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Drought Stress Effects on Vegetable Crop Productivity: Melatonin's Mitigating Role and Rhizobium Inoculants for Enhanced Nitrogen Fixation under Water Scarcity

Elseedy, M. E.[1] ; Dina S. Helaly[1]; M. A. El-Sherpiny [2*] and A. A. Taha [1]

[1]Soils Department, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt.
[2] Soil, Water and Environment Research Institute, Agriculture Research Center, El-Gama St., Giza, Egypt.

ABSTRACT

This review aims to provide a comprehensive overview of the current state of knowledge on the effect of drought stress on the productivity of vegetable crops as well as the impact of applying melatonin to plants exposed to drought stress. Additionally, the study aims to investigate the beneficial role of rhizobium inoculant in nitrogen fixation using different solutions containing molybdenum, cobalt, and copper. By examining the existing literature, this review seeks to synthesize and analyze the key findings, trends, and gaps in research to date. The water scarcity crisis in Egypt (present status and future projections) will be highlighted as well as the effect of water deficit and plant performance, the role of melatonin as a hormone that suppresses the effects of water deficit on plants, biological N-fixation and stimulants of biological N-fixation. Additionally, this review will identify the significant challenges and opportunities in the field, with the ultimate goal of informing future research directions and practical applications.

Keywords: Drought, melatonin, molybdenum, cobalt, copper

INTRODUCTION

Most world countries are suffering from water scarcity, particularly in areas characterized by low rainfall and dry conditions, like Egypt (El-Sherpiny *et al.* 2023). The decline of irrigation water that required disrupts redox homeostasis, leading to an upset in the equilibrium of ROS scavenging (Sharma and Zheng, 2019). Reactive oxygen species (ROS), named free radicals, can be harmful to plant tissues (Jambunathan, 2010). When their production exceeds normal levels or is not properly regulated, they can induce oxidative stress, causing disruption and damage to cellular structures and functions in plants (Das and Roychoudhury, 2014). ROS attack and impair lipid molecules in cell membranes, resulting in lipid peroxidation (Saed-Moucheshi *et al.* 2014). This process generates toxic byproducts that further contribute to oxidative damage (Liu and He, 2016), leading to membrane disruption, loss of integrity, and impaired cellular functions (Singh *et al.* 2016). Similarly, ROS can oxidize and modify proteins within plant cells, altering their structure and function (Waszczak *et al.* 2018). This oxidation process affects enzymatic activity, signaling pathways, and other crucial cellular processes, leading to diminished plant growth and development (Gechev and Petrov, 2020). Moreover, ROS can directly target DNA molecules, leading to oxidative damage in the genetic material of plants (Hasanuzzaman *et al.* 2020). This damage can result in mutations, genetic instability, and impaired mechanisms for DNA repair (Solórzano *et al.* 2020). The accumulation of DNA damage can lead to genetic disorders, decreased plant vitality, and compromised reproductive potential. In addition, excessive ROS can overwhelm the antioxidant defense systems of plants, which are responsible for neutralizing and scavenging ROS (Mittler *et al.* 2022). When there is an imbalance between ROS production and the capacity of antioxidants, it results in oxidative stress (Berrios and Rentsch, 2022). This imbalance further contributes to an overproduction of free radicals and depletion of antioxidants, exacerbating cellular damage. To counteract the detrimental effects of free radicals, plants have developed various mechanisms, including antioxidant defense systems comprising enzymes such as superoxide dismutase, catalase, and peroxidases, as well as non-enzymatic antioxidants like ascorbate and glutathione. These antioxidant systems play a crucial role in maintaining redox homeostasis and safeguarding plant tissues against oxidative damage (Kesawat *et al.* 2023). Inadequate water supply during drought conditions creates a range of physiological challenges for plants. Drought limits the availability of water, which is essential for photosynthesis (Lumactud *et al.* 2023). Consequently, plants undergo a decline in photosynthetic rates, resulting in decreased production of carbohydrates and energy. This, in turn, hinders plant growth and development, ultimately affecting overall productivity (Aslani *et al.* 2023). Due to this world crisis, it is imperative to find effective solutions to enhance plant resilience to drought conditions and achieve a balance between irrigation water supply and demand. Consequently, the conservation of irrigation water has become a crucial objective for sustainable development across all countries (El-Hadidi *et al.* 2020 and Kikuchi *et al.* 2021). Melatonin hormone, also known as N-acetyl-5-methoxytryptamine, offers a promising solution to address the challenges posed by drought stress. It has shown positive effects in enhancing plant resistance against drought, as reported by Sharma *et al.* (2020). The study highlighted that melatonin plays a beneficial role by promoting the production of antioxidants within plants and regulating the functional components of photosynthesis, ultimately inducing drought

[*] Corresponding author.
E-mail address: M_Elsherpiny2010@yahoo.com
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resistance. Furthermore, Rafique *et al.* (2023) found that the application of melatonin hormone helps prevent the accumulation of reactive oxygen species (ROS) in plants. This supports the notion that melatonin can contribute to reducing oxidative stress caused by drought. Melatonin foliar application is considered a safe and environmentally friendly method to enhance plant resistance, as reported by Fleta-Soriano *et al.* (2017). This approach offers a potential strategy for boosting the resilience of plants against drought stress. The symbiotic interaction between rhizobia bacteria and legume plants, enabling the biological fixation of atmospheric nitrogen, holds significant importance for the growth of leguminous plants (Weisany *et al.* 2013 and Ohyama *et al.* 2017). Molybdenum (Mo), cobalt (Co), and copper (Cu) deficiencies are widespread in soils across various regions globally. On other hand, Mo, Co and Cu are essential trace elements for legumes *e.g.*, faba bean, common bean and soya bean and their associated bacteria, where their absence may restrict the development of free-living rhizobia in the rhizosphere and nodulation of host plants and impair the nodule functions(Schulin*et al.*2010). In addition to Mo importance for symbiotic nitrogen fixation bacteria, it is an essential component to convert nitrate in to ammonia for the synthesis of amino acid. Also, Mo helps convert inorganic phosphorus into organic forma available for plants to uptake (Shoaib *et al.* 2020). Co is a crucial element for legumes as it plays a vital role in the formation of nodules and the process of nitrogen fixation. It serves as a component in various enzymes and also enhances the ability of seeds to withstand drought, as mentioned by Sherif *et al. (*2017). Until recently, Co was considered beneficial but not essential for plant growth. However, according to the Official Journal of the European Union, REGULATION (EU) 2019/ 1009, Co has now been recognized as an essential element for the development of various plants. Similarly, Mo acts as a cofactor for nitrogenase, which is responsible for biological nitrogen fixation. Insufficient Mo levels can lead to reduced nodulation and nitrogen fixation, as discussed by Gericó *et al.* (2020). Co and Mo are essential for the vitamin B_{12} required synthesis for hemoglobin formation in nodules, therefore success N fixation (El-Sherpiny *et al.* 2021). The relationship between copper (Cu) requirements for symbiotic nitrogen fixation and its role in plant growth is not well-defined. The question of whether Cu directly influences nitrogen fixation is complex and can only be addressed through specific approaches. Snowball *et al.* (1980) observed that the effects of Cu on clover plant growth and nitrogen fixation occurred simultaneously and at similar levels of Cu supply. Wahab *et al.* (1996) reported that Cu increased nodule mass by 44.7% and absolute N_2-ase by 61.6%, along with elevated leghemoglobin and total nitrogen content in the foliage of faba bean plants. Cu is involved in the production of proteins necessary for N_2 fixation in rhizobia. Cu deficiency has been found to reduce nitrogen fixation in subterranean clover (Weisany *et al.* 2013).

To achieve the goals of this research, an in-depth analysis of pertinent literature has been undertaken, centring on the subsequent pivotal domains:

1.Water scarcity crisis in Egypt: Present status and future projections

Insufficient water resources and drought stress pose significant challenges to crop production in arid and semi-arid environments. Access to fresh water is crucial for economic development, both in Egypt. However, considering the high population growth (approximately 110 billion inhabitants) and limited water supplies, the prospects for future development in Egypt appear bleak (El Bedawy, 2014).

- **Current situation**

Mohamed, (2019) stated that the existing water resources in Egypt can be estimated as follows: (1) 55.5 BCM/year from the Egyptian budget of River Nile, (2) 1.6 BCM/year from rainfall on the northern strip of the Mediterranean Sea, including Sinai, (3) 2.4 BCM/year from deep groundwater resources, and 6.5 BCM/year from shallow groundwater resources. However, the actual water requirements amount to 79.5 BCM/year, revealing a significant deficit in available water resources. This sizable gap highlights the substantial water shortage of approximately 13.5 BCM/year. Apart from the scarcity of water resources and the widening gap between supply and demand, Aziz *et al.* (2019) indicated several additional challenges that pose a threat to the future management of Egypt's water budget. These challenges include evaporation losses, seepage from water canals, the expansion of rice and sugarcane cultivation due to their higher economic value compared to crops such as cotton and maize, and inefficiencies in irrigation practices. Furthermore, limited information is available about the Ethiopian Dam, which is situated in the western region of Ethiopia along the Blue Nile. The dam has a significant capacity to retain hundreds of billions of cubic meters of water. This situation could potentially result in a reduction of Egypt's share of the Nile water budget, impacting future agricultural reclamation plans and potentially leading to an increase in salinity in the northern part of the Nile Delta. (Abd Ellah, 2020; Bozorg-Haddad *et al.* 2020; Christoforidou *et al.* 2022).

- **Future prospects**

In response to the growing scarcity of irrigation water, scientists in Egypt are actively researching and developing innovative approaches to improve water management and ensure the long-term sustainability of crops. One such approach involves the development of alternative techniques for furrow irrigation or the introduction of substances that enhance plants' ability to tolerate water deficit conditions. These initiatives aim to conserve water resources while maintaining agricultural productivity in water-limited environments. Abd El-Dayem *et al.* (2015) investigated the impact of various antioxidants on the growth, yield, and quality of snap bean plants subjected to different levels of water stress. The results of the study demonstrated that the application of exogenous antioxidants led to improvements in the growth, yield, and quality of snap bean plants under water stress conditions. Rakha (2017) studied the response of common bean plants to different irrigation intervals. The irrigation intervals included short, moderate, and long intervals of every 7, 11, and 15 days, respectively. Additionally, foliar application of antioxidants was carried out as a treatment. The findings of the study revealed that the foliar application of antioxidants resulted in an improvement in the performance of the common bean plants compared to the control group (which did not receive any antioxidants), particularly under water deficit conditions. Lopes *et al.* (2019) assessed the efficacy of bioregulators and antioxidants in mitigating the impact of drought stress on common bean plants. The researchers discovered that both bioregulators and antioxidants exhibited promising potential as treatments to enhance the plant's tolerance to water stress. The application of these treatments led to improvements in the plant's defense mechanisms against drought stress, without causing any harmful side effects. These findings suggest that bioregulators and antioxidants can be valuable tools in mitigating the negative effects of water scarcity on common bean plants. Petropoulos *et al.* (2020) explored the impact of applying certain biostimulants on mitigating the detrimental effects of water stress on the yield and chemical composition of green beans. The researchers observed that the application of bio-stimulants positively influenced the nutritional value and chemical composition of both pods and seeds of green beans when subjected to water deficit conditions. These findings suggest that the application of bio stimulants can have a positive impact on improving the quality of green beans. Furthermore, they can mitigate the adverse consequences of water stress on both yield and the chemical composition of the beans. Gaafar *et al.* (2020) assessed the role of ascorbic acid as an antioxidant in alleviating the negative effects of irrigation water deficit on common bean plants. The study included two different irrigation regimes: one at 100% of crop evapotranspiration and the other at 50% of crop evapotranspiration. The researchers concluded that the response of common bean plants to water deficit seemed to be influenced by the presence of antioxidants, with ascorbic acid potentially playing a significant role in mitigating the adverse impacts of water stress on the plants.

2.Water deficit and plants performance

Water is an essential component in plant physiology, playing vital roles in various plant processes such as photosynthesis, transpiration, and nutrient uptake. The following are some of the key importance of water in plant physiology:

Photosynthesis: Water is a crucial component of the process of photosynthesis, which enables plants to produce their food. During photosynthesis, water molecules are split into hydrogen and oxygen, and the hydrogen is used to produce energy in the form of ATP. This energy is then used by plants to convert carbon dioxide into glucose, which they use as food (Taiz *et al.* 2015). Nutrient uptake: Water is the primary medium through which nutrients are transported from the soil to the plant. Nutrients dissolve in water and are then absorbed by the plant's roots. The water also helps to transport these nutrients throughout the plant's tissues (Del Río *et al.* 2015). Transpiration: Water is lost from the leaves of the plant through a process called transpiration. This loss of water helps to regulate the plant's temperature and humidity, and it helps to maintain the plant's shape and structure (Dodd *et al.* 2016). Turgor pressure: Water helps to maintain the rigidity and shape of plant cells through a process called turgor pressure. When a plant cell is fully hydrated, the water inside the cell exerts pressure on the cell walls, which helps to maintain the cell's shape (Chitarra *et al.* 2016).

Overall, water is a crucial component in plant physiology, and plants cannot survive without an adequate supply of water. On the other hand, water deficit, or lack of water, can have significant negative effects on grown plants, ultimately leading to reduced growth, decreased yield, and in severe cases, plant death. Here are some of the effects of water deficit on plants:

Reduced growth: Water deficit can limit a plant's ability to grow and develop, leading to stunted growth and reduced biomass production. Without enough water, plants may not be able to photosynthesize efficiently, which can lead to a reduction in plant size and yield (Salehi-Lisar *et al.* 2016). Wilting: Plants that are not receiving enough water may start to wilt as a result of the loss of turgor pressure in their cells. Wilting can be an early sign of a water deficit, and if the water shortage persists, it can become irreversible (Maimaitiyiming *et al.* 2017). Leaf rolling: In response to water deficit, plants may roll their leaves in an attempt to conserve water. This can lead to reduced leaf surface area, which can further reduce the plant's ability to photosynthesize (Jain *et al.* 2017). Chlorosis: Water deficit can cause the leaves of plants to turn yellow or brown, a condition known as chlorosis. This occurs because water is necessary for the transport of nutrients throughout the plant, and a lack of water can limit nutrient uptake and transport (Ihuoma *et al.* 2017). Reduced yield: Water deficit can significantly reduce the yield of crop plants, leading to economic losses for farmers and food shortages for populations that depend on these crops (Maimaitiyiming *et al.* 2017). Overall, water deficit can have severe consequences for grown plants, and it is essential to provide adequate water to plants to ensure healthy growth and maximum yield. Rakha (2017) examined the response of common bean plants to different irrigation intervals. The irrigation intervals included short (every 7 days), moderate (every 11 days), and long (every 15 days) intervals. The results indicated that as the irrigation interval increased up to 11 days, there was a decrease in various vegetative growth attributes of the plants. These included plant height (cm), plant fresh weight (g), number of branches, leaf number, and leaf area per plant. Additionally, the chemical composition of the leaves, specifically the levels of nitrogen (N), phosphorus (P), and potassium (K), as well as the photosynthesis pigments, were also negatively affected by the longer irrigation intervals. Gaafar *et al.* (2020) investigated the effects of water stress, specifically at 100% and 50% of crop evapotranspiration, on the performance of common bean plants. The results of the study demonstrated that water stress had various impacts on different aspects of the plants. Under water stress conditions, there was a reduction in common bean photosynthetic pigments, carbonic anhydrase activity, and antioxidant activities, including 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) radical cation assay. Additionally, water stress resulted in decreased growth performance and seed yield of the common bean plants. On the other hand, enzymatic antioxidants *e.g.,* peroxidase and secondary metabolites such as phenolic compounds, flavonoids and tannins, as well as MDA, a marker of oxidative stress, were increased under water deficit circumstances. Rai *et al.* (2020) evaluated the impact of different irrigation treatments on dry bean growth traits and yield components. The study involved five irrigation treatments: 25, 50, 75, 100 and 125% of FIT. The results showed that water deficit, particularly during the initial stages of crop growth, had a significant negative effect on the growth and development of dry bean plants. This water deficit resulted in a substantial reduction in seed yield. Specifically, reducing irrigation below the FIT level by 25% led to an average decrease of 30% in seed yield. Alves Souza *et al.* (2020) studied the impact of different irrigation frequencies on the growth, grain yield, and water productivity of beans was investigated. Three irrigation frequencies were considered: one day, four days, and eight days. The results showed that plant growth was not significantly influenced by the different irrigation frequencies. However, the biometric traits and chlorophyll synthesis of the beans were significantly affected by the irrigation frequencies. In terms of water productivity, the treatments with a one-day irrigation frequency were found to be more efficient in water usage compared to the other frequencies. This suggests that irrigating the beans every day resulted in higher water productivity, indicating a more effective utilization of water resources for crop production.

3.Role of melatonin as a hormone that suppresses the effects of water deficit on plants

Melatonin ($C_{13}H_{16}N_2O_2$) comprises an indole heterocycle and two distinct side chains: a 5-methoxy group and a 3-amide group as reported by Tan *et al.* (2002) who also illustrated the chemical structure of melatonin as shown in Fig 1.

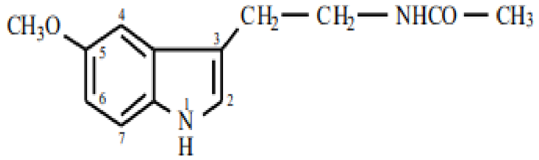

Fig 1. The structure of melatonin (C.F Tan *et al.* 2002)

Applied melatonin as a powerful antioxidant to mitigate the harmful effects of drought stress was done by a few scientists. Melatonin has been found to play a crucial role in combating both abiotic and biotic stresses while enhancing the postharvest lifespan of vegetables and fruits. The external application of melatonin has been shown to mitigate the harmful effects of ROS and cellular damage induced by various stresses. This is achieved through the repair of mitochondria, a vital organelle in cells. Furthermore, melatonin exhibits diverse functions within plant systems, which can contribute to the advancement of environmentally friendly crop production and the assurance of food safety (Sharif *et al.* 2018). Nonetheless, there is a necessity for additional investigation to comprehensively comprehend the involvement of melatonin in regulating stress reactions across diverse plant species and various growth phases. Sustained exploration in this field will contribute to unveiling the potential advantages of melatonin in augmenting stress resilience and the overall efficacy of plants. To our knowledge, no research has been done to evaluate the role of melatonin as a hormone that suppresses the effects of water deficit on red kidney bean plants (*Phasuolas vlugaris* L.) or its family. However little research has been done to evaluate the role of melatonin in suppressing the harmful effects of water deficit on other plants. According to the findings of Sharma and Zheng (2019), melatonin has demonstrated the ability to safeguard plants from the detrimental impacts of drought stress by enhancing the efficiency of ROS scavenging. By doing so, melatonin assists in shielding the photosynthetic apparatus of plants and reducing oxidative stress caused by drought. Furthermore, melatonin plays a crucial role in organizing plant processes at a molecular level, ultimately leading to improved resistance against the adverse effects of drought stress. These observations highlight the potential of melatonin as a protective agent in mitigating the negative consequences of drought stress on plants. Campos *et al.* (2019) investigated the impact of exogenous melatonin on the promotion of water deficit tolerance in *Coffea arabica* L. Two concentrations of melatonin, specifically 300 μM and 500 μM, were applied to the plants. The results revealed that lower concentrations of melatonin (300 μM) had a positive effect on the root system, leading to its enhancement, and also contributed to the protection of the photosynthetic apparatus. However, higher concentrations of melatonin (500 μM) exhibited negative effects on stress tolerance. These findings suggest that melatonin plays a crucial role in mediating the signaling pathways involved in responses to water deficit. At lower concentrations, melatonin acts as an inducer of tolerance, promoting stress resilience. Conversely, higher concentrations of melatonin may have adverse effects on the plant's ability to cope with water deficit. Overall, this study demonstrated that melatonin can act as a protective agent against water deficit stress, but the optimal concentration for achieving beneficial effects may vary depending on the specific plant species and environmental conditions. Sadak *et al.* (2020) studied the physiological effects of melatonin on Moringa plants under drought stress. The researchers applied foliar applications of melatonin at three different concentrations (0.0, 50, 100, and 150 mM).The results indicated that the application of melatonin at various concentrations had a positive impact on the growth criteria, qualitative and quantitative yield of *Moringa oleifera* plants under both normal irrigation and drought stress conditions. Additionally, melatonin treatment resulted in improvements in phenolic, element contents, photosynthetic pigments (IAA) and antioxidant enzyme systems. At the same time, it led to a decrease in MDA contents compared to the untreated control plants. Among the different melatonin concentrations tested, the foliar application of 100 mM showed the most significant positive influences on the growth performance and yield components, regardless of whether they were subjected to normal irrigation or drought stress. Sadak and Bakry (2020) assessed the role of melatonin (2.5, 5.0, and 7.5 mM) in enhancing plant tolerance to drought stress. The studied melatonin treatments were applied to two varieties of flax plants under normal irrigation (100% of traditional irrigation) and drought stress circumstances (75% and 50%). The results illustrated that drought stress led to a reduction in growth performance and chlorophyll pigment as well as the yield and its components. However, the external application of melatonin positively influenced the two flax varieties, counteracting the negative effects of drought stress. Likewise, in a study by Imran *et al.* (2021), the possible impact of applying external melatonin (via foliage or root zone) to enhance drought stress resilience in soybean seedlings was investigated. Melatonin was administered at concentrations of 50 and 100 μM, and its influence in the rhizosphere was also explored. The outcomes demonstrated that the application of melatonin at both concentrations notably alleviated the adverse consequences of drought stress on factors pertaining to plant growth and chlorophyll levels. Furthermore, Sadak and Ramadan (2021) investigated the effects of melatonin spraying at different concentrations on the growth performance as well as the biochemical aspects and yield traits of white lupine plants under water deficit stress conditions. Melatonin was applied at rates of 0.0, 50, 100, and 150 μM under varying water irrigation conditions (100%, 75%, or 50% of water irrigation requirements). The results revealed that under conditions of water deficit (at 75% or 50% of the required irrigation), there was a noteworthy decline in growth, indole acetic acid, photosynthetic pigments and yield when compared to plants that received complete water irrigation (100%). Nevertheless, the external administration of melatonin effectively mitigated the detrimental impacts of water deficit. This was evident through heightened phenolic contents, increased levels of hydrogen peroxide, lipid peroxidation, and certain antioxidant enzymes. Altaf *et al.* (2022) conducted a study to investigate the defensive role of melatonin in tomato seedlings under drought-stress conditions. The researchers observed that drought stress had a significant negative impact on the growth and biomass production of tomato seedlings. It inhibited photosynthesis, resulting in reduced chlorophyll content and adversely affecting the root morphology. However, when melatonin was applied as a pretreatment at a concentration of 100 µM, it effectively counteracted the detrimental effects of drought stress. The application of melatonin improved photosynthetic activity by restoring chlorophyll content and enhancing root architecture. Furthermore, the addition of melatonin also ameliorated the performance of antioxidant enzymes such as CAT, DHAR, APX, POD, and SOD, along with augmenting the activities of non-enzymatic antioxidants like DHA and AsA. Neamah and Jdayea, (2022) investigate the effects of melatonin (0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1}) on the sustainable production of biomass and secondary antioxidant metabolites in *Hyoscyamus pusillus* under both normal and water deficit circumstances. The findings indicated an escalation in oxidative stress, notably demonstrated by a significant rise in the levels of H_2O_2 and MDA. However, the introduction of melatonin treatment correlated with a reduction in oxidative stress. This reduction was attributed to the amplified presence of antioxidant enzymes, such as SOD and CAT, within the plant samples subjected to melatonin treatment. Consequently, this increase in antioxidant defense mechanisms led to heightened MSI (secondary antioxidant metabolites) levels and ultimately contributed to enhanced plant growth.

4.Biological N-fixation

Biological nitrogen fixation denotes the procedure during which specific categories of bacteria transform nitrogen gas (N_2) from the atmosphere into a usable form for plants, like ammonia (NH_3) or ammonium ions (NH_4^+). Plants such as legumes, encompassing crops such as beans, peas, and lentils, have the capacity to establish a mutually beneficial connection with these nitrogen-fixing bacteria. This alliance enables them to draw nitrogen from the air and integrate it into their own tissues (Raza *et al.* 2020). Biological nitrogen fixation within legumes takes place within distinct formations referred to as root nodules. These nodules are generated by the plant as a reaction to the existence of particular strains of bacteria, commonly from the Rhizobium genus. These bacteria invade the plant's root hairs, creating a mutually beneficial association with the plant cells. In this partnership, the plant cells offer the bacteria a supply of energy in the shape of carbohydrates, while the bacteria reciprocate by supplying the plant with nitrogen that has been converted into a usable form (Romanyà and Casals, 2020). Inside the nodules, the bacteria use an enzyme called nitrogenase to convert atmospheric nitrogen gas into ammonium ions. The ammonium ions are then used by the plant to synthesize amino acids, nucleotides, and other nitrogen-containing compounds that are essential for growth and development (Salgado *et al.* 2021). Overall, biological nitrogen fixation is an important process for maintaining soil fertility and supporting crop production, especially in systems where synthetic fertilizers are not used. Legumes play a critical role in this process by forming symbiotic relationships with nitrogen-fixing bacteria, allowing them to access nitrogen from the atmosphere and incorporate it into their tissues (Palmero *et al.*, 2022).

5.Stimulants of biological N-fixation

a.Molybdenum

Molybdenum plays a critical role as a vital micronutrient in the process of biological nitrogen fixation. This significance stems from its integral role within the nitrogenase enzyme, responsible for the conversion of atmospheric nitrogen into a plant-usable form. More precisely, molybdenum functions as a cofactor for the nitrogenase enzyme. This cofactor function entails aiding in the activation of the enzyme and assisting in the chemical transformation that changes nitrogen gas into ammonia (Reed *et al.* 2013). Almeida *et al.* (2013) examined the influence of molybdenum (Mo) in seeds on the growth and nitrogen (N) acquisition of bean plants under varying fertilization conditions, either through symbiotic N fixation or mineral N application. This investigation encompassed the assessment of nitrogenase and nitrate reductase activities, as well as the role of biological nitrogen fixation at different developmental stages. The experimentation involved planting Mo-enriched or standard seeds in soil-filled pots, with each pot containing 10 kg of soil. Two different sources of nitrogen were employed: one through inoculation with rhizobia for symbiotic N fixation, and the other through direct N-fertilization. Two experiments were carried out. In the first experiment, an added treatment involved Mo-enriched seeds along with molybdenum applied directly to the soil. In the second experiment, the extent of N_2 fixation was estimated through the use of the 15N isotope dilution technique. The outcomes of this study revealed that bean plants originating from seeds with elevated Mo concentrations exhibited an advancement in the flowering stage by one day. Seeds with heightened Mo concentrations contributed to an increase in leaf area, shoot mass, and N-accumulation, regardless of the nitrogen source employed. Moreover, seeds enriched with Mo demonstrated a notable enhancement in nitrogenase activity during the vegetative growth stage of plants inoculated with rhizobia, and an increase in nitrate reductase activity during the later stages of growth, irrespective of the nitrogen source. The investigation also determined that the contribution of N_2 fixation to plant nitrogen content was 17% and 61% for plants originating from seeds with low and high Mo concentrations, respectively. Adhikari *et al.* (2017) highlighted the intricate role of molybdenum in supporting nodule development and function. They found that Molybdenum's significance extends to the formation and operation of root nodules in leguminous plants engaged in a symbiotic association with nitrogen-fixing bacteria. This importance arises from molybdenum's necessity in the creation of specific enzymes pivotal for the synthesis of plant hormones and other essential compounds that contribute to both the advancement and effectiveness of nodules. In a study conducted by Da Silva *et al.* (2017), the impact of different rates of molybdenum (Mo) [0.0, 40, 80, 120 & 140 g ha^{-1}] on the growth and biological nitrogen fixation of two common bean cultivars was investigated. The findings demonstrated that applying Mo and employing rhizobia strains for inoculation led to enhanced nitrogen fixation and increased grain weight. The combination of Rhizobium inoculation and Mo supply effectively contributed to biological nitrogen fixation and positively influenced grain production. Darnajoux *et al.* (2017) highlighted that molybdenum plays a pivotal role in the proper functioning of nitrogenase, the enzyme responsible for initiating biological nitrogen fixation. The stability of nitrogen gas and its triple bond structure necessitates significant energy to convert it into a reactive form beneficial for plants. Nitrogenase, facilitated by molybdenum, is one of the rare enzymes capable of achieving this conversion. Adjetey and Ntombela (2019) illustrated that the application of molybdenum at a concentration of 0.5 g L^{-1} significantly promoted the formation of nodules in soybean plants. However, this molybdenum application did not have a discernible impact on the concentration and uptake of the measured nutrients. In the research by Biswas *et al.* (2020), the combined effect of biofertilizers and chemical fertilizers on protein content, nodulation, and bean production was explored. This study involved two strains of Rhizobium species, varying levels of nitrogen-N application (at 75%, 50%, and 25% of the recommended dose), and two levels of ammonium molybdate-Mo (at 50% and 100% of the recommended dose). The outcomes indicated that molybdenum treatments, particularly at lower nitrogen doses, led to increased productivity due to enhanced nodulation. Ahmad *et al.* (2022) conducted a study to examine the influence of molybdenum application at various rates (0.0, 0.5, 1.5, and 2.5 kg ha^{-1}) on the performance of mung bean plants. The findings of the research demonstrated that when molybdenum was applied at a rate of 1.5 kg ha^{-1}, it led to significant enhancements in several plant attributes. Specifically, the application of molybdenum at this rate resulted in improved metrics such as the number of nodules per plant, the number of seeds per pod, the number of pods per plant, the mass of a thousand seeds, as well as elevated levels of biological and grain production. Additionally, positive effects were observed in terms of plant height, protein content, carbohydrate content, and seed nitrogen content.Collectively, these studies emphasize the crucial role of molybdenum in biological nitrogen fixation, its impact on plant growth, and its potential to enhance nitrogen fixation and crop production when appropriately applied.

b. Cobalt

Cobalt is another essential micronutrient for biological nitrogen fixation. It plays a critical role in the synthesis of the nitrogenase enzyme, which is responsible for converting atmospheric nitrogen gas into ammonia. Specifically, cobalt is required for the biosynthesis of the iron-molybdenum cofactor (FeMo-co), which is a critical component of the nitrogenase enzyme. The FeMo-co is composed of iron, molybdenum, and sulfur atoms, and cobalt is required for the assembly of the sulfur atoms into the cofactor (Pattanayak *et al.*, 2000).Without cobalt, the nitrogenase enzyme cannot be fully synthesized and its activity will be severely limited. This can result in reduced biological nitrogen fixation, which can have negative impacts on plant growth and soil fertility (Bakken *et al.* 2004).Cobalt is also important for the formation and function of root nodules in legumes. This is because it is required for the synthesis of enzymes that are involved in the metabolism of carbohydrates and other compounds that are important for nodule development and functioning (Weisany *et al.*, 2013).Overall, cobalt plays a crucial role in biological nitrogen fixation by being a key component in the synthesis of the nitrogenase enzyme and supporting nodule development and functioning in legumes. In a study by Reyes *et al.* (2016), the effects of cobalt (Co) application were investigated on common bean plants under two fertilization systems: inorganic fertilization and inoculation with Rhizobium. Cobalt was applied through seed impregnation at doses of 0.26 g Co kg^{-1} seeds. The outcomes indicated that the application of cobalt resulted in an increase in the number and weight of nodules, as well as nitrogen content, specifically when combined with Rhizobium inoculation. The study concluded that cobalt application was most effective when beans were inoculated with rhizobia. Gad *et al.* (2018) reported that the addition of cobalt at various rates (4, 8, 12, 16, and 20 mg L^{-1}) significantly improved growth and yield parameters, along with the mineral content in the leaves of green bean plants. These effects were observed across different levels of water regimes (100%, 80%, and 60%) in comparison to untreated plants. Vaseer *et al.* (2020) explored the impact of cobalt application on bean seeds treated with inoculation slurry. Cobalt was applied as cobalt nitrate at rates of 10, 20, and 30 mg L^{-1} in combination with inoculation using three bean varieties. The results demonstrated that applying cobalt at a rate of 30 mg L^{-1} yielded the most favorable outcomes in terms of growth, yield attributes, nodule number, nodule weight, and grain yield. In the research by Baddour *et al.* (2021), the combined effect of Rhizobium inoculation, nitrogen application at different rates (100%, 75%, and 50% of the recommended dose), and cobalt supplementation at varying concentrations (0.0, 8.0, and 12.0 mg cobalt L^{-1}) was examined using a combined method of soaking and foliar application. The best results were achieved when plants were inoculated with a specific Rhizobium strain, nitrogen was applied at a rate of 15 kg N fed^{-1}, and cobalt was treated at rates of 8.0 and 12.0 mg cobalt L^{-1}. Hatamman (2021) investigated the effects of cobalt addition on the growth performance and yield of *Phaseolus vulgaris* using three concentrations of cobalt (0.0, 5.0, and 10.0 mg Co L^{-1} as cobalt sulfate). The study demonstrated that cobalt addition at concentrations of 5 and 10 mg L^{-1} yielded the most favorable outcomes in terms of leaf number, plant height, leaf area and number of root nodes as well as pod weight and yield.

c. Copper

Wahab *et al.* (1996) reported that copper (Cu) promoted nodule mass by 44.7% and absolute N_2-ase by 61.6% as well as it increased leghaemoglobin and total nitrogen content of in foliage of faba bean plants. Copper (Cu) is an essential micronutrient that plays a crucial role in many biological processes, including biological nitrogen fixation. Copper plays a role in proteins that are required for N_2 fixation in rhizobia. Cu deficiency decreased nitrogen fixation in subterranean clover (Klevay, 2002). Copper is a cofactor for the enzyme nitrogenase, which is responsible for the conversion of N_2 to ammonia in many nitrogen-fixing bacteria. Nitrogenase contains two metalloclusters: the

FeMo-cofactor and the P-cluster. The P-cluster contains a copper ion, which is essential for the proper functioning of nitrogenase (Weisany *et al.* 2013).The copper ion in the P-cluster helps to transfer electrons during the reduction of nitrogen, allowing the enzyme to convert N_2 to NH_3. Without copper, the electron transfer process would be impaired, and nitrogen fixation would be severely limited or non-existent (Luo *et al.* 2014). In addition to its role in nitrogen fixation, copper is also involved in other metabolic processes in bacteria, such as respiration and oxidative stress defense. Thus, a deficiency in copper can have detrimental effects on the growth and survival of nitrogen-fixing bacteria (Murtaza *et al.* 2017). Overall, copper plays a critical role in biological nitrogen fixation by supporting the activity of nitrogenase, the enzyme responsible for converting atmospheric nitrogen into forms that can be used by plants. Fatnassi *et al.,* (2015) investigated the impact of dual inoculation with both Rhizobium and plant growth-promoting bacteria (PGPR) strains on the growth of Vicia faba plants exposed to copper. Copper (Cu) was applied at various rates: 0.0, 0.2, 0.5, 1.0, and 2.0 mM. The application of copper above a concentration of 1.0 mM was found to be detrimental to plant growth. However, the inoculation with copper appeared to alleviate its harmful effects. In particular, the co-inoculation of plants treated with 1.0 and 2.0 mM copper led to increased dry weights compared to copper-treated plants without inoculation. Notably, this co-inoculation approach also resulted in a reduction of copper uptake by up to 80% in the roots of plants treated with 1.0 mM copper. Furthermore, the treatment with 1.0 mM copper positively impacted the activities of antioxidant enzymes in the plant. Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities were enhanced in the roots. In shoots, only SOD and peroxidase (POX) were activated. Chou *et al.* (2019) investigated the response of heavy metal-tolerant legumes when exposed to varying concentrations of copper. Copper was applied at different rates: 0.0, 50, 100, 150, and 200 mg kg^{-1}. The research outcomes indicated several significant findings regarding the impact of copper on the growth and development of these legumes. At copper concentrations of 100 mg kg^{-1} and higher, the growth parameters of the legumes were negatively affected. Plant height, root length, and lateral root number per plant were suppressed when the copper concentration reached 100 mg kg^{-1} or more. Furthermore, the number of nodules per plant and nitrogenase activity displayed a declining trend at copper levels of 100 mg kg^{-1} and above. However, at a copper concentration of 50 mg kg^{-1}, the number of nodules per plant and nitrogenase activity increased. It was also observed that overall, legumes that were inoculated with rhizobia showed better growth and development compared to those that were not inoculated, particularly under the same copper treatment conditions.

CONCLUSION

In conclusion, this study highlights the potential of nutrient solutions in enhancing nitrogen fixation under drought stress as well as melatonin application in mitigating the impacts of water deficit on plants. However, further research and field trials are recommended to fine-tune the application methods and understand the complex interactions between these interventions for sustainable and effective crop management.

RECOMMENDATION

1. Since water deficit significantly affects plant performance and yield, it is recommended to implement irrigation strategies that ensure an adequate supply of water to the plants, especially during critical growth stages.
2. Considering the positive effects of molybdenum, cobalt, and copper solutions on nodulation and growth, incorporating these nutrients through appropriate fertilization strategies could aid in improving the plant resilience to water deficit conditions. However, further studies should focus on determining optimal application rates and timings.
3. The use of melatonin as a foliar application demonstrated its potential to enhance the plant's ability to cope with water deficit stress. Further research is recommended to delve into the underlying mechanisms of how melatonin exerts its protective effects.
4. While nutrient solutions and melatonin application appeared to reduce the plant's inherent production of enzymatic antioxidants, a delicate balance needs to be maintained. Future studies should explore ways to maintain adequate antioxidant levels while benefiting from the growth-enhancing effects of nutrient solutions and melatonin.

REFERENCES

Abd El-Dayem, H. M., Mady, M. A., Abd El-All, M. M., & Eid, R. S. (2015). Effect of some antioxidants, potassium and arbuscular mycorrhiza on growth, yield and quality of snap bean plants grown under water stress levels. Annals of Agric. Sci., Moshtohor, 53, 15-30.

Abd Ellah, R. G. (2020). Water resources in Egypt and their challenges, Lake Nasser case study. The Egyptian Journal of Aquatic Research, 46(1), 1-12.

Adhikari, L., & Missaoui, A. M. (2017). Nodulation response to molybdenum supplementation in alfalfa and its correlation with root and shoot growth in low pH soil. Journal of Plant Nutrition, 40(16), 2290-2302.

Adjetey, J.A., & Ntombela, Z. (2019). Lime and foliar application of molybdenum affects nodulation, nutrient uptake and pod production in soybean grown in acid soils. Bots. J. Agric. Appl. Sci., 13., 57–65.

Ahmad, J ; Anwar, S ; Shad, A.A ; Souri, S.S ; Amina, B ; Noor, W ; Abidullah & Adil , M. (2022). Influence of molybdenum and phosphorus application on yield, yield components, carbohydrates and protein content of moonbeam Pakistan Journal of Agricultural Research., 35(1)., 93-104.

Almeida, F. F. D., Araújo, A. P., & Alves, B. J. R. (2013). Seeds with high molybdenum concentration improved growth and nitrogen acquisition of rhizobium-inoculated and nitrogen-fertilized common bean plants. Revista Brasileira de Ciência do Solo, 37, 367-378.

Altaf, M. A., Shahid, R., Ren, M. X., Naz, S., Altaf, M. M., Khan, L. U., & Ahmad, P. (2022). Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. Antioxidants, 11(2), 309.

Alves Souza, S., Higino Vieira, J., Bispo dos Santos Farias, D., da Silva, G. H., & Cabral Aleman, C. (2020). Impact of irrigation frequency and planting density on bean's morpho-physiological and productive traits. Water, 12(9), 2468.

Aslani, Z., Hassani, A., Mandoulakani, B. A., Barin, M and Maleki, R. (2023). Effect of drought stress and inoculation treatments on nutrient uptake, essential oil and expression of genes related to monoterpenes in sage (*Salvia officinalis*). Scientia Horticulturae, 309, 111610.

Aziz, S. A., Zeleňáková, M., Mésároš, P., Purcz, P., & Abd-Elhamid, H. (2019). Assessing the potential impacts of the Grand Ethiopian Renaissance Dam on water resources and soil salinity in the Nile Delta, Egypt. Sustainability, 11(24), 7050.

Baddour, A. G., El-Sherpiny, M. A., & Sakara, H. M. (2021). Effect of rhizobium inoculant, nitrogen starter and cobalt on stimulation of nodulation, n fixation and performance of faba bean (*Vicia faba* L.) grown under salinity stress. Journal of Soil Sciences and Agricultural Engineering, 12(2), 61-69.

Bakken, A. K., Synnes, O. M., & Hansen, S. (2004). Nitrogen fixation by red clover as related to the supply of cobalt and molybdenum from some Norwegian soils. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 54(2), 97-101.

Berrios, L., & Rentsch, J. D. (2022). Linking reactive oxygen species (ROS) to abiotic and biotic feedbacks in plant microbiomes: The dose makes the poison. International Journal of Molecular Sciences, 23(8), 4402.

Biswas, S., Banerjee, A., Acharyya, P., & Chakraborty, N. (2020). Response of French bean (*Phaseolus vulgaris* L. cv. Arka Arjun) to Rhizobium inoculation under varied levels of nitrogen and molybdenum. Int. J. Curr. Microbiol. App. Sci., 9(3)., 2759-2767.

Bozorg-Haddad, O., Zolghadr-Asli, B., Sarzaeim, P., Aboutalebi, M., Chu, X., & Loáiciga, H. A. (2020). Evaluation of water shortage crisis in the Middle East and possible remedies. Journal of Water Supply: Research and Technology-Aqua, 69(1), 85-98.

Campos, C. N., Ávila, R. G., de Souza, K. R. D., Azevedo, L. M., & Alves, J. D. (2019). Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* L. plants. Agricultural water management, 211, 37-47.

Chitarra, W., Pagliarani, C., Maserti, B., Lumini, E., Siciliano, I., Cascone, P., & Guerrieri, E. (2016). Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. *Plant Physiology*, *171*(2), 1009-1023.

Chou, M., Sun, Y., Yang, J., Wang, Y., Li, Y., Yuan, J., Zhang, D., Wang, J., & Wei, G. (2019). Comprehensive analysis of phenotype, microstructure and global transcriptional profiling to unravel the effect of excess copper on the symbiosis between nitrogen-fixing bacteria and *Medicago lupulina*. Science of the Total Environment, 656 ., 1346–1357.

Christoforidou, M., Borghuis, G., Seijger, C., van Halsema, G. E., & Hellegers, P. (2022). Food security under water scarcity: a comparative analysis of Egypt and Jordan. *Food Security*, 1-15.

Da Silva, A., Franzini,V.I., Piccolla,C.D ., & Muraoka,T.(2017). Molybdenum supply and biological fixation of nitrogen by two Brazilian common bean cultivars. Revista Brasileira de Engenharia Agrícolae Ambiental, 21, 100-105.

Darnajoux, R., Zhang, X., McRose, D. L., Miadlikowska, J., Lutzoni, F., Kraepiel, A. M., & Bellenger, J. P. (2017). Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications for current biological nitrogen fixation estimates. New Phytologist, 213(2), 680-689.

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in environmental science, 2, 53.

Del Río, L. A. (2015). ROS and RNS in plant physiology: an overview. Journal of Experimental Botany, 66(10), 2827-2837.

Dodd, I. C., & Ryan, A. C. (2016). Whole-plant physiological responses to water-deficit stress. eLS, 1-9.

El Bedawy, R. (2014). Water resources management: alarming crisis for Egypt. J. Mgmt. & Sustainability, 4, 108.

El-Hadidi, E.M., Meleha, A.M.I., El-Tobgy, S.M.M., & Abo El-Ezz, S.F. (2020). Response of rice (*Oriza Sativa* L.) to some antitranspiratiors under water stress in North Nile Delta, Egypt. Plant Archives, 20(2): 2210-2220.

El-Sherpiny, M. A., El-Bakry, F.A., Ibrahim, N.R., & El-Shaboury, H.A. (2023). Role of compost, biochar and sugar alcohols in raising the maize tolerance to water deficit conditions. Egyptian Journal of Soil Science, 63(1), 67-81.

El-Sherpiny, M.A., Baddour, A. G., & El-Kafrawy, M. M. (2020). Effect of zeolite soil addition under different irrigation intervals on maize yield (*Zea mays* L.) and some soil properties. Journal of Soil Sciences and Agricultural Engineering, 11(12):793-799.

Fatnassi, I.C., Chiboub, M., Saadani, O., Jebara, M., & Jebara, S.H.(2015). Impact of dual inoculation with Rhizobium and PGPR on growth and antioxidant status of *Vicia faba* L. under copper stress. C. R. Biologies .,338 ., 241–254.

Fleta-Soriano, E., Díaz, L., Bonet, E. and Munné-Bosch, S. (2017). Melatonin may exert a protective role against drought stress in maize. Journal of Agronomy and Crop Science, 203(4): 286-294.

Gaafar, A. A., Ali, S. I., El-Shawadfy, M. A., Salama, Z. A., Sękara, A., Ulrichs, C., & Abdelhamid, M. T. (2020). Ascorbic acid induces the increase of secondary metabolites, antioxidant activity, growth, and productivity of the common bean under water stress conditions. Plants, 9(5), 627.

Gad, N., Abd El Zaher Fatma, H., Abd El Maksoud, H. K., & Abd El-Moez, M. R. (2011). Response of faba bean (*Vicia faba* L.) to cobalt amendements and nitrogen fertilization. The African Journal of Plant Science and Biotechnology. Global Science Books, 41-45.

Gad, N., Ali, M. F., Abbas, M. M., & Abdel-Moez, M. R. (2018). Maximization of drought tolerance of bean plants using cobalt supplementation A-Growth, Yield and nutritional status. Middle East Journal, 7(4):1819-1826.

Gad, N., Ali, M.E.F., Abbas, M.M., & Abdel-Moez, M.R. (2018). Maximization of drought tolerance of bean plants using cobalt supplementation A-Growth, Yield and nutritional status. Middle East Journal of Agriculture Research.,7(4)., 1819-1826.

Gechev, T., & Petrov, V. (2020). Reactive oxygen species and abiotic stress in plants. International Journal of Molecular Sciences, 21(20), 7433.

Gericó, T. G., Tavanti, R. F. R., de Lima, J. P., Ribeiro, R. P., dos Santos, L. C. C., da Silva, M. S ., & dos Reis, A. R. (2020). Cobalt and molybdenum stimulate compounds of primary metabolism, nitrogen forms, and photosynthetic pigments in peanut plants (*Arachishypogaea* L.). Journal of Plant Nutrition, 43(12): 1907-1922.

Hardy, R. W., Holsten, R. D., Jackson, E. K., & Burns, R. C. (1968). The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant physiology, 43(8): 1185-1207.

Hasanuzzaman, M., Bhuyan, M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants, 9(8), 681.

Hatamman, F.D., & Abdullah A.A.A. (2021). Effect of soil mulching and cobalt on growth and yield of (*Phaseolus Vulgaris* L.) under protected conditions. Earth Environ. Sci., 910.

Heshmat, K., Asgari Lajayer, B., Shakiba, M. R., & Astatkie, T. (2021). Assessment of physiological traits of common bean cultivars in response to water stress and molybdenum levels. Journal of Plant Nutrition, 44(3): 366-372.

Huber, D., Römheld, V., & Weinmann, M. (2012). Relationship between nutrition, plant diseases and pests. In Marschner's mineral nutrition of higher plants (pp. 283-298). Academic Press.

Ihuoma, S. O., & Madramootoo, C. A. (2017). Recent advances in crop water stress detection. Computers and Electronics in Agriculture, 141, 267-275.

Imran, M., Latif Khan, A., Shahzad, R., Aaqil Khan, M., Bilal, S., Khan, A., & Lee, I. J. (2021). Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. Plants, 13(4).

Jain, V. K. (2017). Fundamentals of plant physiology. S. Chand Publishing.

Jambunathan, N. (2010). Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. Plant stress tolerance: methods and protocols, 291-297.

Kandil, H., Gad, N., & Abdelhamid, M. T. (2013). Effects of different rates of phosphorus and molybdenum application on two varieties common bean of (*Phaseolus vulgaris* L.). Journal of Agriculture and Food Technology, 3(3): 8-16.

Kesawat, M. S., Satheesh, N., Kherawat, B. S., Kumar, A., Kim, H. U., Chung, S. M., & Kumar, M. (2023). Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—Current perspectives and future directions. Plants, 12(4), 864.

Kikuchi, M., Mano, Y., Njagi, T. N., Merrey, D., & Otsuka, K. (2021). Economic viability of large-scale irrigation construction in Sub-Saharan Africa: What if Mwea Irrigation Scheme Were Constructed as a Brand-new Scheme?. The Journal of Development Studies, 57(5): 772-789.

Klevay, L. M. (2002). Copper in legumes may lower heart disease risk. Archives of internal medicine, 162(15), 1780-1781.

Liu, Y., & He, C. (2016). Regulation of plant reactive oxygen species (ROS) in stress responses: learning from ATRBOHD. Plant Cell Reports, 35, 995-1007.

Lopes, L. S., Nobre, D. A. C., & Macedo, W. R. (2019). Effect of foliar application of 24-epibrassinolide and salicylic acid on common bean plants grown under drought stress. *Emirates Journal of Food and Agriculture*, 635-644.

Lumactud, R. A., Dollete, D., Liyanage, D. K., Szczyglowski, K., Hill, B., & Thilakarathna, M. S. (2023). The effect of drought stress on nodulation, plant growth, and nitrogen fixation in soybean during early plant growth. Journal of Agronomy and Crop Science, 209(3), 345-354.

Luo, Y. W., Xie, W. H., Jin, X. X., Wang, Q., & He, Y. J. (2014). Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes. CyTA-Journal of Food, 12(1), 22-26.

Maimaitiyiming, M., Ghulam, A., Bozzolo, A., Wilkins, J. L., & Kwasniewski, M. T. (2017). Early detection of plant physiological responses to different levels of water stress using reflectance spectroscopy. Remote Sensing, 9(7), 745.

Malhi, S. S., & Karamanos, R. (2014). Feasibility of copper fertilization for optimum crop yield in the Canadian Prairies. Maszynopis. Dostępne na stronie: http://www. neag. ca/ResearchResults/Articles. aspx (1.04. 2014).

Menzies, J. G., & Turkington, T. K. (2015). An overview of the ergot (*Claviceps purpurea*) issue in western Canada: Challenges and solutions. Canadian Journal of Plant Pathology, 37(1), 40-51.

Mishra, P. K., Tripathi, J., Gupta, S., & Variyar, P. S. (2017). Effect of cooking on aroma profile of red kidney beans (*Phaseolus vulgaris* L.) and correlation with sensory quality. Food chemistry, 215, 401-409.

Mittler, R., Zandalinas, S. I., Fichman, Y ., & Van-Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. Nature Reviews Molecular Cell Biology, 23(10), 663-679.

Mohamed, N. N. (2019). Egyptian food insecurity under water shortage and its socioeconomic impacts. Conventional Water Resources and Agriculture in Egypt, 245-273.

Murtaza, G., Javed, W., Hussain, A., Qadir, M., & Aslam, M. (2017). Soil-applied zinc and copper suppress cadmium uptake and improve the performance of cereals and legumes. International Journal of Phytoremediation, 19(2), 199-206.

Neamah, S. I., & Jdayea, N. A. (2022). Positive response of *Hyoscyamus pusillus* callus cultures to exogenous melatonin on biochemical traits and secondary metabolites under drought conditions. International Journal of Agronomy. Vol (2022).

Official Journal of the European Union. Regulation (Eu) 2019/1009 of the European Parliament and of the Council of 5 June 2019 Laying down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003. L 170. 25 June 2019, Volume 62. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2019:170:FULL&from=EN (accessed on 22 January 2020).

Ohyama, T., Tewari, K., Ishikawa, S., Tanaka, K., Kamiyama, S., Ono, Y a., & Takahashi, Y. (2017). Role of nitrogen on growth and seed yield of soybean and a new fertilization technique to promote nitrogen fixation and seed yield. The Basis of Yield, Biomass and Productivity (pp. 153-185).

Palmero, F., Fernandez, J. A., Garcia, F. O., Haro, R. J., Prasad, P. V., Salvagiotti, F., & Ciampitti, I. A. (2022). A quantitative review into the contributions of biological nitrogen fixation to agricultural systems by grain legumes. European Journal of Agronomy, 136, 126514.

Pattanayak, S. K., Dash, D., Jena, M. K., & Nayak, R. K. (2000). Seed treatment of green gram with molybdenum and cobalt: effect on nodulation, biomass production and N uptake in an acid soil. Journal of the Indian Society of Soil Science, 48(4), 769-773.

Petropoulos, S. A., Fernandes, Â., Plexida, S., Chrysargyris, A., Tzortzakis, N., Barreira, J. C., & Ferreira, I. C. (2020). Biostimulants application alleviates water stress effects on yield and chemical composition of greenhouse green bean (*Phaseolus vulgaris* L.). Agronomy, 10(2), 181.

Rafique, N., Ilyas, N., Aqeel, M., Raja, N. I., Shabbir, G., Ajaib, M., & Ansari, M. J. (2023). Interactive effects of melatonin and salicylic acid on *Brassica napus* under drought condition. Plant and Soil, 1-20.

Rai, A., Sharma, V., & Heitholt, J. (2020). Dry bean (*phaseolus vulgaris* L.) growth and yield response to variable irrigation in the arid to semi-arid climate. Sustainability, 12(9), 3851.

Rakha, M. K. A. (2017). Response of common bean (*Phaseolus vulgaris* L) to foliar application with some antioxidants under different irrigation intervals. Curr. Sci. Int, 6(4), 920-929.

Raza, A., Zahra, N., Hafeez, M. B., Ahmad, M., Iqbal, S., Shaukat, K., & Ahmad, G. (2020). Nitrogen fixation of legumes: Biology and Physiology. The Plant Family Fabaceae: Biology and Physiological Responses to Environmental Stresses, 43-74.

Reed, S. C., Cleveland, C. C., & Townsend, A. R. (2013). Relationships among phosphorus, molybdenum and free-living nitrogen fixation in tropical rain forests: results from observational and experimental analyses. Biogeochemistry, 114, 135-147.

Reyes, J.O., Bendezú, S.G ., & Joaquín, A.H.(2016). Molybdenum and cobalt application in bean (*Phaseoluvulgaris* L.) with two fertilization systems under No-Tillage. Journal of Agricultural Science and Technology B., 6., 72-77.

Romanyà, J., & Casals, P. (2020). Biological nitrogen fixation response to soil fertility is species-dependent in annual legumes. Journal of Soil Science and Plant Nutrition, 20(2), 546-556.

Sadak, M. S., & Bakry, B. A. (2020). Alleviation of drought stress by melatonin foliar treatment on two flax varieties under sandy soil. Physiology and molecular biology of plants, 26, 907-919.

Sadak, M. S., & Ramadan, A. A. E. M. (2021). Impact of melatonin and tryptophan on water stress tolerance in white lupine (*Lupinus termis* L.). Physiology and Molecular Biology of Plants, 27, 469-481.

Sadak, M. S., Abdalla, A. M., Abd Elhamid, E. M., & Ezzo, M. I. (2020). Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress. Bulletin of the National Research Centre, 44(1), 1-13.

Saed-Moucheshi, A., Shekoofa, A., & Pessarakli, M. (2014). Reactive oxygen species (ROS) generation and detoxifying in plants. Journal of Plant Nutrition, 37(10), 1573-1585.

Salehi-Lisar, S. Y., & Bakhshayeshan-Agdam, H. (2016). Drought stress in plants: causes, consequences, and tolerance. Drought Stress Tolerance in Plants, Vol 1: Physiology and Biochemistry, 1-16.

Salgado, G. C., Ambrosano, E. J., Rossi, F., Otsuk, I. P., Ambrosano, G. M. B., Santana, C. A., & Trivelin, P. C. O. (2021). Biological N fixation and N transfer in an intercropping system between legumes and organic cherry tomatoes in succession to green corn. Agriculture, 11(8), 690.

Schulin, R., Johnson, A., & Frossard, E. (2010). Trace element deficient soils. Trace elements in soils, 175-197.

Sharif, R., Xie, C., Zhang, H., Arnao, M. B., Ali, M., Ali, Q. & Li, Y. (2018). Melatonin and its effects on plant systems. Molecules, 23(9), 2352.

Sharma, A. and Zheng, B. (2019). Melatonin mediated regulation of drought stress: Physiological and molecular aspects. Plants, 8(7): 190.

Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., Yan, D., & Zheng, B. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted Carya cathayensis plants. Science of the Total Environment, 713, 136675.

Sherif, A., ElKhalawy, S., & Hegab, E. (2017). Impact of nitrogen and cobalt rates on faba bean crop grown on clayey soil. Journal of Soil Sciences and Agricultural Engineering, 8(9): 459-465.

Shoaib, R.M., Bhantana, P., Imran, M., Saleem, M. H., Moussa, M. G., Khan, Z., & Hu, C. (2020). Molybdenum potential vital role in plants metabolism for optimizing the growth and development. Annals of Environmental Science and Toxicology, 4(1), 032-044.

Sidra, Y., Khan, A., Saleem, M. H., Ali, B., Qureshi, K. A., & Jaremko, M. (2022). Melatonin as a foliar application and adaptation in lentil (*Lens culinaris Medik.)* crops under drought stress. Sustainability, 14(24), 16345.

Singh, R., Singh, S., Parihar, P., Mishra, R. K., Tripathi, D. K., Singh, V. P., & Prasad, S. M. (2016). Reactive oxygen species (ROS): beneficial companions of plants' developmental processes. Frontiers in plant science, 7, 1299.

Snowball, K., Robson, A. D., & Loneragan, J. F. (1980). The effect of copper on nitrogen fixation in subterranean clover (*Trifolium subterraneum*). New Phytologist, 85(1): 63-72.

Solórzano, E., Corpas, F. J., González-Gordo, S., & Palma, J. M. (2020). Reactive oxygen species (ROS) metabolism and nitric oxide (NO) content in roots and shoots of rice (*Oryza sativa* L.) plants under arsenic-induced stress. Agronomy, 10(7), 1014.

Sourour, A., Afef, O., Mounir, R., & Mongi, B. Y. (2017). A review: morphological, physiological, biochemical and molecular plant responses to water deficit stress. International Journal of Engineering Science Journal, 6(1): 1-4.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development (No. Ed. 6). Sinauer Associates Incorporated.

Tan, D. X., Reiter, R. J., Manchester, L. C., Yan, M. T., El-Sawi, M., Sainz, R. M., & Hardeland, R. (2002). Chemical and physical properties and potential mechanisms: melatonin as a broad-spectrum antioxidant and free radical scavenger. Current topics in medicinal chemistry, 2(2), 181-197.

Tunley, D. R. (1971). Book Review: Enzymes and the Determination of Enzyme Activity.

Vaseer, S.G., Rasheed, M; Ansar, M., Bibi, Y., Shah, S., Hassan, A., Durani, L.A., Asif, A ., & Zuhair Husnain. (2020). Cobalt application improves the growth and development of mung bean. Pakistan Journal of Agricultural Research, 33(2), 303-310.

Wahab, A. A., Abd-Alla, M. H., & El-Enany, A. E. (1996). Stimulation of nodulation, nitrogen fixation and plant growth of faba bean by cobalt and copper additions. In Fertilizers and environment (pp. 127-130). Springer, Dordrecht.

Waszczak, C., Carmody, M., & Kangasjärvi, J. (2018). Reactive oxygen species in plant signaling. Annual review of plant biology, 69, 209-236.

Weisany, W., Raei, Y., & Allahverdipoor, K. H. (2013). Role of some of mineral nutrients in biological nitrogen fixation. Bulletin of Environment, Pharmacology and Life Sciences, 2(4), 77-84.

Yu, G. H., Kuzyakov, Y., Luo, Y., Goodman, B. A., Kappler, A., Liu, F. F., & Sun, F. S. (2021). Molybdenum bioavailability and asymbiotic nitrogen fixation in soils are raised by iron (oxyhydr) oxide-mediated free radical production. Environmental Science & Technology, 55(21), 14979-14989.

تأثير إجهاد الجفاف على إنتاجية محاصيل الخضروات: دور الميلاتونين المخفف وكذلك الريزوبيوم في تعزيز تثبيت النيتروجين في ظل ندرة المياه

مدحت عصام الصعيدي[1]، دينا شعبان لطفي عوض هلالي[1]، محمد عاطف الشربيني[2] و احمد عبد القادر طه[1]

[1]قسم الأراضي -كلية الزراعة – جامعة المنصورة -مصر.
[2]معهد بحوث الأراضي والمياه والبيئة – مركز البحوث الزراعية – الجيزة – مصره

الملخص

هذه المراجعة تهدف إلى تقديم نظرة شاملة للحالة الحالية للمعرفة حول تأثير إجهاد الجفاف على إنتاج محاصيل الخضروات، بالإضافة إلى تأثير تطبيق الميلاتونين على النباتات المعرضة لإجهاد الجفاف. بالإضافة إلى ذلك، تهدف الدراسة إلى التحقيق في الدور المفيد للريزوبيوم في تثبيت النيتروجين مع استخدام محفزات مختلفة مثل الموليبدينوم والكوبالت والنحاس. من خلال فحص المراجع الحالية، تسعى هذه المراجعة إلى تجميع وتحليل النتائج الرئيسية والاتجاهات والثغرات في البحث حتى الآن. سيتم التركيز أيضًا في هذه المراجعة على أزمة ندرة المياه في مصر، بما في ذلك الحالة الحالية والتوقعات المستقبلية. ايضا تم استعراض تأثير نقص المياه على أداء النباتات، وتم التحقق أيضًا من دور الميلاتونين كهرمون يخفف من تأثيرات نقص المياه على النباتات. بالإضافة إلى ذلك، تم استعراض تثبيت النيتروجين البيولوجي والمحفزات له. وبالإضافة إلى ذلك، ستقوم هذه المراجعة بتحديد التحديات والفرص الكبيرة في هذا المجال، بهدف توجيه اتجاهات البحث المستقبلية وتطبيقاتها العملية.