

# Project Report of 2023: SVP-2318

"Arbuscular mycorrhization: A silent but yet effective approach of phytoremediation of soil contaminated with heavy metal Cadmium (Cd)"

IQAC Sri Venkateswara College University of Delhi Benito Juarez Road, Dhaula Kuan, New Delhi New Delhi -110021

# **SRIVIPRA PROJECT 2023**

**Title:** Arbuscular mycorrhization: A silent but yet effective approach of phytoremediation of soil contaminated with heavy metal Cadmium (Cd).



List of students under the SRIVIPRA Project

S.No	Photo	Name of the student	Roll number	Course	Signature
1		Pawani Pandey	1421034	B.Sc. Hons Botany	Pawani

2	Chandranshu Bhushan	1421020	B.Sc. hons Botany	. Chundranshu
3	Priya	1421038	B.Sc. Hons Botany	Thomas

Showtony Mondal

Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2318 titled "Arbuscular mycorrhization: "A silent but yet effective approach of phytoremediation of soil contaminated with heavy metal Cadmium (Cd)". The participants have carried out the research project work under my guidance and supervision from 15 June, 2023 to 15<sup>th</sup> September 2023. The work carried out is original and carried out in an online/offline/hybrid mode.

Showtony Mandal

Signature of Mentor

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Pawani Pandey, Chandranshu Bhushan and Priya

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Arbuscular mycorrhization: A silent but yet effective approach of phytoremiadiation of soil contaminated with heavy metal Cadmium (Cd)

The contamination of soils, plants, sediments, and surface water by heavy metals is a significant environmental concern (Jung, 2008). Heavy metals with a density exceeding 5 g/cm<sup>3</sup> are often considered as contaminants, although the exact definition may vary (Abdelatey et al., 2011). Various human activities contribute to the release of these pollutants into the environment. Mining, smelting, fertilizers, pesticides, coal combustion, medical waste disposal, combustion of leaded gasoline, and improper disposal of batteries are some of the primary sources of heavy metals (Memon et al., 2001; Thangavel et al., 2004; Khan et al., 2007; Wuana et al., 2011; Rodrigues et al., 2012). These activities introduce heavy metals into the ecosystem, leading to widespread contamination that are detrimental to the environment and living species, such as plants, animals, and microbes (Doble et al., 2005; Rajkumar et al., 2009).

Cadmium (cd) is a toxic heavy metal that poses significant risks to the environment and human health (Gupta et al., 1998). It is found naturally in soils at levels ranging from 0.07 to 1.1 mg kg - 1 soil (WHO 2007), which has raised considerable environmental and occupational concerns (Wang et al., 2013). With an extremely long biological half-life of over 20 years, Cd accumulates in plants, animals, and humans, making it a priority contaminant according to the US-EPA (IARC 1994). In fact, it ranks seventh among the top 20 toxins and is classified as a probable human carcinogen by UNEP, 2008.

The threshold range of Cd, particularly in agricultural soils is approximately around 100 mg Cd kg1 soil (Salt et al., 1995). The rise in Cd levels can be attributed to various manmade activities, such as the use of phosphate fertilizers in agriculture and the application of municipal sewage sludge for soil amendment (Sanita di Toppi et al.,1999; Polle et al., 2003; DalCorso et al. 2010; Gill et al. 2012). These practices have inadvertently introduced large amounts of Cd into the environment. Studies have estimated that out of the 30,000 tonnes of Cd added annually, around 13,000 tonnes are contributed by human activities alone (reviewed by Gallego et al., 2012).

In plants, Cd can disrupt essential physiological processes such as photosynthesis, nutrient uptake, and water balance. This can lead to reduced growth, chlorosis, necrosis, and even death of the affected plants. Animals that consume Cd-contaminated plants or prey on other animals that have accumulated Cd may experience reproductive disorders, organ damage, and

impaired immune function. Additionally, Cd can also enter the human body through the consumption of contaminated food and water. Once inside the body, it can accumulate in various organs such as the liver and kidneys, leading to long-term health issues such as kidney damage and increased risk of cancer.

The increase concentration of Cd also has inhibitory effects on mitochondrial enzymes and can disrupt the tricarboxylic acid (TCA) cycle, leading to a decrease in ATP production and impaired energy metabolism. Furthermore, Cd-induced inhibition of key enzymes involved in the TCA cycle can also result in the accumulation of toxic intermediates, such as aketoglutarate and succinate, which can further contribute to cellular damage. In addition to its impact on photosynthetic and mitochondrial processes, Cd has been shown to interfere with various other cellular mechanisms. For instance, it can disrupt calcium homeostasis by interfering with calcium channels and transporters, leading to an imbalance in intracellular calcium levels. This disruption can have cascading effects on numerous cellular processes that rely on calcium signaling, including cell division, gene expression, and enzyme activation.

Furthermore, Cd accumulation in plants can disrupt the uptake and transport of essential nutrients such as iron, zinc, and calcium. This interference with nutrient uptake can lead to nutrient deficiencies and further hinder plant growth and development. Additionally, Cd exposure has been shown to induce oxidative stress in plants by generating reactive oxygen species (ROS) and disrupting the balance between ROS production and antioxidant defense mechanisms. This oxidative stress can cause damage to cellular components such as lipids, proteins, and DNA, leading to cellular dysfunction and ultimately plant death. Moreover, Cd toxicity can also disrupt hormonal regulation in plants, affecting processes such as seed germination, root development, flowering, and fruit set. The detrimental effects of Cd on plant physiology extend beyond individual plants to ecosystem-level impacts. Cd-contaminated soils can lead to reduced biodiversity and impaired ecosystem functioning as sensitive plant species are unable to survive or thrive in these conditions. Overall, the consequences of Cd accumulation in plants are multifaceted and have far-reaching implications for both plant health and human health. This highlights the urgent need for effective measures to mitigate Cd pollution and prevent further damage to our ecosystems. Strategies such as improved waste management, sustainable farming practices, and stricter

regulations on fertilizer usage are crucial in addressing this issue and safeguarding both human and environmental health. One such sustainable strategy is the use of arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota and form symbioses with the roots of over 80% of terrestrial plant species (Smith et al., 2008). AM fungi have been found to play a crucial role in enhancing crop production and promoting environmental cleanliness. They achieve this through various mechanisms, including nutrient uptake, soil structure improvement, disease suppression, and interaction with other soil microbes. Studies have shown that AM fungi are particularly effective in helping plants grow in soils contaminated with heavy metals. This is attributed to their unique abilities and the positive impact they have on plant growth. By facilitating nutrient absorption and improving soil conditions, AM fungi contribute to the overall health and resilience of plants in polluted environments. In return, the plant provides carbohydrates to the fungus, fueling its growth and reproduction. This mutualistic relationship benefits both parties involved, as it enhances nutrient uptake for the plant while providing a steady supply of carbon for the fungus.

Additionally, AM fungi have been found to play a crucial role in improving soil structure and promoting overall ecosystem health. Their extensive hyphal network helps to bind soil particles together, reducing erosion and increasing water infiltration capacity. Furthermore, AM fungi have been shown to enhance plant tolerance to various environmental stresses such as drought and heavy metal toxicity. Overall, their symbiotic association with host plants highlights their importance in nutrient cycling and ecosystem functioning. The resistance mechanisms of mycorrhizal plants to abiotic stress, such as drought, (Miransari et al., 2011; Porcel et al., 2012; Seguel et al., 2013; Wu et al., 2013a,b). linity and pollution, have been recently reviewed in depth by different authors (Abdel Latef et al, 2014; Cicatelli et al., 2014).

This is attributed to their unique abilities and the positive impact they have on plant growth. By facilitating nutrient absorption and improving soil conditions, AM fungi contribute to the overall health and resilience of plants in polluted environments. **Table 1** provides an overview of AMF-assisted phytoremediation of various heavy metals (particularly Cd) from the polluted sites. This review highlights the potential of AM fungi as a sustainable solution for mitigating the negative effects of heavy metal pollution on agriculture and ecosystem health.

# Mycorrhizal plant tolerance to metals promotes AMF-induced increases in plant biomass.

High levels of heavy metal deposition in the soil have a deleterious impact on microbial diversity and activity. AMF, microorganisms that directly interact with plant roots in the soil and live in symbiosis with them, have a significant role in the mobility and immobility of metal cations in soil. The usual physical and chemical procedures for removing heavy metals from polluted soils are very expensive, and the likelihood of success is quite low (Benavides et al., 2005; Shrama et al, 2005). Therefore, methods that effectively and affordably combat heavy metal pollution must be developed. According to Meier et al. (2015), mycorrhizal fungi are crucial for the remediation of soil contaminated with heavy metals. By promoting nutrient uptake, shielding plants from metal toxicity, absorbing metals, and also enhancing phytostabilization and phytoextraction, AMF can help plants survive on heavy metal-contaminated soils (Gaur et al , 2004; Jahromi et al., 2008; Javaid et al, 2009).

With the ability to fix heavy metals in the cell wall and store them in the vacuole or to chelate with other substances in the cytoplasm (Punamiya et al., 2010), fungal hyphae of internal and external origin can immobilise heavy metals, reducing their toxicity to plants. According to Moghadam (2016), plants grown in soils enriched with Cd and Zn exhibit significant suppression in shoot and root growth, leaf chlorosis, and even death. According to Kapoor et al. (2013) and Audet (2014), increased growth or chelation in the rhizospheric soil may also result in metal dilution in plant tissues. According to AMF, Cd and Zn are bound in the cell walls of mantle hyphae and cortical cells, preventing them from being absorbed and enhancing growth, yield, and nutritional status (Andrade et al, 2008; Garg et al, 2012).

AMF uses hyphal "metal binding" to remove heavy metals by lowering the bioavailability of elements like Cu, Pb, Co, Cd, and Zn . Both transpiration and photosynthetic rate are decreased by increasing Cd levels in plants (Inouhe et al , 2005; Bazzaz et al., 1974).

Experimental plant	Arbuscular Mycorrhizal fungi used	Heavy metal used	Inference of the stu
Eucalyptus grandis, Ailanthus altissima	Glomus mosseae	Cu, Cd, Zn	<i>Ailanthus altissima</i> has exper although Eucalyptus grandis growt
Canna indica L.	Glomus sp.	Zn, Cu, Cd	A greater buildup of pollutants
Helianthus annuus L.	Funneliformis caledonium,	Cu, Pb, Cd, Zn, Ni, Cr	Increased P uptake and plant grow metals in the shoots
Vetiveria zizanioides	Glomus mosseae	Fe, Cu, Cd, Pb, Zn	Increased biomass and less stress
Acacia mangium, Sorghum bicolor, Urochloa brizantha	Glomus macrocarpum, Paraglomus occultum, Glomus sp.	Cd, Pb, Cu, Zn	More chlorophyll and greater root
Zea mays	Acaulospora laveis, Glomus caledonium, and Glomus manihotis	Cd and Cu	The plants that had been vaccinat increased metal concentrations in t
Oryza sativa	Glomus versiforme, Glomus mosseae, and Glomus diaphanum	Cu, Zn, Pb and Cd	AMF-inoculated rice collected mo roots, but there were less of the roots to the shoots
Panicum virgatum (switchgrass)	Glomus mosseae, Glomus fasciculatum, Gigaspora margarita	Pb, Cd	Accumulation of high concentration of Pb and Cd

Table1. Role of arbuscular mycorrhizal fungi in remediation of soil polluted with the heavy metal copper

Oryza sativa	Funneliformis mosseae	Cd	Reduced Cd uptake
Sorghum bicolor	Funneliformis caledonium	Cd	Increased soil enzyme activities, phosphate nutrition, and overall pla growth
Solanum melongena L.	No description	Pb, Cd, As	Improved antioxidant defense syste and biomass
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Rhizophagus irregularis	Cd	Alleviated Cd toxicity in all organs
Daucus carota L., Corchorus olitorius L.	Glomus mosseae, Glomus lamellosum, Gigaspora margarita	Ni, Cd	Reduced metal accumulation, improved plant growth
Lycopersicon esculentum L.	Funneliformis mosseae	Cd	Improved plant growth, hindered Cd distribution from root to shoot
Onopordum acanthium	Glomus fasciculatum, Glomus mosseae, Glomus intraradices	Cd	Improved plant performance, higher Cd accumulation
<i>Limonium sinuatum</i> Mill.	Glomus intraradices, Glomus mosseae	Pb, Cd	Higher metal accumulation in roots, less metal translocation to shoots
Medicago sativa	Glomus aggregatum, Glomus versiforme, Glomus etunicatum, Glomus intraradices	Cd	Increased N, P in shoots, reduced Cd in shoots

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Cajanus cajan	Funneliformis mosseae	Cd, Pb	Phytostabilization
* Nicotiana tabacum	Rhizophagus intraradices	Cd	Phytostabilization



Fig. 1- (A) Cd induced stress in the host plant. (B) Role of arbuscular mycorrhizal fungi in amelioration of stress induced by cadmium

less carbon dioxide absorption because Cd has a greater concentration played a crucial part in affecting how quickly plants were able to synthesise oxygen (Larbi et al., 2002).

Chlorosis resulted from a considerable decrease in iron uptake caused by higher cadmium intake and mobility in plants (Genchi et al., 2020; Larbi et al., 2002). Chlorosis and necrosis are the two main signs that plants with Ni poisoning display (Ahmad et al, 2014). Ni also demonstrates the conflict between calcium and magnesium. The structure of the chlorophyll in the leaves deteriorates when magnesium absorption is reduced. The low rate of photosynthesis in the plants was eventually caused by the low chlorophyll content of the Leaves (Piccini et al, 1992). Ni<sup>+2</sup> stress inhibits the development of the branching system, shoot and root growth, biomass production, germination, aberrant flower morphology, mitotic root tip disruption, leaf spotting, and foliar necrosis (Gajewska et al.,2006). Additionally, too much Ni2+ impairs photosynthesis, transpiration, and the transport of photoassimilates from leaves (Seregin IV et al., 2006) and alters how nutrients are absorbed by roots (Hasinur et al.,2005).

The fungus uses tactics that mirror those of its host. These include chelation of metals within the fungus, precipitation in soil polyphosphate granules, adsorption to fungal cell walls, and immobilisation of metals by chemicals released by the fungus (Gaur et al., 2004). According to Gonzalez-Chavez et al. (2004), one example of an insoluble glycoprotein that is generated and released by AM fungus and binds HM in the soil is glomalin. Heavy metal-induced stress has been shown to be significantly reduced by colonisation with AM-fungi (Gildon et al , 1981; Dehn et al., 1989; Diaz et al., 1996; Hall, 2002). Mycorrhizal colonisation of pea plants has recently been shown to have a Cd-stress buffering effect, as indicated by Rivera-Becerril et al. (2005). Mycorrhizal plants have fungal vesicles similar to plant vacuoles, which are thought to be responsible for storing poisonous substances. Due to its larger surface area, the external mycelium of roots is more effective than plant roots at absorbing metals and has a major effect on the immobilisation of metals in stressed soil.

By keeping them in roots, this retention mechanism by AMF reduces the translocation of HMs and guards against damage to leaf tissues, contributing to phytostabilization. As metals continue to be accommodated in the fungal Mycelium, the HM tolerance capability in mycorrhizal plants is associated with the biomass yield and fungal growth (Fig.1). Additionally, the fungi's vesicles grow together with the concentration of HMs (Yang et al.,2015). Aster tripolium roots were colonised by AMF, which allowed the fungus to retain Cd in the mycelium and increased plant tolerance to Cd (Carvalho et al.,2006).

#### Increased nutrients acquisition in the host plant colonized by AMF

AM fungi colonise roots of dominant plant species even in highly contaminated soils ( Pawlowska et al., 1996; Leung et al., 2007). These soil microorganisms are generally acknowledged as plant growth promoters, mainly due to the participation of their extensive extraradical mycelium (ERM) in plant nutrient acquisition, phosphorus in particular (Smith et al., 1997). One of the mechanisms of plant HM-tolerance has been proposed as symbiosis with mycorrhizal fungi (Hall et al., 2002; Hildebrandt et al., 2007; Joachim et al., 2009). (Hall et al., 2002; Kramer et al., 2005; Peuke et al, 2005; Joachim et al., 2009) AM have been found to evolve mechanisms that can lessen heavy metal hazards in mixed culture systems and, consequently, from the food chains. By improving the intake of essential nutrients (such as phosphorus) or by affecting the symbiotic relationship between legumes and rhizobia, arbuscular mycorrhizal symbiosis can enhance root nodulation and N2 fixation (Barea et al.,2005).By growing beyond the limitations of the plant roots and expanding the absorption region, AMF increase the concentration of phosphorus and other key mineral nutrients in plants with augmentation in the uptake from soil (Porras-Soriano et al.,2009). In host plants under HM stress in the presence of AMF, higher levels of nitrogen, potassium, and phosphorus have been found (Elhindi et al.,2018) (Fig.1).

#### Activation of antioxidant defences

The antioxidant system is a crucial line of defence that plants employ to deal with elevated amounts of harmful metals, especially Cd, in the growing media. Under Cd exposure, several changes of this system are seen that give Cd tolerance and Cd detoxification abilities. Enzymatic and non-enzymatic antioxidants are both a part of the antioxidant defence mechanism. Several ascorbate enzymes, including catalase. peroxidase. monodehydroascorbate reductase, and superoxide dismutase, are examples of enzymes that act as antioxidants. While non-enzymatic antioxidants include glutathione, a-tocopherols, phenolic compounds, ascorbate, non-protein amino acids, and alkaloids, enzymatic antioxidants include dehydroascorbate reductase, glutathione reductase, glutathione S-transferase, and glutathione peroxidase. By transforming ROS into less harmful chemicals, this intricate mechanism is triggered to defend the plant against ROS and help maintain the plant cell redox equilibrium (Huang, et al., 2019; Song et al., 2019). The plant membrane structure and its activities are safeguarded by the antioxidant defence system, which also eliminates free radicals (Chen et al., 2010).

In plant cells, ROS are continuously produced and used in a balanced manner (Kamran et al., 2019b; Riaz et al., 2018a; Yan et al., 2018). However, when HM toxicities are present, significant levels of ROS are produced. Because they are immobile and unable to escape harsh atmospheric conditions, plants perceive and process environmental changes to activate signal transduction and induce changes in cellular and molecular (down or upregulated gene expression) through ROS (signalling) to adjust and respond quickly to these stress conditions (Lin et al , 2012; Bin Luo et al., 2016). According to Miller et al. (2008) and Lenoir et al. (2017), increased ROS concentrations in the cell cause oxidative stress, organelle damage, and even cell death.

Malondialdehyde (MDA) is a measure for the level of lipid and plasma membrane peroxidation as well as the severity of membrane damage. By converting hydrogen peroxide  $(H_2O_2)$ , superoxides, and free radicals to oxygen  $(O_2)$  and then expelling them from the cell, ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), and catalase (CAT) play significant roles in the anti-oxidative enzymatic defence mechanism. Plants may also detoxify oxidative stress and excessive ROS accumulation caused by HMs by regulating gene transcription (Khanna et al., 2018).

According to research done on maize, the ZmWRKY4 gene controls the activity of antioxidant enzymes (SOD and APX) while the plant is under Cd stress (Hong et al., 2017). Overexpression of the gene found in the mesophyll protoplasts of maize increases in order to identify the differentially expressed genes and the transcription patterns of the antioxidant enzyme-encoding genes (CAT, POD, APX), several primers were used to measure SOD and APX production. Results demonstrated that exposure to Cd, Pb, and Cr significantly increased the expression of mRNA. POD and CAT both had the lowest expression levels in the presence of Cd, but APX's expression increased just moderately. There have also been reports of numerous other genes increasing plant tolerance to Cd stress. Due to the overexpression of the ZmVTE4 gene, which encodes c-tocopherol-methyl-transferase, the levels of c-tocopherol and a-tocopherol were raised in transgenic tobacco leaves exposed to Cd, enhancing the tolerance of tobacco plants to Cd stress (Zhu et al., 2019). In response to exposure to HMs and pollutants, AMF has been shown to activate the host plants' defence mechanisms (Lenoir et al., 2017; Debiane et al., 2009; Sharma et al., 2017; Devi et al., 2019). AMF (Glomus mosseae) boosted the activation of the defence system (CAT, POD, and SOD) and decreased membrane lipid peroxidation in Dysosma versipellis under Cu stress (Luo et al., 2020).

Ni2+ concentrations that are too high sharply increase the levels of hydroxyl radicals, superoxide anions, nitric oxide, and hydrogen peroxide (MadhavaRao KV et al.,2000). Since Ni2+ is not redox-active, it cannot directly produce these reactive oxygen species but instead affects several antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPOX), glutathione reductase (GR), peroxidase (POD), guaiacol peroxidase (GOPX), and ascorbate peroxidase (APX).

It has been demonstrated that exposing plants to Ni2+ at low concentrations and/or for brief periods of time enhances the activation of other antioxidant defences by increasing the activities of SOD, POD, GR, and GOPX and, in turn, results in the elimination of ROS through scavenging (Freeman JL et al.,2004). The oxidative stress that Ni2+ causes in tissues may be significantly influenced by lipid peroxidation.

The competition between Ni and Fe in biochemical and physiological processes, as well as Nimediated modulation of the activity of antioxidant Fe enzymes (such as Fe SOD and CAT), may also play a role in Ni2+-induced oxidative stress in plants (Pandey N et al.,2005 Gajewska E et al., 2007 and Nishida S, et al., 2011). It has been discovered that an increase in Ni2+ concentration decreases the activity of numerous cellular antioxidant enzymes, both in vitro and in vivo, as well as a plant's ability to scavenge ROS. This results in an accumulation of ROS and, eventually, oxidative stress in plants (Bhalerao SA et al.,2015).

A wide variety of enzymatic antioxidants consisting of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione-s-transferase (GST) which may efficiently convert the superoxide radicals into hydrogen peroxide and subsequently water and oxygen whereas low molecular weight nonenzymatic antioxidants consisting the proline, ascorbic acid and glutathione which may directly detoxify the ROS (Yadav G et al.,2014).

These two groups of antioxidants may successfully quench a wide range of toxic oxygen derivatives and prevent the cells from oxidative stress. (Gajewska et al.,2005) studied SOD, APOX, CAT and GST activity in leaves and roots of 14 days old pea plants treated with 10, 100, 200  $\mu$ M NiSO4. Ni2+ caused decrease in total SOD activity in both leaves and roots. Gajewska E et al., (2005) concluded that stimulation of GST activity in tissue is mainly involved in response of pea plants under the Ni2+ stress.



FIG 2- Genes expressed in arbuscular mycorrhizal plant that prevent from Cd uptake.

### Protein chaperone manufacturing

In order to maximise the output of refolding, experiments on in vitro refolding are often carried out with a pure protein at low concentration. In contrast, in vivo folding takes place at a protein concentration of 300–400 mg/mL in the packed interior of a cell, considerably increasing the likelihood of intermolecular interactions, including the aggregation of still partially folded or misfolded proteins. As soon as they leave the ribosome's exit tunnel, many nascent polypeptide chains need help folding. This help comes from molecular chaperones. In particular, chaperonins (Hsp60) and molecular chaperones (heat shock proteins of the Hsp70 family) are active. By preventing hydrophobic intra- and intermolecular contacts that could lead to misfolding and aggregation on the one hand, and by unfolding misfolded and

aggregated proteins and giving them a second chance to continue on their proper folding pathway on the other, chaperones increase the yield of folding of the nascent chains. Proteins that are irreversibly misfolded are removed by quality control systems (Tyedmers, J et al.,2010). Both routes lead to the specific 3D structure of the supplied protein, even though the intracellular mechanism of protein folding is significantly more complex than that of in vitro folding.

It has been demonstrated that the mechanism of thermotolerance in yeast and bacterial cells relies on chaperone-mediated protein rescue rather than the degradation of aggregated proteins (Sanchez et al.,1990). Although heavy metals and metalloids induce 4 260 heat-shock proteins, chaperones and other Biomolecules 2014, it is unclear how much protein rescue influences tolerance. Hsp104p, a disaggregating chaperone, and other heat-shock proteins are expressed more frequently in yeast after exposure to arsenite (Jacobson et al.,2012; Thorsen et al.,2007); however, while yeast cells lacking Hsp104p are 100–1,000 times more sensitive to heat than wild-type cells, Hsp104p only offers a two-threefold survival advantage when exposed to lethal concentrations of arsenite (Sanchez et al.,1992).

Similar to Hsp104p, cadmium substantially induces it while making little to no difference in cadmium tolerance (Sanchez et al). These observations can be interpreted in a number of different ways. Aggregates made by heat and those made of cadmium/arsenite may be fundamentally different from the perspective of aggregation. From the perspective of toxicity, the primary lesion caused by arsenite and cadmium might not be accessible to Hsp104p (due to different compartments, for example) or might not even be a protein. Arsenite and cadmium may directly block Hsp104p or other chaperones necessary to maintain folding homeostasis from the perspective of chaperones. The latter mechanism might be responsible for Hsp104p's subpar arsenite tolerance (Sanchez et al.,1992) and its incapacity to dissolve aggregates in the presence of this metalloid (Jacobson et al.,2012).

#### Sequestration

Various anthropogenic activities, such as industrial pollution, mining, and the use of specific fertilizers or pesticides, can cause the harmful heavy metal cadmium (Cd) to be present in soils.

In microbiology it is the process of forming coordination complexes of an ion in solution. It often involves the formation of chelate complexes, and is used to prevent the chemical effect of an ion without removing it from the solution.

The fixation of HM in the soil is best accomplished by HM-tolerant fungi because they have a higher affinity for HM (2-4 times greater than roots) (Joner et al. 2000).

AMF is said to have evolved a sequestration mechanism in response to pollution stress. According to several studies (Cornejo et al., 2008; González-Chávez et al., 2004; Vodnik et al., 2008), AMF can produce glomalin (GRSP; glomalin related soil protein), a waterinsoluble and temperature-resistant glycoprotein that is primarily found in the spore and hyphal wall (80%) rather than secreted (Wright et al, 1996; Driver et al., 2005) and has the ability to immobilise large amounts of metals (Cornejo et al., 2008; GonzálezChávez et al., 2004; Vodnik et al., 2008).

GRSP is recalcitrant and has a long persistence in soil and performs a pivotal role in longterm C/N storage (Malekzadeh et al., 2016; Gonz'alez-Ch'avez et al., 2004; Wang et al., 2018b).. Some of the previous studies documented that 1 g of glomalin secreted by certain strains of AMF could effectively chelate up to 1.12 mg Pb, 0.08 mg Cd, and 4.3 mg Cu from heavy metal contaminated soils indicating the variable capacity of glomalin under different metal species and concentration (Gonz'alez-Ch'avez et al., 2004). Gonzalez-Chavez et al. (2004) found that up to 0.08 mg Cd per gram glomalin could be extracted from polluted soils that had been inoculated with laboratory cultures of AM fungi.

A method of compartmentalising lipophilic pollutants, such as polycyclic aromatic hydrocarbons (PAH), has also been developed in addition to mechanisms for metal sequestration. According to research by Verdin et al. (2006), *R. irregularis* spores and hyphae had anthracene in their lipid bodies. Since the amount of HM bound and the amount of glomalin in the soil are correlated, glomalin-secreting fungi should be more suited for biostabilization efforts. The local concentration of HM in the soil is decreased when it is bound to chitin in the fungal cell wall.

According to Wu (Wu et al., 2014c), after 140 days of experimental treatments, glomalin absorbed 4% of the soil's total Pb and Ni by absorbing 0.21–1.78% of Pb and 0.38–0.98% of Ni. As a protective mechanism, AMF in Cd-contaminated soil raises the concentration of glomalin in the rhizosphere, which reduces the translocation of Cd in *Sorghum* roots and limits the amount of Cd in plant tissues (Babadi et al., 2019). By releasing glomalin (total GRSP), which chelated heavy metals in soil, the inoculation with mycorrhizal fungi, notably with *Glomus aggregatum*, demonstrated a notable decrease in Cd, Pb, Zn, and Cu

concentrations in the shoot (Nafady et al, 2018). Glomalin's capacity to chelate heavy metals under varied circumstances demonstrates how adaptable and efficient this natural chelating agent is. Heavy metal contamination is a major global concern, this might have considerable effects on agricultural practises and environmental cleanup initiatives.

Several remediation strategies can be used to improve the sequestration of Cd ions in the soil. Like adding soil amendments ( lime ,organic matter, substance based on phosphate) that's help to raise pH of soil to promote the complexation of Cd ion, Phytoremediation , and soil washing(involve use of chemicals or surfactants ).

#### Transporters

Translocation of solutes, ions, or drugs across biological membranes is mediated by transporters, which are crucial transmembrane proteins. They serve a variety of puposes ,including cell nutrition, stress tolerance, and homeostasis.

In addition to (CPx-type) ATPases, which perform a crucial role in heavy metal transport in higher plants and draw a strategy for heavy metal tolerance in addition to their role in metal ion homeostasis, the natural resistance linked macrophage protein family (Nramp) and the cation diffusion facilitator (CDF) family also play crucial roles in this regard (Williams et al., 2000).

ZIP stand for zinc regulated / iron regulated transporter- like protein . It has been discovered that the transporters from the ZIP gene family are responsible for transportation of several different cations, including cadmium, iron, manganese, and zinc (Guerinot 2000).

Numerous bacteria, plants, fungi, and mammals contain the metal transporter NRAMP. Numerous metal ions can be translocated by them (Nevo et al., 2006). In plants, they are expressed in both the plasma membrane and the tonoplast membrane of roots and shoots. (Thomine et al., 2000) successfully figured out that there exists a homology between plant metal transporter family in *Arabidopsis* meant for cadmium (Cd) and iron (Fe) transport to Nramp genes. It was stated that AtNramp is responsible in the relocation of both metal Fe and a poisonous metal Cd, and the Nramp genes in plants code for metal transporters.

A low molecular weight protein known as metallothioneins (MTs) can bind to and move metal ions, including Cd. They are produced by a wide range of organisms that are subjected to harmful levels of metals including Cu, Zn, and Cd (Kumar et al., 2005; Folli-Pereira et al.

2012). Numerous studies have demonstrated that MTs play a complex function in the regulation of trace element homeostasis as well as in the defense against the oxidative stress brought by trace elements (Maret et al., 2003).

Plants commonly compartmentalise metals and transport them to vacuoles in an effort to detoxify or sequester metal ions. This kind of transport occurring in the tonoplast is carried out by a number of extremely specialised transporters. Two subfamilies, MRP and PDR, of the ATP Binding Cassette (ABC) transporter family are involved in the chelation and sequestering of metal ions and transport them to the vacuole. ABC transporter are wide family of membrane transport proteins that play role in the active transport of several substates. Most common transport in the vacuole is of the phytochelatin and cadmium complex, which is efficiently transported by ABC transporters. The phytochelatin-cadmium complexes are reported transporter to be discovered in plants (Ortiz et al., 1995). The CDF or MTP transport family is a different family of transporters involved in the movement of metal from the cytosol to the vacuole, endoplasmic reticulum, or apoplast.

AMF play a crucial role in the uptake and transport of essential metals in plants. The versatility of ABC transporters in metal homeostasis is demonstrated by GintABC1 has capacity to carry both Cu and Cd. 131 open-reading frames (ORFs) encoding for ABC transporters, which are potential candidates for detoxification ,have been discovered in the *Arabidopsis* genome (Martinoia et al., 2002; SanchezFernandez et al., 2001). Additionally, the discovery of 30 ORFs in the *R. irregularis* genome that encode potential metal transporters reveals that AMF have a sophisticated system for metal uptake and transport. Transporters involve in Cd ion transport can vary in different organisms.

**Table 2.** Genes encoding Natural Resistance-Associated Macrophage Proteins (Nramp) for Cd transport in plants.

Plant Species	Genes	Expression Sites	Function
	AtNramp1	Roots	
	AtNramp3	Roots and aerial parts	Cd transmost
Arabidopsis thaliana	AtNramp4	Roots and aerial parts	Ca transport
	AtNramp6	Seed embryo, lateral roots and young leaves	
	OsNramp1	Roots and leaves	Cd uptake and transloc
Oryza sativa L.	OsNramp2	Embryo of germinating seeds, roots, leaf sheaths and leaf blades	Cd retranslocation
	OsNramp5	Roots epidermis, exodermis, outer layers of cortex and tissues around xylem	Cd uptake
Triticum polonicum L.	TpNramp3	leaf blades and roots at the jointing and booting stages, first nodes at the grain filling stage	Cd accumulation
	TpNramp5	Roots and basal stems of DPW	
Triticum turgidum L.	TtNramp6	Roots	Cd accumulation

Hordeum vulgare	HvNramp5	Roots	Cd uptake
Fagopyrum esculentum Moench	FeNramp5	Roots	Cd uptake
Brassica rapa L.	BcNramp1	Roots	Cd uptake
	SaNramp1	Young tissues of the shoots	Cd translocation
Sedum alfredii Hance	SaNramp3		Cd translocation
	SaNramp6	Roots	Cd uptake or transloca
Malus xiaojinensis	MxNramp1	Roots	
	MxNramp3	Roots and leaves	Cd uptake and translocati
Crotalaria juncea	CjNramp1	juncea Leaves, stems, and roots Plasma membrane	Cd uptake and translocati
	NtNRAMP1	Roots	Cd uptake
Nicotiana tabacum			

NIND A MD2	Conductive tissue of	Cd efflux
INUNIXAINIT 5	leaves	Cuemux



Fig. 2 - Genes encoding Natural Resistance-Associated Macrophage Proteins (Nramp) for Cd transport in non AMF plants.

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## Project Report of 2023: SVP-2318

"Integration between plant and microbe for the amelioration of biotic stress"

IQAC Sri Venkateswara College University of Delhi Benito Juarez Road, Dhaula Kuan, New Delhi New Delhi -110021

## **SRIVIPRA PROJECT 2023**

**Title** : Integration between plant and microbe for the amelioration of biotic stress

Name Name Design <u>List o</u>	of Mentor of Depart nation: As of students	r: Dr. Shantanu Mandal ment: Botany sistant Professor <i>under the SRIVIPRA Pro</i>	oject			
	S.No	Photo	Name of the student	Roll number	Course	
	1		Anshika Aggarwal	1321019	B.Sc (Hons) Biological Sciences	
	2		Anshu Yadav	1421011	B.Sc(Hons) Botany	
	3		Deepti Khandelwal	1421024	B.Sc(Hons) Botany	

Shortony Mandal

**Signature of Mentor** 

## **Certificate of Originality**

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2318 titled "Integration between plant and microbe for the amelioration of biotic stress". The participants have carried out the research project work under my guidance and supervision from 15 June, 2023 to 15<sup>th</sup> September 2023. The work carried out is original and carried out in an online/offline/hybrid mode.

Showtony Mondal

# Signature of Mentor

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#### Introduction

In the past few decades with the increase in population a greater demand for food security by an increase in global food production has come to the forefront. However with the ever increasing prices of the fertilizers, pesticides, weedicides accompanied by the consequences of using such chemicals regularly on soil has made it necessary to look for alternatives which are not only eco-friendly but also not too heavy on the farmers pockets. Keeping such ordeals in view usage of Arbuscular mycorrhizal symbiosis provides a much needed opportunity to bridge the gap between the much needed nutrient supply and the healthy growth of plants in the changing climatic conditions (Mitra et al., 2021)

This AMF is capable of establishing a mutually beneficial relationship with more than 100,000 plant species, in a typical set up the mycelial fungal network of the fungal partner spreads along the host plant roots supplying it with the suitable nutrients such as phosphorus and nitrogen in return for the carbohydrates and starch.

During the pre-symbiotic process the host plant releases certain root exudates in order to attract the nearby fungal partners to facilitate root colonization and subsequent formation of internal edifices for easy transport of material. One of the most important of these root exudates is a newly found class of phytohormones known as strigolactone which act as the main modulators in the face of nutrient deficit conditions to ensure the proper and coordinated growth of the plant species. Strigolactone are secreted by the roots of the host plants in very small amounts into the rhizosphere as a means of communication between the

plant and its environment. They are carotenoid derivatives produced by a conversion mediated by  $\beta$ -carotene isomerase (D27) where *trans*- $\beta$ -carotene is transformed to 9-*cis*- $\beta$ -carotene by a series of reactions. (Mitra et al., 2021)

Strigolactone once released help detect the nearby fungal partner arbuscular mycorrhiza along with some root parasitic weeds such as *Orobronche* and *striga spp*.(Wang et al., 2022). Once detected the symbioses relationship formed helps the plants not only in better acquisition of non-mobile nutrients specially phosphorus but also imparts additional benefits such as better tolerance towards both biotic and abiotic stresses for example water scarcity , salinization , drought conditions and weed removal (Andreo-Jimenez et al., 2015).

Apart from this the studies have showcased the enhanced root development in plants and change in the architecture of plants above ground with respect to nutrient deficit conditions due to this association hence helping the plant to optimise its resources and undergo complete growth. Strigolactone promote the growth of adventitious roots and seminal roots while repressing the growth of lateral roots and tiller buds in case of rice. This is done simultaneously with the promotion of AMF symbioses to activate hyphal branching to better reach the nutrient. The benefits associated with this this relationship to mitigate the consequences of various biotic and abiotic stresses are summarized in the present chapter.

#### Strigolactone, a key regulator of nutrient allocation in plants

Strigolactones (SLs) are a novel class of plant hormones that have undergone extensive study in recent years. The broad range of SLs actions, which include the regulation of shoot/root architecture, the stimulation of interactions between roots and fungi or bacteria, the stimulation of the germination of parasitic plants, and others, suggest that this group of hormones may be crucial in the mechanisms that regulate soil exploration and the root-mediated uptake of nutrients.

The amount of macronutrients in the soil is a significant regulator of the production and exudation of SLs, according to studies on several plant species. The first studies examining the relationship between nutritional insufficiency and increased SL secretion were published in Matusova. (Matusova et al.,2007) *Trifolium pretence* L., which is known to be a host for the parasitic plants and AMF, was used to study the effects of nutritional deficiencies on the biosynthesis and exudation of SLs.

The understanding that SLs function as the branching factor for arbuscular mycorrhizal (AM) fungus was a significant development (Akiyama et al., 2005; Parniske 2008). According to Akiyama and Hayashi (2006), mycorrhizae are symbiotic relationships between soil, fungi, and plant roots. According to Brachmann and Parniske (2006), this relationship is likely the most significant and pervasive symbiosis in all of nature. A suitable host is required for AM fungus to complete their life cycle, making them obligate symbionts. The importance of endogenous SLs in the regulation of plant architecture was then shown. Examples of typical examples include preventing bud development and preventing shoot branching (Gomez-Roldan et al. 2008; Umehara et al. 2008).

It has long been understood that auxin and cytokinines, two other groups of plant hormones, play a role in regulating shoot branching. SLs are now acknowledged as a third class of novel plant hormones.

Inorganic nutrient deficiencies, such as those in nitrogen and Pi (Lopez-Raez et al., 2008; Umehara et al., 2008; Yoneyama et al., 2007a; Yoneyama et al., 2007b), cause an increase in SL levels. The host plant can receive both Pi and nitrogen from AMF (Govindarajulu et al., 2005). SL is exclusively produced by some leguminous plants in response to Pi deficit, such as red clover and Lotus japonicus, which can interact symbiotically with root nodule bacteria.

In times of a shortage, plants rely on AMF to acquire both nitrogen and Pi. However, many plants commonly increase their SL levels in response to Pi shortage. High endogenous SL levels under Pi deprivation suggest that SLs may be crucial for the effective uptake of Pi by plants. Additionally, shoot branching is typically reduced by nitrogen deficiency in addition to endogenous SL levels enhanced by Pi deficiency in roots. It is currently unknown, nevertheless, whether SL synthesis in response to nitrogen deficit impacts shoot branching. Both root and shoot branching are regulated by SLs. Contrary to Arabidopsis, white lupin does not experience an increase in SL levels in response to Pi shortage (Yoneyama et al., 2008). Auxins are crucial for the development of lateral roots and cluster roots in both Arabidopsis and white lupin, although SL production under Pi deprivation differs significantly between the two species, both of which are not AMF hosts. Figure 1 showcases the phosphorus utilisation and acquisition in case of its deficiency.



Fig. 1. Utilisation and Acquisition of phosphorus by plants by Arbuscular Mycorrhizal and host plant symbiosis

#### The relationship between SL production and AMF Colonization

Many plant species' roots manufacture SLs in large quantities when Pi is insufficient (Lopez-Raez et al., 2008; Umehara et al., 2008; Yoneyama et al., 2007a; Yoneyama et al., 2007b). AMF's hyphal branching, which provides the host with vital inorganic nutrients, particularly Pi, is induced by SLs (Akiyama et al., 2005). Therefore, lowering SL levels lowers the rate of AMF colonisation.

Both root parts exhibit low levels of SL and AMF colonisation comparable to roots under high-Pi conditions when the pea root system is divided into two parts, with one part placed in low-Pi solution and the other in high-Pi solution but both still connected to the same shoot (Balzergue et al., 2011). This suggests that by systemic signalling, the higher Pi side of these split-root plants negatively restricted SL production on the lower Pi side. It's interesting to note that the Pi concentration of these split-root plants is higher in the leaves than in the roots. These findings imply that plants react to Pi levels in the shoot.

The physiological roles of SLs are thought to be crucial for effective nutrient allocation in plants, especially Pi acquisition. In low nutrient conditions, the limited supply of mineral

nutrients is needed for new leaf development or plants like growing seeds and leaves when leaves begin to senescee of their delayed leaf senescence.

The axillary buds can develop using the Pi that was translocated from the roots when SL levels are still low. Contrarily, when Pi is deficient, which prevents tiller bud formation in shoots, SL levels in roots are dramatically increased.

By promoting hyphal branching, SLs released into the soil improve symbiotic interactions with AMF. Pi is given to the host plants by AMF, which also gets carbon from them. Additionally, root-parasitic plants extract carbon from the host plants by recognising the SL signals. Thus, a process that at first glance seemed to be harmful actually serves the plant in many ways.

SLs will work in conjunction with other plant hormones rather than independently. . The action and production of SL are controlled by other hormones. For example, in the regulation of axillary bud outgrowth (Dun et al., 2012) and in the regulation of mesocotyl elongation in darkness (Hu et al., 2014), cytokinins function as SLs' antagonists. In addition to being one of the primary regulators of SL production, auxins may function as antagonists since SLs may improve auxin transport (Hayward et al., 2009; Al- Babili and Bouwmeester 2015). Abscisic acid, one of the fundamental regulators of plant response to abiotic stress, plays a role in SL biosynthesis, as Lopez-Raez et al., (2010) shown, but SLs can also have an impact on abscisic acid biosynthesis (Al-Babili and Bouwmeester 2015). In addition to phytohormones, it is known that phosphate influences SL biosynthesis, which means that a phosphate deficit enhances the production of SL (Koltai 2015 and references therein).

**Table 1.** A set of the gene encoding proteins involved in the biosynthesis and signaling of SLs

PROTINE	ARABIDOPSIS	RICE	PEA	PETUNIA	PROCESS	
Iron-containing protein	AtD27	D27				
CCD7	MAX3	HTD1/D17	RMS5	DAD3	Biosynthesis	
CCD8	MAX4	D10	RMS1	DAD1		
cytochrome P450	MAXI				-	
F-box protein	MAX2	D3	RMS4		Signaling	
α/β hydrolase	AtD14	D14/D88/ HTD2		DAD2		

#### Strigolactone and its role in biotic stress

Strigolactones (SLs) are plant secondary metabolites generated from carotenoids that are crucial for different aspects of plant growth and development as well as for communication with symbiotic microorganisms and root parasitic weeds in the rhizosphere. The symbiosis of beneficial bacteria, such as arbuscular mycorrhizal (AM) fungi, is therefore anticipated to be promoted by sophisticated control of SL biosynthesis, perception, and functions. It is also anticipated that this will delay parasitism by destructive root parasitic weeds. Weak but significantly increased plant resistance to diseases was the consequence of the AM colonisation. SL mimics can be employed to encourage AM symbiosis and lower the pace at which systemic inducers of acquired resistance, which are often phytotoxic to horticulture crops, are applied.

*Striga* and *Orobanche* species of root parasitic weeds were initially shown to respond favourably to Strigolactones (SLs) as germination stimulants. However, it has been demonstrated that SLs are produced and released into the rhizosphere by both their hosts and

non-hosts. This is due to the fact that SLs serve as crucial cues for the colonisation of arbuscular mycorrhizal (AM) fungi (Akiyama et al., 2005). Plants develop and release SLs for AM fungus rather than root parasites. .(Xie et al.,2010; Al-Babili S and Bouwmeester HJ et al.,2015). In addition to serving as rhizosphere signalling agents, SLs or their subsequent metabolites are a group of plant hormones that control things like shoot and root structures, secondary growth, and leaf senescence.(Xie et al.,2010; Al-Babili S and Bouwmeester HJ et al.,2015).

Communication between the AM fungus and the plant host begins in the rhizosphere with the generation and exudation by the host plant of signalling molecules that are recognised and encourage hyphal development by the AM fungus. While other substances, including flavonoids and hydroxy fatty acids, have also been reported to stimulate hyphal growth (Scervino et al., 2005; Nagahashi and Douds 2011), Strigolactones have emerged as crucial cues among these signalling molecules (Akiyama et al., 2005; Bouwmeester et al., 2007). By operating alone or in concert with other related microbes inhabiting in areas that are directly influenced by the plant, AM defends host plants against a variety of biotic stressors. Plants that have been colonised by AM have improved growth and tolerance to plant diseases. . According to the research results, host defence induction, rhizosphere modification, and competition for resources, space, and photosynthates are the most common conceivable processes.

To obtain the photosynthates generated by their host, pathogens also infect a plant. In the case of soil-borne diseases, they first infiltrate the roots, which is a vital location for the virus to travel further throughout the body. The presence of photosynthates at this location is also significant (Wheatley and Poole 2018). Higher carbon demand may have a deleterious impact on pathogens when AMF have main access to photosynthates (Poveda et al., 2020). As a generalised method for pathogen biocontrol through AMF symbiosis, competition for photosynthate is not supported by any evidence. Actually, under nutrient-limited circumstances, ectomycorrhizal hyphae are known to be important in the quick transfer of plant-derived carbon to maintain bacterial populations in root- distant locations (Gorka et al., 2019).

Under the conditions of our experiment, AM colonisation increased the pathogen resistance of tomato and rice (Kusajima et al., unpublished). Because it fills the SL binding pocket and blocks SL signalling, it is likely that the SL-signalling inhibitor KK094 upregulated SL production to further increase pathogen resistance brought on by AM colonisation. Additionally, pathogen resistance brought on by AM colonisation had no impact on SAR, indicating that both promoting AM colonisation and bringing on SAR can help plants become more resistant to infections. However, neither the generation of phytoalexins nor the induction of SAR was impacted by AM symbiosis, which increased plant resistance to infections (Kusajima M et al., unpublished). Instead, AM symbiosis would prepare plants for pathogen assault such that mycorrhizal plants respond to pathogen infections far more promptly and effectively. (Pozo et al.,2007)

In the mycorrhizal symbiosis between plants and Glomeromycota fungus, SLs have been found to benefit plants. With the roots of terrestrial plants, the fungi in these associations produce arbuscular mycorrhizas. Arbuscular mycorrhizal fungus (AMF) symbiosis, which originated around 460 million years ago and is attributed with the development of terrestrial plants and making them more resilient to abiotic and biotic stressors, is regulated by SLs, which control hyphal branching. (Harrison, 1999; Liu et al., 2007)

Abiotic and biotic stressors are brought on by the constantly shifting and frequently adverse environmental circumstances that plants must contend with. As a result, plants have developed complex systems to quickly adjust to changing environmental circumstances. Significant progress has been made in recent years in identifying the essential elements of plant responses to biotic stress. The phytohormones salicylic acid (SA), jasmonic acid (JA) and derivatives—known as jasmonates (JAs)-and ABA are the major players in the signalling networks regulating plant defence responses against pathogens and pests, although others, such as ET, brassinosteroids, gibberellins and auxins, are now known to contribute to modulate the response (Robert-Seilaniantz et al., 2011; Tonne et al., 2009).

#### Arbuscular mycorrhizal and strigolactone biosynthesis in abiotic stress conditions

Plants especially agricultural crops unremittingly encounter changing environmental conditions which quite often results in both biotic and abiotic stresses. These stresses may include pathogen, waterlogging, nutrient deficiency, soil salinity and drought. Such conditions hamper the proper development of the plant and its productivity thus adversely affecting the global crop production. (Ruiz-Lozano et al., 2016) Among the abiotic stress the most dangerous ones are the draught and salt stresses. Water scarcity increases the risk of

many other water stresses while salinity of the lands can affect the 20% of the land which needs to be irrigated for agriculture and produces over 40% of the world's food. Reduced rainfall is high temperatures and over exploitation of the natural water resources only adds to the problem further. (López-Ráez and Juan, 2016)

Owing the two above ordeals a paradigm shift in the new agriculture is required which is based on the environment resilience and sustainability. Ecofriendly alternatives promoting both plant and soil fitness by decreased use of chemical fertilizers and insecticides is the need of the hour. One of the greenest ways of doing this is by using the mutually beneficial plant symbiotic interactions taking place in the rhizosphere. AM symbiosis is one of such plant – microbe interactions which not only helps in the plant growth and fitness but also acts as bioprotective agents that helps alleviate the stress symptoms including the slat and draught stresses by stimulating the production of certain plant hormones which positively impacts the ability of the plant to overcome the these stresses .These obligate biotrophs facilitate the plants in its nutrient and water acquisition via the arbuscules hence acting like biofertilizers used for increasing the biomass production and plant growth. (Saxena et al., 2022)

To begin this plant fungus interaction, the host plant produces and exudates a type of phytohormone known as strigolactone into the rhizosphere. These are detected by the uncharacterized receptor of AM fungi encouraging hyphal growth and branching to come in contact with the host plants. Strigolactone thus play and important role in establishing this symbiosis arrangement and in its subsequent functioning which is beneficial to the host plant especially in conditions of stress. Figure 2 demonstrates the elevation of multiple abiotic stress by the biosynthesis and release of Strigolactones which helps form a symbioses relationship with the Arbuscular mycorrhizal fungi. As previously mentioned, water related stresses presents with a dangerous hurdle that the plants face owing to the increased scarcity of water. So, elevations of these stresses for the proper growth of the crops becomes the primary concern. In such a time the symbiosis relationship which can be established by almost 80% of the terrestrial plants becomes an important alternative way for crop and plant sustainability as opposed to the increasing use of potentially destructive insecticides and pesticides which are not only expensive but also do a lot of harm to the soil health in the long run. (López-Ráez and Juan A., 2016)

#### **Draught stress**

Water scarcity has an enormous impact on the crop productivity as a result of which improving yield under these antagonistic conditions becomes the top priority. AM symbiosis works to mitigate such affects by regulating various response mechanisms such as morphological adaptations, growth reduction, stomatal closure, photosynthetic reduction and induction of stress responsive genes and proteins such as ABA (abscisic acid) whose increased production helps in the elevation of stress symptoms and in return also further enhance the AM symbiosis. (López-Ráez and Juan A., 2016) Fungal hyphae provided by the mycorrhiza partner increases the accessibility to the water usually inaccessible to the roots as it thinner than roots and hence increases the capacity of soil exploration by plants significantly. (López-Ráez and Juan 2016). This highly expansive AM hyphal network can contribute up to 20% of the plant water uptake further indicating the importance of this rhizosphere interaction. AM symbiosis also helps in the absorption of water via the aquaporins through the passive movement of water thereby regulating the root hydraulic conductivity. (López-Ráez and Juan, 2016).

Finally arbuscular mycorrhizal interaction with plants also induces the production of the phytohormone strigolactone which helps mitigate the draught stress symptoms. Recent experiments performed with tomato and lettuce have shown increased root colonization by mycorrhiza when plants when subjected to draught stress treatments with an increment of 17% in case of lettuce and 8% for tomatoes after the completion of 4 weeks. These values only increased over time establishing the significance of this symbiosis. Further even though the draught stresses conditions significantly reduced the harvest for both plants, the AM tomato and lettuce still exhibited better growth and photosystem efficiency as compared to the non-AM tomato and lettuce with the difference being more evident for the tomato. (Ruiz-Lozano et al., 2016). AM plants also displayed an upregulation of SICCD7 and SICCD8 which are the two tomato genes involves in the biosynthetic pathway of strigolactone which suggests the induction of SLs to possibly improve the root colonization and carry out its others functions like nutrient uptake in case of nutrient deficiency.

Lastly AM tomato and lettuce upregulating the encoding of LSNCED2 which is an ABA synthesis gene that is a stress hormone that promotes symbiosis establishment and stress tolerance. The AM plants steadily increased their ABA accumulation b 400 and 760% under

moderate and severe stress treatments respectively (Ruiz-Lozano et al., 2016). Advantageous effects have also been observed in a no. of other species of plants such as rice, barley, maize and pistachio. The AM symbiosis helps the pants grow better in stressful environments and adjust to the changing outside environment sustainably with encourages strategies to optimize the water consumption of the plant.

#### **Salt Stress**

Salinization of soils has been on a gradual increase since the past decade and is expected to result in 30% land loss within the next two decades increasing up to 50% by the middle of the 21<sup>st</sup> century. This makes salt stress on plants one of the major threats towards a food security of the world (Saxena et al., 2022). The accumulation in high concentration of these dissolved salts hinders the plant growth and eventually its survival by its ionic osmotic and cell water homoeostasis. It also affects other physic chemical properties of soil which creates a toxic environment for the plants thereby decreasing their nutrients and water uptake sodium and chlorine are the two ions which when enter into the plant cell disturb the proper function of the plant membranes and prevent the plant from developing properly (Saxena et al., 2022). Under such stressful condition the symbiotic association between the plant and the AM fungi helps in enhancing the plants phenotypic, biochemical and physiological response thereby promoting salinity tolerance and the production of growth hormones the also boosting the conditions of the rhizosphere the help the plant. Signaling pathways involved in detoxification and ion homeostasis get activated in response to the salt stress in order to counter its ill effects such as increased ROS generation, stomatal closure, increased photorespiration, membrane rupturing, decreased photosynthesis and lower water potential. Associated mycorrhizal partner further reduces these damaging effects by up regulating the jeans for the synthesis of LEA and Osmolytes like proline having the capability of ROS scavenging, cell's osmoprotection and maintenance of the membrane integrity. Cellular hydration and Na+ influx used for maintaining the osmotic potential of the cell is also regulated by mycorrhizal partner by the activation of SOS and AQP genes of the plant. Lastly the AM symbiosis could also help in the activation of various ion channels and carriers specially the Ca2+ channel which increases the concentration of Ca<sup>+</sup>2 ion in the cell further activating the kinases responsible for regulating the stress control gene of the plant (Saxena et al., 2022).

AM symbiosis also regulates the expression of multiple aquaporin genes which play an important role in restoring the plant osmotic balance in salt stress condition. AMF colonized plants instigate the production of the phytohormones such as salicylic acid (SA), abscisic acid (ABA) and strigolactone (SL) which upon accumulation facilitate certain mechanisms in the plant that modulate stomatal conductance and improve the plant's responses to salt stress (Saxena et al., 2022)

Experiments conducted using Triticum Durum (wheat) further shed light on the involvement of arbuscular mycorrhiza in bettering the plants health when subjected to salt stress. The results obtained concluded that while salt stress significantly reduced the wheat growth the plant having AMF partners had significantly improved growth parameters like an increment in the shoot dry weight by 67 grams and that of root by 36 grams. Salt stress inflicted plants showed a substantial increase in the root colonization. An improves stomatal conductance was also observed along with an increased soluble protein and sugar content in the shoots and the production of antioxidant enzymes for excess ROS removal. It also boosts the uptake of essential minerals like P which enhances the plant growth. (Ikan et al., 2023). AMF as a biofertilizer imparts many advantages to plants under adverse conditions thereby ensuring their survival and sustainable growth in the future. It also opens the gate to many possible green alternatives with the potential to significantly reducing the use of artificial fertilizers which are major contributors of soil degradation.



Fig. 2. Arbuscular mycorrhizal fungi mediated protection of plants from various abiotic stresses by strigolactone biosynthesis under stress conditions.

#### Arbuscular Mycorrhizal fungi as ecosystem engineers

Symbiotic interactions shared between two species can be both mutualistic and antagonistic and can promote a paradigm shift in the structure and function of host plant communities. For example, it has been known for quite some time that the parasitic plants have the ability to cause such shifts in the floristic diversity by suppressing the competitive dominants in order to free the sub dominant species from competitive exclusion. This suppression further causes the competitive hierarchies of the given community to reorder and restore the degraded plant communities thus showcasing the potential of the parasitic plants to act as 'ecosystem agroengineers' (Cameron, 2010). Consequently, AM fungi can also very well fit under this category of ecosystem agroengineers as it can also induce such shifts in the plant communities. AM fungi upon interacting with its host plant forms a mutually beneficial symbiotic relationship with its host plant by increasing its nutrient uptake and providing it protection against various kinds of biotic and abiotic stresses whereas it also showcases antagonistic behavior towards less compatible species and reduce their biomass in the vicinity drastically. (Cameron, 2010)

Such antagonistic interactions can be used to harness the potential of AM fungi as a biocontrol for weed suppression in agriculture by either the inhibiting the growth of a variety of non-mycorrhizal weed families or by increasing the competitive ability of the given crop species against both the mycorrhizal and non-mycorrhizal weeds. Considering that weed poses a serious problem towards the crop production and could reduce the global yield by 34% it has become quite important to explore this arena further. Many experiments have been conducted along these lines to check the efficiency of AM fungi as a weed control one of which was to judge the impact of AM fungi on weed biomass and crop productivity were *Helianthus annuus*—sunflower was grown alongside six different weed species of temperate zone some on which were quite aggressive in nature and the effect of the AM fungi on these microcosms was observed. The results showed high levels of root colonization by AMF in the sunflower ranging from 56.5% to 88% in presence of different weed species whereas for weed themselves these levels ranged from 0.7% to 55% indicating their low compatibly with weeds. (Rinaudo et al., 2009)

Although no significant increase was seen in the biomass of sunflower in the presence of AMF the weed biomass however was negatively affected with its average dropping by 47%. This reinforced the antagonistic interactions shared between the two species. Lastly it was also seen that the amount of phosphorus obtained by the AMF sunflower was increased by 48% whereas for 3 weed species this phosphorus uptake was significantly reduced in the presence of AMF. This could possibly be the result of AMF using indirect mechanisms to allocate the nutrients to the host species and depriving the non-host competitors of the same (Rinaudo et al., 2009).

Apart from such indirect methods some direct methods have also been suggested that could be used by the AMF to supress the weed by inducing the plant defence systems and releasing of the toxic allopathic compounds by the AMF having detrimental effects on the weeds. Over the years many such experiments have been conducted which further prove the potential of the AM fungi to be used as a possible replacement for the chemical herbicides which have degrading effect on both crops and the soil. A substantial change in the modern agricultural practices in required to enhance the populations of AMF and integrate the benefits associated with them into domestic agricultural gains.

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