype of the AMF strain used. AMF strains isolated from arid land confer different metabolic pathways and compounds in response to stress (Rivero *et al.* 2018). Hence, additional studies are required to compare the mechanisms of the plasticity in ROS generation and elimination in host plants inoculated with different AMF species.

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